A LABORATORY STUDY OF THE SIMILARITIES AND DIFFERENCES OF RHIZOCTONIA CULTURES

by

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INTRODUCTION

The fungi belonging to the genus Rhizoctonia are among the most common and universally distributed soil fungi, a number of them being aggressive plant pathogenes. The most common species of Rhizoctonia in the United States is R. solani Kühn which was found by Rolfs (1904) to produce a basidial stage that is named Corticium vagum B. & C. A number of strains of Rhizoctonia solani occur and each have their preferred host plants. When Rhizoctonias are cultured, differences in the cultural characteristics commonly occur and these differences, if sufficiently great, are indicative of strains. The purpose of this investigation has been to separate strains of Rhizoctonia by noting differential cultural characteristics of various pure lines.

REVIEW OF LITERATURE

Many and varied reports have been made on the diameters of the hyphae of Rhizoctonia. Matz (1921) and Gadd and Bertus (1928) recorded the diameter of R. solani as being 8 to 12 microns, while Wellensiek (1924) reported this to be from 9 to 10 microns. Briton-Jones (1924) found that in cultures of R. solani of all ages, the hyphae of all forms vary within the same limits. Shaw (1912) found the hyphae of 24-hour cultures to be 8 to 9 microns in diameter, but those of 10-day cultures to be only 6 to 8 microns in diameter and stated

that the morphological characteristics of the fungus appear to vary with the age of the cultures. Peltier (1916), after making many measurements, concluded that the strains can not be distinguished by the dimensions of the hyphae because of the great variation that exists within each strain.

The size of the hyphae has been found to vary with their age. Monteith and Dahl (1928) avoided this variability by measuring the diameters of the hyphae just back of the most recently formed lateral branches in 2-day cultures.

It was noted by Monteith and Dahl (1928) that size of hyphal diameter varies according to the temperatures at which the cultures are grown and because of temperature relations, strains that normally produce the largest hyphae may easily be mistaken for strains that normally produce smaller hyphae. Matz (1921) also noted variations in the size of young mycelium due to different temperature relations.

Duggar (1915) and Gadd and Bertus (1928) found that young hyphal branches incline in the direction of mycelial growth and are always somewhat constricted at the point of union with the main hyphae. Upon maturing, the hyphae were found to become more deeply colored, more uniform and rigid, and the constrictions less marked at the base of the branches which, at this age, extend more at right angles than previously from the main hyphae.

There is considerable variation in the sclerotia of different strains of Rhizoctonia solani as can be seen in a review of the literature. Sclerotia of Rhizoctonia may vary in size, shape, texture, and color. According to Gadd and Bertus (1928) young sclerotia of R. solani appear as small tufts or fluffy aggregations of hyphae that increase in size up to 5 mm. in diameter. Bertus (1928) found sclerotia varying in diameter from 0.5 to 4 mm. Shaw (1912) found that the R. solani cultures he was studying produced sclerotia that were 1 to 1.5 mm. in diameter, while the sclerotia produced by some Corticium vagum cultures were 1 to 5 mm. in diameter. He gave the name "microsclerotia" to the sclerotia of Rhizoctonia solani and the name "macrosclerotia" to the sclerotia of Corticium vagum.

Thomas (1925) used the term "pseudosclerotia" to describe structures from 0.5 to 4 mm. in diameter. Schweizer (1927) used the term "pseudosclerotia" but it was not determined to what type and size of structures he referred.

Wiant (1929) described sclerotial formations that differ from normal sclerotia. He found that two of his many strains of R. solani differed on certain media from the others in that they produced papery sclerotia which were light in color and were loosely fused with the abundant aerial mycelium. Sclerotial formation intermediate between those described above and normal sclerotia also occurred in certain of his cultures. He also described sclerotia of another strain that

were profusely scattered over the surface of two media that were usually less than one millimeter in diameter, indefinite in structure and consisted for the most part only of denser tufts of fairly abundant aerial mycelium. These last described structures are apparently the same as those which Stevens (1925) described as "sclerotia-like tufts" with broad, more or less triangular cells which function as chlamydospores.

Peltier (1916) from observations on four strains that rarely produced sclerotia, concluded that the absence of sclerotia was the first sign of degeneration and loss of virulence.

The reaction of Rhizoctonia to changes of temperature has been studied by many investigators. Matsumoto (1921) found the maximum, minimum, and optimum temperatures for his B 1 strain to be 42° to 44°, 13° to 15°, and 27° to 33° C., respectively. Müller (1923) found these temperatures to be 30° C. or slightly over, 7° C., and 25° C., respectively. The maximum, minimum, and optimum temperatures for mycelial development of R. solani were found by Gratz (1925) to be about 31° C., 9° C., and between 22° and 26° C. Lauritzen (1929) found these temperatures to be 31.5° to 34.5° C., 0° to 2° C., and 23° C.; Weber (1931) found them to be 34° C., 5° C., and 25° C.; and Walker (1928) stated them to be 38° C., 7° to 11° C., and 27° to 29° C. for Corticium vagum attacking cotton.

Richards (1923) found the maximum, minimum, and optimum temperatures for mycelial development to be 32.6° C., 4.6° C., and 25° C.,

respectively, but states that since the rate of growth on hard medium decreases with the age of the culture on the medium, the optimum temperature for continued growth is perhaps much lower. The decrease in the rate of growth taking place in several day old cultures of Rhizoctonia on hard medium was noted earlier by Balls (1905), who thought that accumulations of by-products of metabolic processes were responsible for the inhibition of mycelial growth.

Dickinson (1930) in his work with Rhizoctonia solani on turf, discovered that it was necessary to chill sclerotia to get them to start growth when the temperature is warmer than 35° C. Newton (1931) reported the lethal temperature period for vegetative cultures of R. solani to be one hour at 50° C.

Zonation of mycelial growth on agar media is not uncommon in cultures of R. solani. Peltier (1916) and Tilford (1930) noted zonation occurring in cultures of R. solani isolated from potatoes.

Matsumoto (1921) found that the most favorable hydrogen-ion concentration for vegetative growth of several strains of Rhizoctonia solani was about pH 3.8. Subsequently (1922), he found that the effect of the H-ion concentration varies with the nature of the medium and states that it is almost impossible to name a definite optimum H-ion concentration for growth of this fungus. No growth occurred on any medium at a pH of 2.5 but mycelial growth was fairly luxuriant on Czapek's solution at pH 9.8 and scanty on turnip and potato decoction at pH 8.5. Gratz (1925) found that the minimum H-ion concentration

for mycelial growth of R. solani is approximately pH 2.0, the optimum about 6.2, and the maximum 10.4. Weber (1931) found the minimum, optimum, and maximum H-ion concentration to be pH 2.5, 6.5 to 7, and 10, respectively. Monteith and Dahl (1928) noted that variations in the acidity of the culture medium produced marked variations in the color of different cultures of R. solani. Color variations from hyaline to dark brown, occurred in the mycelium, the darker color usually being present on a medium near the neutral point.

A number of investigators have stated that while differences between Rhizoctonia solani cultures have been stressed by many workers, these differences are not sufficient to warrant grouping the cultures into different strains. Corsaut (1915) compared a large number of cultures of Rhizoctonia from potato and found their cultural characters to be similar but showing some variation. Duggar (1915) found that the different strains from 23 hosts possess minor differences in culture which are not sufficient to be considered of specific importance, except in the case of a certain culture from rhubarb. This form differed from the other strains mainly in the type and amount of aerial mycelium, color of the cultures and the rapidity of sclerotia formation. He states that the differences between most cultures are such as might be due to permanent differences in the pathological strains or temporary differences due to present environment.

Peltier (1916) concludes from his investigations with R. solani that the differences in various cultural characters which are shown

by strains from different hosts are no greater than differences that may be manifested by two different strains isolated from the same host or by the same strain at different ages. The differences revealed by strains of R. solani in culture are said by Briton-Jones (1924) to be too slight to warrant specific recognition.

Shaw (1912) noted considerable difference in the mycelium of Corticium vagum and Rhizoctonia solani, the differences, however, being no greater than those found by other investigators between strains of R. solani. Gadd (1928) also found differences between cultures of Corticium vagum and Rhizoctonia solani, but considered them as being of minor importance.

Matsumoto (1921) grew Rhizoctonia solani in flasks sealed with paraffin and noted a suppression of mycelium and sclerotia formation. Gadd and Bertus (1928) stated that Rhizoctonia solani demands good aeration for optimum growth and for this reason growth is usually slow and feeble on liquid media. Kotila (1929) stated that this fungus requires an abundant supply of air and that it will not remain alive for more than four days when cultured in sealed test tubes.

EXPERIMENTAL WORK

The experimental work of this investigation is divided into two parts. Investigations regarding oxygen supply, temperature relations, and constancy of cultural characteristics of Rhizoctonias were made

in the first part and comparisons of ten pure-lined cultures of Rhizoctonia grown on a number of different media were made in the second part.

The cultures of Rhizoctonia used in this investigation originated from a number of different substrates and from widely different parts of this country (Table 1). All cultures used, except culture R 4, were pure lined through single mycelial tip isolations. Ten of the cultures had been pure lined before this thesis problem was begun. The mycelial single tip isolations were obtained by excising isolated hyphal tips and transferring them to sterile nutrient media in Petri plates. From the mycelium that developed, two additional generations of hyphal tips were transferred and grown on nutrient media before the cultures were considered as pure lines.

Table 1.--Source of Rhizoctonia cultures used.

Culture	Substrate from which isolated	Person making isolation	Geographical origin
R 20	Pansy	P. Peterson	Cottonwood Co., Minn.
R 6	Sugar Beet	E. L. LeClerg	Colorado.
R 33	Flax	H. H. Flor	Fargo, North Dakota.
R 14	Potato	O. H. Elmer	Tribune, Kansas.
R 2	Potato	J. B. Kendrick	Delta region in California.
R 3	Bean	J. B. Kendrick	Terminus, California.
R 40	Cotton	V. H. Young	Hummok, Arkansas.
R 15	Soil	O. H. Elmer	Manhattan, Kansas.
R 19	Turf	J. Monteith, Jr.	Madison, Wisconsin.
R 1	Potato	O. H. Elmer	Manhattan, Kansas.
R 4	Tomato root	J. B. Kendrick	Woodland, California.
R 5	Horseradish roots	.....	California.
R 7	.....	.....	Maine.

Effect of Various Factors upon Cultures of Rhizoctonia

Effect of Various Incubation Temperatures and the Effect of Continued Culturing on Artificial Media. The object of this experiment was to determine: first, if changes occur in the growth rate of cultures of Rhizoctonia after they have been grown continually on artificial media for an extended period; and second, the effect of various temperatures on the growth rate of these cultures.

Preliminary plantings of the stock and reisolated cultures of the following Rhizoctonias were started Dec. 24, 1931 on potato dextrose agar.

Stock culture of R 1 from potato lesions, in culture since Sept. 30, 1929.

Reisolation of R1 from potato lesion, Dec. 5, 1931.

Reisolation of R 1 from soybean lesion, Dec. 5, 1931.

Stock culture of R 4 from tomato root in culture since Aug. 24, 1927.

Reisolation of R 4 from potato sclerotia, Nov. 1, 1931.

Stock culture of R 5 from horseradish in culture since Aug. 24, 1927.

Reisolation of R 5 from soybean sclerotia, Dec. 5, 1931.

Stock culture of R 14 from potato sclerotia in culture since Oct. 11, 1929.

Reisolation of R 14 from potato sclerotia, Dec. 5, 1931.

Stock culture of R 15 from soil, in culture since Oct. 21, 1929.

Reisolation of R 15 from potato stem lesion, Dec. 5, 1931.

Reisolation of R 15 from vetch lesion, Dec. 5, 1931.

Stock culture of R 7 in culture previous to Oct. 14, 1929.

Reisolation of R 7 from potato, Nov. 1, 1931.

When the temporary cultures were about seven days old, transfers from the growing edge of each were made to sixteen freshly poured plates of potato dextrose agar. These cultures were incubated at room temperature and examined twice a day. As the newly developing colonies became about one fourth of an inch in diameter, the perimeter of the growth was traced on the bottom of the dish with a wax pencil. The plates were then placed in a refrigerator and held at 2° C. until the colonies in all were of similar size, at which time they were removed to eight different incubators thermostatically controlled to maintain, within a degree or two, the following temperatures: 12°, 15°, 21°, 24°, 27°, 30°, and 33° C.

After the first day the plates were examined every 12 hours and as the growing cultures approached the sides of the plates their new perimeters were traced and dated on the bottom of the plates with a wax pencil. The hourly rates of growth were later calculated by dividing the distance between the two traced perimeters by the number of hours required by the mycelium to develop from one to the other.

Every set of duplicates was carefully examined and the following cultural characteristics noted: color of mycelium, density of mycelium, elevation of mycelium, size and abundance of sclerotia, and presence or absence of zonated areas of growth. No differences could be noted between the stock and reisolated cultures of each Rhizoctonia when thus examined.

Some degree of brown was present in all the R 4, R 5, R 7, and R 14 cultures incubated above 12° C. The mycelium remained hyaline during the 8-day period at 12° C. All of the R 1 cultures and all but a few of the R 15 cultures were entirely white. A very slight brown tinge occurred in some of the R 15 cultures incubated at 21°, 24°, 27°, and 30° C.

Sclerotial developments were entirely absent in the R 4 and R 14 cultures incubated at 12° C., although they occurred abundantly in both cultures when incubated at the higher temperatures. Although sclerotial development occurred at all the temperatures in the other cultures, there was a noticeable decrease at the two lower temperatures. The presence or absence of zonated areas in the mycelial growth was not associated with temperature relations.

The optimum incubation temperature for the R 4, R 5, R 7, and R 14 cultures was found to be 24° C. The optimum incubation temperature for the R 1 and R 15 cultures was found to be about 27° C. and these two cultures were able to grow much more rapidly at 33° C. than were the above four cultures.

In this experiment no significant difference occurred in the cultural characteristics of stock and reisolated cultures of Rhizoctonia. The optimum temperature of four of the six cultures was found to be close to 24° C., and for the other two cultures, close to 27° C.

Effect of Limited Aeration on Rhizoctonias in Culture. An experiment was performed to determine what effect a restricted oxygen supply has on Rhizoctonia.

Approximately 8 cc. of potato dextrose agar was sterilized and slanted in 100 test tubes of 22½ cc. capacity and the tubes plugged with cotton. Ten pure-line cultures of Rhizoctonia, including R 1, R 2, R 3, R 6, R 14, R 15, R 19, R 20, R 33, and R 40 were planted on ten potato dextrose agar slants. Seven of these were later sealed with paraffin while the remaining three were held as controlls. The checks and the sealed tubes were then held at laboratory temperature.

On January 8, forty-one days later, the sealed and unsealed culture tubes were examined and the appearance of the cultures noted. Only 12 of the paraffined tubes were perfectly sealed, as the media in the others was shrunken due to evaporation, and the fungous growths were well developed and, with the exception of the white R 33 culture, were all dark in color. The mycelia of the cultures in the sealed tubes were white, without sclerotia, and had the appearance of being only two to four days old. The substrate in these tubes was not shrunken. One tube each of R 3, R 6, R 14, R 15, and R 19 was opened

and mycelial transfers made to plates of potato dextrose agar. New mycelial growth developed from all of these transfers, indicating that the fungi were still alive.

On March 2, ninety-one days after the tubes were inoculated, mycelial transfers were made to potato dextrose agar plates from the remaining tubes which were sealed. The cultures represented were two slants of R 1 and one slant each of R 2, R 14, R 15, R 19, and R 40. Mycelial growth developed on these plates, indicating that the cultures had remained alive in the sealed tubes for 91 days.

Cultural Comparisons of Ten Rhizoctonias

Rhizoctonia Cultures on Three Agar Media. An experiment was planned to make a comparison of the cultural responses of ten pure-lined cultures of Rhizoctonia when these are grown on Richard's, potato dextrose, and carrot agar. The comparisons were made to secure information on which of these cultures were similar and which varied so widely that, by this method of differentiation, strains may possibly be recognized.

Three liter quantities of the three different media were prepared. Richard's agar was prepared by first making one and a half liters of a solution containing the following ingredients:

Potassium dihydrogen phosphate	15.00 gms.
Potassium nitrate	30.00 "
Magnesium sulphate	7.50 "
Iron chloride	.09 "
Cane sugar	150.00 "
Water (distilled)	1500.00 cc.

The above concentrated solution was then diluted with 1500 cc. of a 4 per cent solution of agar agar.

Potato dextrose agar was prepared according to the following formula.

Sliced peeled potatoes	600 gms.
Agar agar	60 "
Dextrose	60 "
Distilled water	3000 cc.

The potatoes were cooked in 1500 cc. of water over a direct flame until soft and the broth decanted and filtered through cheesecloth. The agar agar was melted in the other 1500 cc. of water and the two solutions were mixed, after which the dextrose was added.

A carrot decoction was prepared by cooking carrots at the rate of 390 grams per liter of water and then adding agar. The ingredients used for this medium were as follows:

Carrot decoction	750 cc.
Distilled water	2250 cc.
Agar agar	60 gms.

The agar agar was melted in the water and the decoction then added.

All of the three media were strained through absorbent cotton while still hot and were then transferred in 15 cc. quantities to test tubes. This media was later poured into Petri dishes in which the cultures to be compared were planted.

Earlier experiments had indicated that the ten cultures used in this test vary in their rates of growth on nutrient media. The slow growing cultures were, therefore, planted some time previous to the faster growing ones in order to afford all the cultures an even start in the test. They were all held at room temperature until definite growth appeared. When the cultures were about one-fourth inch in diameter, their perimeters were traced on the bottom of the Petri dishes with a wax pencil and the plates were then placed in the refrigerator at 2° C. and held there until all the cultures had started growth. When all the cultures had started growth, the plates were placed in an incubator and held, in the first test, at temperatures between 22.5° and 27.75° C., with an average of 23.75° C. as recorded by a recording thermograph. The recording thermograph was not available for the second test and the extremes and average temperatures, based on 14 temperature readings during the ten-day period, were 23.5°, 27.5°, and 25.9° C., respectively.

After the plates had been in the incubator for 24 hours they were examined and additional examinations were made at twelve hour intervals. As the Rhizoctonia colonies approached the outer edge of the dish, the perimeters were again traced in order that distances of growth could be measured and rates of growth could be computed. At the end of ten days the plates were examined and the cultural characteristics of the various Rhizoctonias were recorded.

Three types of sclerotia or sclerotia-like formations were noted in the study of the ten cultures of Rhizoctonia. The most common and the most conspicuous sclerotia were small hard bodies 1 to 12 mm. in size which at maturity varied in color from gray to dark brown or black. In this experiment these will be referred to as "normal sclerotia." A second type of sclerotia noted was a thin mat-like development approximately 1 mm. thick that was observed to vary from 0.5 to 5 cm. in diameter and from hyaline to dark brown in color. This type of sclerotia will be referred to as "mat-like sclerotia." A number of the cultures produced small weft-like sclerotia which appeared as aggregates of mycelium, remained white, and were typically less than 1 mm. in diameter. These sclerotia-like bodies will be referred to as "pseudosclerotia."

The maximum sizes of the sclerotia produced by the different cultures in this experiment are as follows: R 2, 12 mm.; R 19, 4 mm.; R 20, 4 mm.; R 6, 3 mm.; R 3, 3 mm.; R 1, 3 mm.; R 40, 2mm.; and R 15, 1 mm.

Mat-like sclerotia were produced on Richard's media by cultures R 6, R 15, R 3, R 1, R 14, R 40, R 20, and R 2. The last four cultures also produced mat-like sclerotia on potato dextrose agar. Culture R 33 produced mat-like sclerotia on potato dextrose agar. Culture R 19 produced no sclerotia of this type on any of the three media.

All of the ten Rhizoctonias except R 20 produced pseudosclerotia in one or more of the plates in this experiment. Most of the pseudo-

sclerotia occurred on Richard's and potato dextrose agar. Culture R 40 was the only one of the Rhizoctonias producing them on carrot agar.

The rates of mycelial growth of the ten cultures were found to vary considerably. (figs. 1 to 3; Table 2.) Culture R 19 developed most rapidly when grown on potato dextrose and carrot agar. Culture R 1 was the second fastest growing culture on these media and was followed in decreasing order of growth rate by cultures R 15, R 40, R 3, R 2, R 33, R 14, R 6, and R 20. The growth rates on Richard's agar were in decreasing order from R 1, the fastest grower on this medium, to R 3, R 15, R 19, R 40, R 2, R 14, R 6, R 33, and R 20.

Considerable variation occurred at the end of the 10-day period in the color of the ten Rhizoctonia cultures. The colors of mycelium on potato dextrose agar, beginning with the lightest and progressing to the darkest, are: R 33, white; R 1 and R 15, light gray; R 19, R 6, R 3, R 2, and R 40, brownish gray; R 20, light reddish brown; and R 14, brown. Cultures R 40, R 3, R 2, and R 6, which were all brownish gray on potato dextrose agar, were all light gray to gray in color on carrot and Richard's media.

A number of differences in the cultural characteristics of the ten Rhizoctonia cultures have been noted by growing them on three agar media. Differences were noted in such characteristics as color of mycelium, hourly rate of growth, and type and size of sclerotia.

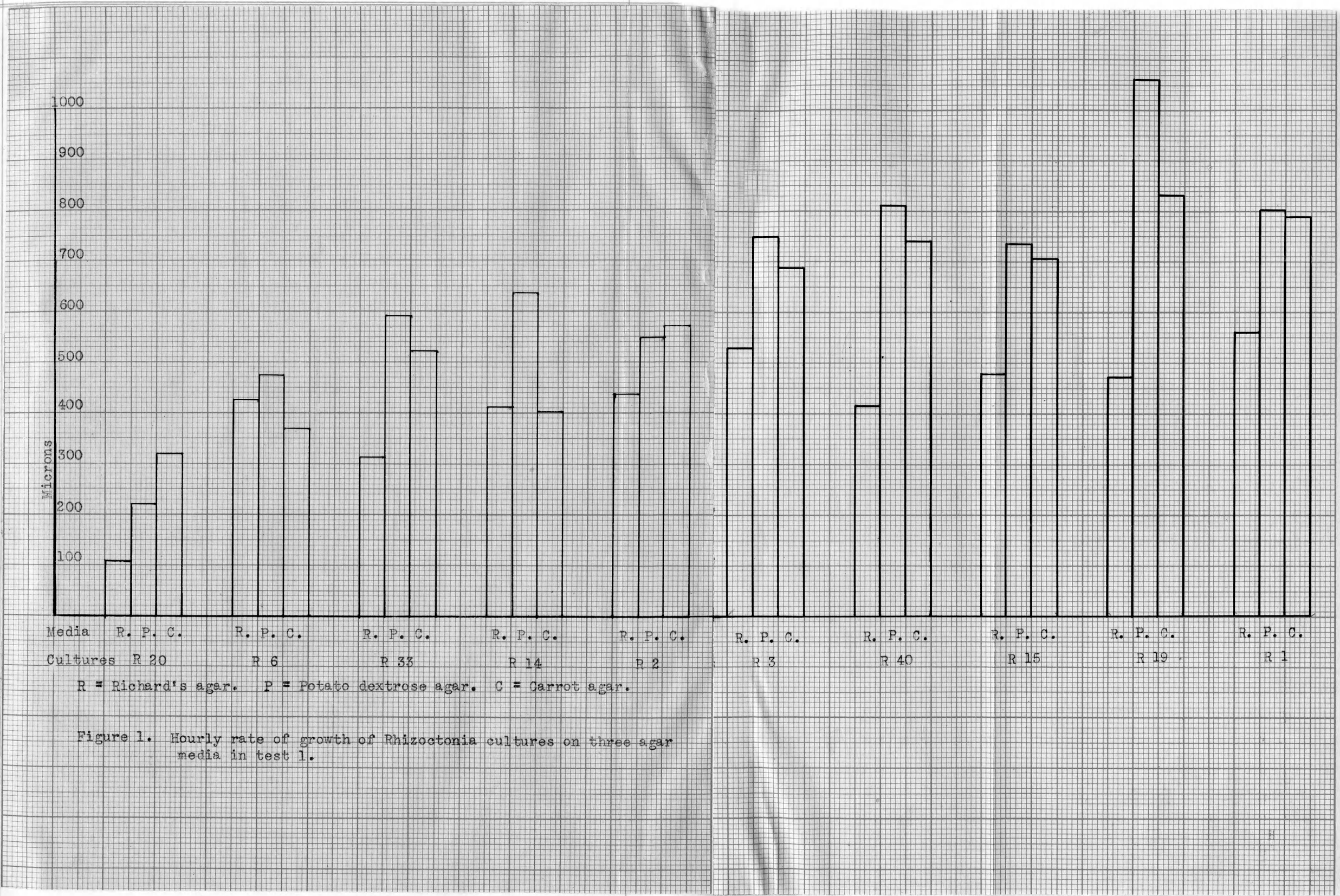
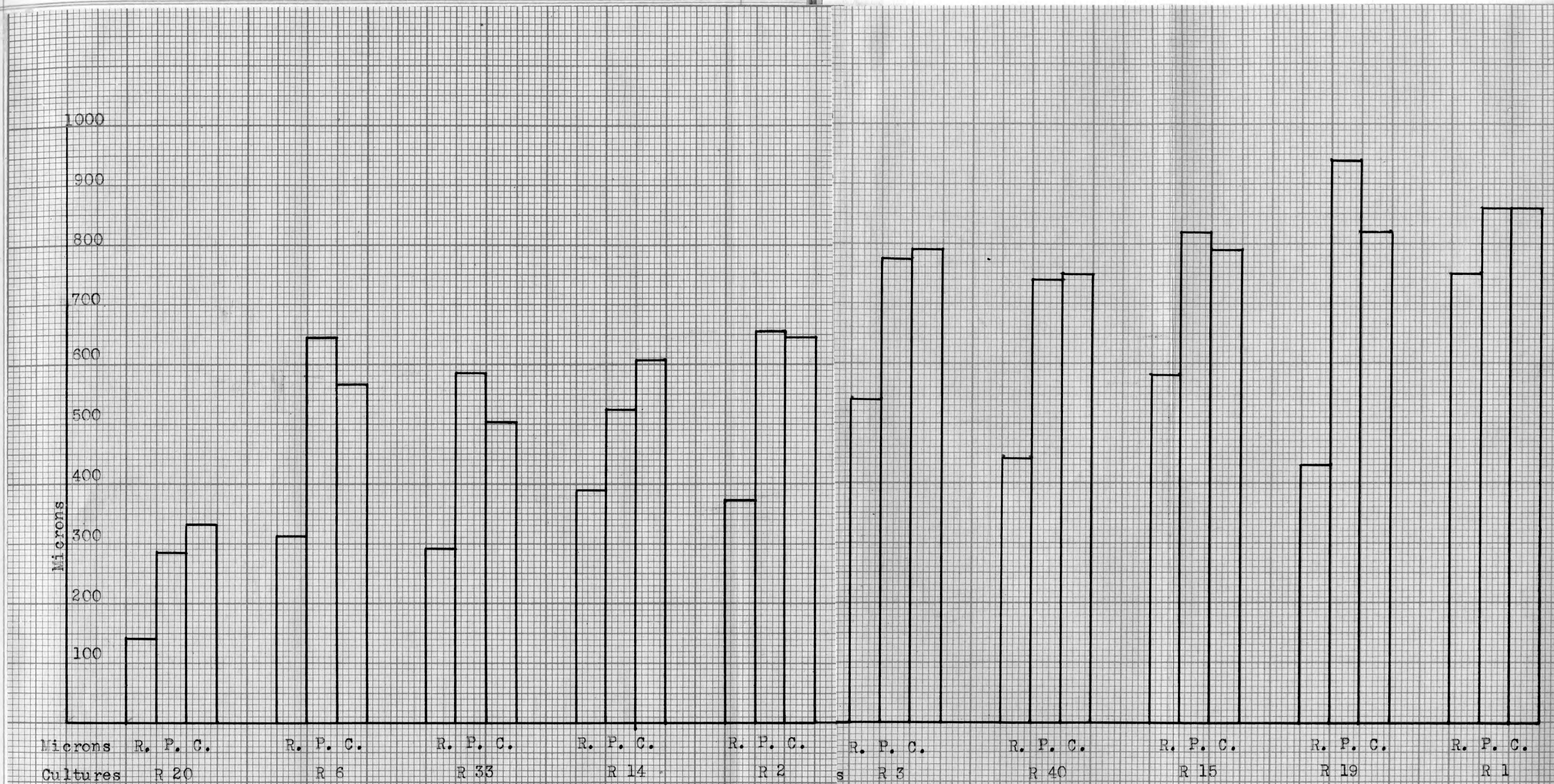
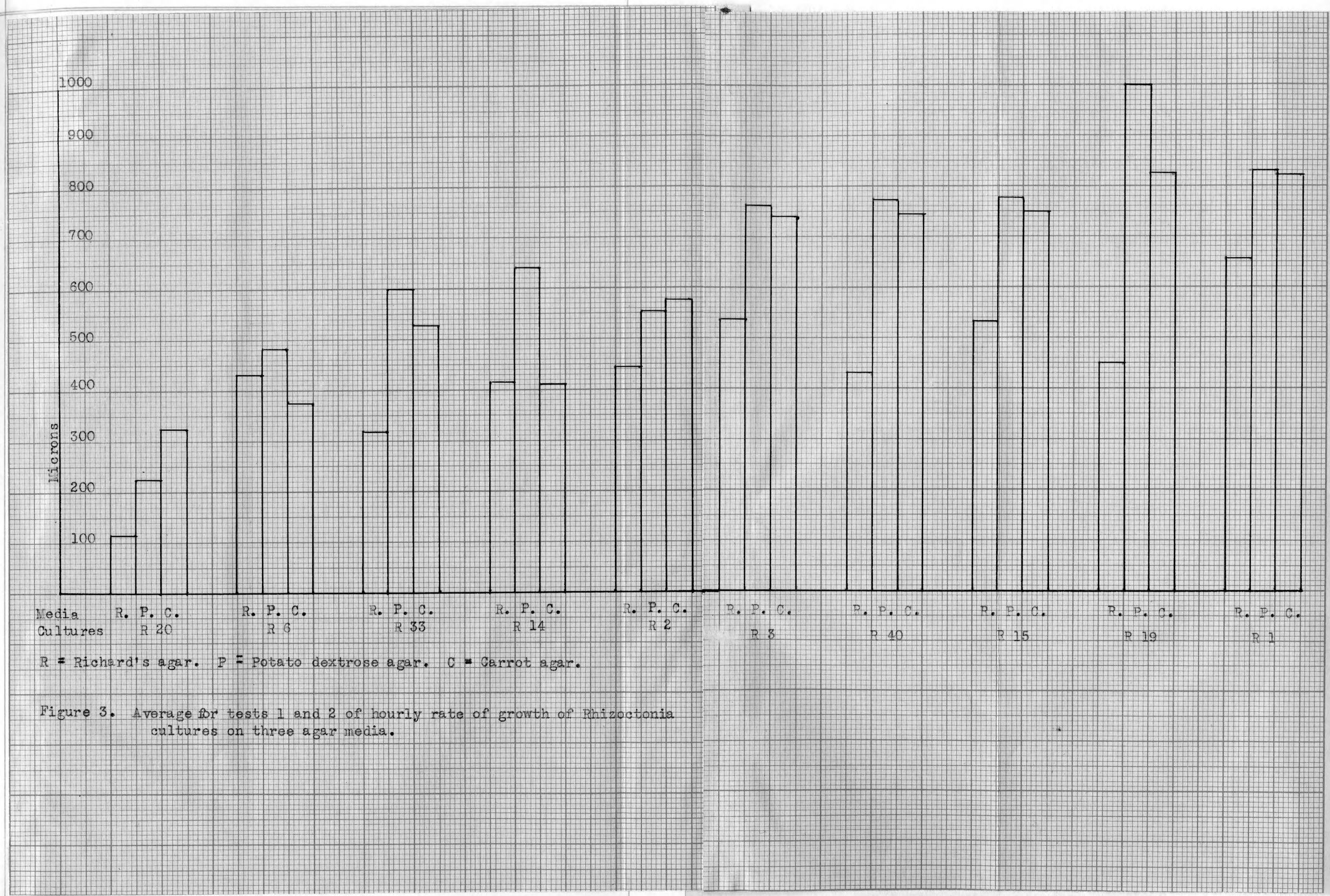


Figure 1. Hourly rate of growth of Rhizoctonia cultures on three agar media in test 1.



R = Richard's agar. P = Potato dextrose agar. C = Carrot agar.

Figure 2. Hourly rate of growth of Rhizoctonia cultures on three agar media in test 2.



R = Richard's agar. P = Potato dextrose agar. C = Carrot agar.

Figure 3. Average for tests 1 and 2 of hourly rate of growth of Rhizoctonia cultures on three agar media.

Table 2.--Growth rates of ten Rhizoctonia cultures on three agar media. Manhattan, Kans., 1932.

Strain	Kind of media	Test 1, Sept. 26 to Oct. 6: Hourly rate of growth in microns: Duplicate No. 1	Test 1, Sept. 26 to Oct. 6: Hourly rate of growth in microns: Duplicate No. 2	Test 1, Sept. 26 to Oct. 6: Ave. hourly rate of growth in microns	Test 2, Oct. 19 to Oct. 29: Hourly rate of growth in microns: Duplicate No. 1	Test 2, Oct. 19 to Oct. 29: Hourly rate of growth in microns: Duplicate No. 2	Test 2, Oct. 19 to Oct. 29: Ave. hourly rate of growth in microns	Grand Ave. hourly rate of growth in microns
R 19	Richard's	472	472	472	440	413	427	450
	Potato dextrose	1000	1125	1062	944	944	944	1003
	Carrot	1000	666	833	861	778	819	826
R 40	Richard's	417	417	417	467	417	442	430
	Potato dextrose	889	833	861	771	709	740	801
	Carrot	708	771	740	750	750	750	745
R 1	Richard's	550	569	560	562	562	562	561
	Potato dextrose	833	771	802	861	861	861	831
	Carrot	717	861	789	833	889	861	825
R 3	Richard's	528	528	528	521	563	542	535
	Potato dextrose	750	750	750	806	750	778	764
	Carrot	729	646	688	806	778	792	740
R 15	Richard's	500	458	479	583	583	583	531
	Potato dextrose	666	806	736	861	778	819	778
	Carrot	683	729	706	778	806	792	749
R 2	Richard's	431	458	445	369	411	390	418
	Potato dextrose	483	629	556	657	646	652	604
	Carrot	657	500	579	625	667	646	613

R 33	Richard's	317	323	320	282	292	287	304
	Potato dextrose	583	617	600	583	583	583	592
	Carrot	555	500	528	507	493	500	514
R 14	Richard's	375	458	417	426	350	388	403
	Potato Dextrose	625	667	646	458	583	521	584
	Carrot	416	583	489	667	542	605	547
R 6	Richard's	431		431	300	316	308	369
	Potato dextrose	528	444	486	625	657	641	569
	Carrot	375	375	375	542	583	563	469
R 20	Richard's	92	141	117	138		138	128
	Potato dextrose	257	275	266	291	275	283	275
	Carrot	333	316	325	347	308	328	327

Rhizoctonia Cultures on a Medium of Eight Different Acidities. The purpose of this experiment was to compare the cultural characteristics of ten pure-lined cultures of Rhizoctonia when they are grown on media with various H-ion concentrations.

The method used by Webb and Fellows (1926) in their work with Ophiobolus graminis Sacc. was employed with several modifications. One-fifth normal solutions of NaOH and HCl were prepared in the usual manner by titration against a standardized solution of acid. Two liters of a double concentration of potato dextrose decoction was prepared by using the following ingredients: broth from 1200 gms. cooked potatoes, dextrose 120 gms., and water 2000 cc. The potato broth was strained through several thicknesses of cheese cloth. The dextrose was then added and the solution strained through absorbent cotton, after which 80 grams of powdered agar agar were dissolved in the hot solution.

One hundred and seventy-five cubic centimeter portions of the solution were measured out and poured into one-half liter flasks. The medium was then sterilized in flowing steam for one hour periods on three successive days.

The use of concentrated potato dextrose agar in the method as used by Webb and Fellows permits the addition of regulated quantities of acid or alkali for the adjustment of the hydrogen-ion concentration without disturbing the concentration of the various constituents.

The quantities of medium, the amounts of water used in effecting the proper dilutions, and the quantities of acid or alkali added, together with the resultant pH values are given in Table 3.

The pipetting of the proper amounts of hot steril distilled water and proper quantities of NaOH or HCl into the remelted medium and the pouring of the medium into the Petri dishes were done in a culture chamber. The interior parts of the chamber were washed with a 1-1000 $HgCl_2$ solution and the dust precipitated by injecting live steam into the chamber. After the water and the acid or base were added to the flask, the contents were well mixed by rotating the flasks for a few seconds. A sample was then removed with a steril pipette and the pH value was determined colorimetrically by the use of a Model 5B LaMotte Hydrogen-Ion Testing Set.

Immediately after each pH determination was made, approximately 15 cc. portions of the medium were poured into 20 Petri plates and allowed to harden on a level surface. The plates were inoculated by transferring small portions of the medium and cultures from the edge of 7 to 10-day-old Rhizoctonia cultures to the center of the poured plates. From earlier observations, R 20 was known to require a long time to develop visible growth. For this reason it was planted first in order that it would have about an even start with the faster growing cultures. Cultures R 6, R 14, R 33, and R 2 were known to grow faster than does R 20, but slower than the remaining cultures, so these were planted about 12 hours after the former and about 12 hours before the latter.

Table 3.--Solid medium adjusted to various hydrogen-ion concentrations by regulated additions of N/5 hydrochloric acid or N/5 sodium hydroxide.

Concentrated potato dextrose medium	HCl or NaOH N/5	H_2O	Total	Resultant pH value
cc.	cc.	cc.	cc.	
		HCl		
175	35.00	140.00	350	3.2
175	21.00	154.00	350	3.7
175	5.25	169.75	350	4.9
175	0.00	175.00	350	5.6
		NaOH		
175	3.50	171.50	350	6.8
175	12.25	162.75	350	8.4
175	14.00	161.00	350	8.6
175	35.00	140.00	350	9.8

As soon as the plates were planted, they were placed in an incubator that was kept at approximately 22° C. At 2 P.M., December 13, the perimeters of all but the very smallest colonies were traced on the bottoms of the Petri plates with a wax pencil. The plates were then placed into another incubator where the temperature varied between 24.25° and 27.25° C.

The next morning and at 12-hour intervals following, the cultures were examined. As the later appearing colonies developed, their perimeters were traced and dated and as the growing colonies approached the sides of the plates the new perimeters were likewise traced and dated. The rate of growth in each plate was later calculated by dividing the distance the mycelium grew by the time required for the growth.

The cultures were examined on December 23 after a ten-day incubation period and the color of the mycelium, presence or absence of zonation, type of sclerotia, and size of sclerotia were noted. Hydrogen-ion concentration determinations of the substrate in the plate were made colorimetrically. Only about half of the determinations were completed on Dec. 23, the remaining cultures being placed in a refrigerator and kept at 2° C. until Dec. 28, when they were completed.

The Rhizoctonia cultures showed a tendency to change the H-ion concentration toward neutrality when the medium was distinctly acid or distinctly alkaline. (Table 4.)

Table 4.--Changes in reaction induced by growth, growth rate, and cultural characteristics of ten Rhizoctonias on potato dextrose medium of eight different initial acidities. Manhattan, Kans., 1932.

Cultures	pH value of medium: Initial	pH value of medium: Final in duplicate plates		Hourly rate of growth in microns: Duplicate plates		Hourly rate of growth in microns: Average	Color of mycelium(a)	Zonation(b)	Sclerotia(c): Type	Sclerotia(c): Size mm.
R 20	3.2	2.6		88	102	95	W.	0	Nor. I.	1
	3.7	6.0	5.9	171	154	163	W.	4	Nor. A.	
	4.9	6.2		245	245	245	L.R.B.	4	do.	
	5.6	6.6	6.6	246	245	246	L.R.B.	4	do.	
	6.8	5.8	6.0	258	268	263	L.R.B.	4	do.	
	8.4	6.6		277		277	L.R.B.	4	do.	
	8.6			258	248	253	L.R.B.	4	do.	
	9.8						L.R.B.	4	Nor. I.	1
R 6	3.2	3.7	3.7	333		333	W.	0	Nor. I.	2
	3.7	5.5	6.6	441	448	445	B.G.	2	do.	1
	4.9	7.3	6.6	493	493	493	B.G.	1	do.	1
	5.6	6.7	6.8	574	648	611	B.G.	1	do.	1
	6.8	7.3	7.3	721	648	685	B.G.	0	do.	1
	8.4	6.8	6.7	611	630	621	B.G.	0	do.	8
	8.6	7.3	6.7	593	567	580	B.G.	1	do.	8
	9.8	7.3	7.4	409	419	414	B.G.	1	do.	1
R 33	3.2	3.6		108		108	W.	0	Weft.	
	3.7	6.8	6.8	344		344	W.	1	do.	
	4.9	7.7		433	463	448	W.	1	do.	
	5.6	7.8		556	556	556	W.	0	do.	
	6.8	8.0	8.0	537	574	556	W.	0	do.	
	8.4	8.3	7.5	448		448	W.	0	do.	
	8.6	7.6	7.6	344	366	355	W.	0	do.	
	9.8	8.0	8.3	276	245	261	W.	0	do.	

Table 4 Cont.

R 14	3.2	3.7	3.7	209	252	231	W. and B.G.	0	Nor. I. and A.	2
	3.7	6.0	6.0	495	516	506	D.B.	2	do.	2
	4.9	6.4	6.4	574		574	D.B.	2	do.	2
	5.6	6.5	6.4	574	628	601	D.B.	0	do.	1
	6.8	6.5	6.5	698	698	698	D.B.	3	do.	2
	8.4	6.5	6.4	537	522	528	D.B.	2	do.	1
	8.6	6.7		479	344	412	D.B.	2	do.	1
	9.8	6.6		353		353	D.B.	1	Nor. A.	1
R 2	3.2	3.2	3.6	336	312	324	W.	2	Nor. I. and A.	1
	3.7	5.4	5.7	567	552	558	B.G.	0	do.	1
	4.9	6.7	6.7	651	558	605	B.G.	2	do.	1
	5.6	6.8		721	909	815	B.G.	1	do.	2
	6.8	6.5	6.7	727		727	B.G.	0	do.	2
	8.4	6.7	6.7	648	522	585	B.G.	1	do.	2
	8.6	7.1	6.5	628		628	B.G.	1	do.	2
	9.8	6.5		409	462	436	B.G.	1	do.	2
R 3	3.2	3.8	3.9	433	467	450	W.	0	Nor. I.	1
	3.7	6.7	6.7	537	567	552	B.G.	1	Nor.I. and Mat.	1
	4.9	7.7		648		648	B.G.	0	do.	1
	5.6	7.2	7.2	721	672	697	B.G.	1	do.	1
	6.8	8.0	8.1	797	741	769	B.G.	2	do.	1
	8.4	7.3	6.8	702	741	722	B.G.	1	do.	1
	8.6	7.0		648	685	667	B.G.	2	do.	1
	9.8	7.4		493		493	B.G.	1	do.	1
R 40	3.2	3.9		520	427	474	W.	1	do.	2
	3.7	6.2	6.4	759	759	759	B.G.	1	do.	2
	4.9	6.0	6.2	721	767	744	B.G.	2	do.	3
	5.6	6.6		796	704	750	B.G.	2	do.	2
	6.8	7.5	7.6	741	759	750	B.G.	2	do.	2
	8.4	6.3	6.1	778	704	741	B.G.	2	do.	2
	8.6	7.2		741	685	713	B.G.	1	do.	2
	9.8	6.2		533	495	514	B.G.	1	do.	2

Table 4 Cont.

R 15	3.2	3.7	3.9	398		398	W.	1	Weft.
	3.7	6.9	6.8	685	722	704	L.G.	2	Nor.I. and Weft 2
	4.9	7.4	7.4	837	814	826	L.G.	2	do. 2
	5.6	7.5	7.6	879	885	884	L.G.	2	do. 1
	6.8	7.5	7.9	860	860	860	L.G.	2	do. 1
	8.4	7.5	7.2	814	814	814	L.G.	1	do. 1
	8.6	7.4	7.4	767	778	773	L.G.	0	do. 1
	9.8	7.4		478	452	465	L.G.	1	No sclerotia.
R 19	3.2	3.6	3.8	258	245	252	W.	0	Nor. I. 1
	3.7	6.5	6.0	667	685	676	L.G.	0	do. 6
	4.9	6.0	6.2	884	837	861	B.G.	1	do. 6
	5.6	6.8	6.6	1110	1106	1108	B.G.	2	do. 6
	6.8	6.5	6.5	1127	1127	1127	B.G.	1	do. 6
	8.4	6.4	6.4	837	907	872	B.G.	2	Nor. I. and A. 6
	8.6	6.3	6.7	837	860	849	B.G.	2	Nor. I. 4
	9.8	7.4		478	389	434	B.G.	2	do. 3
R 1	3.2	3.9		461		461	W.	1	do. 1
	3.7	6.6	6.6	556	611	584	L.G.	1	do. 1
	4.9	7.4	6.9	814	814	814	L.G.	1	do. 1
	5.6	7.0	7.0	814	837	826	L.G.	1	do. 1
	6.8	7.5	7.4	837	814	826	L.G.	2	do. 1
	8.4	7.2	6.9	698	698	698	L.G.	1	do. 1
	8.6	7.4	7.3	741	741	741	L.G.	1	do. 1
	9.8	7.3	7.2	430	430	430	L.G.	2	do. 1

(a) W. = white; L.G. = light gray; B.G. = brownish gray; B. = brown; L.R.B. = light reddish brown; D.B. = dark brown.

(b) 0 = absent; 1 = very faint; 2 = faint; 3 = distinct; 4 = very distinct.

(c) Nor. I. = normal individuals; Nor. A. = normal sclerotia in aggregations; Mat. = mat-like sclerotia formation; Weft. = weft-like sclerotia.

The effect of various H-ion concentrations on the mycelial growth rates of the ten Rhizoctonia cultures indicates that a slightly acid medium is more favorable for the most rapid growth than is a slightly alkaline medium. Seven cultures, R 1, R 2, R 14, R 15, R 19, R 33, and R 40, grew more rapidly on a medium of pH 5.6 which is 1.4 pH units below neutrality, than on a medium of pH 8.4, 1.4 pH units above neutrality. The other three cultures, R 3, R 6, and R 20, all grew slightly faster on a medium at pH 8.4 than at pH 5.6.

An H-ion concentration of the medium at pH 3.2 had a stronger inhibitory effect on the mycelial growth of nine of the ten cultures than did an H-ion concentration of pH 9.8. Culture R 1, the only exception, grew slightly faster on the medium at pH 3.2 than at pH 9.8. An H-ion concentration of pH 3.2 reduced the growth rate on potato dextrose agar of cultures R 3 and R 40 approximately 1/3, R 1, R 15, and R 2 approximately $\frac{1}{2}$, R 19, R 14 approximately 2/3, R 20 approximately 3/4, and R 33 approximately 5/6 from that which occurred on this medium at the various optimum H-ion concentrations. An H-ion concentration of pH 9.8 reduced the growth rate on potato dextrose agar of cultures R 3 and R 40 approximately 1/3, of R 1, R 33, R 15, R 14, and R 2 approximately 1/2, and of R 19 approximately 3/4 from that which occurred on this medium at the various optimum H-ion concentrations.

All the R 20 cultures were of a light reddish brown color excepting those on media at pH 3.2 and 3.7, in which case the mycelium was white. Cultures R 2, R 3, R 6, and R 40 produced brownish-gray

colored mycelia on potato dextrose agar at all the various H-ion concentrations except pH 3.2 in which case the mycelium remained white. The R 19 cultures were of a similar color on the medium with the six highest pH values, but were of a light gray and white color respectively on the medium at pH 3.7 and 3.2.

An examination of the R 20 cultures showed that the sclerotia were distributed over the plates in a series of distinct rings or zones. Less distinct zones were observed on many of the plates planted with the other cultures. These zoned areas were primarily due to differences in the density of mycelial growth and not to the location of the sclerotia. Some of these zones were very faint and could be seen only when the plates were held up to a light. The zonated areas in the other plates varied from faint to distinct and could be seen in reflected light. Distinct zonation was produced only by the R 14 culture and only when this culture was grown on the medium at pH 6.8. Faint zonation was common in the R 14, R 15, R 19, and R 40 cultures and very faint zonation occurred in all the Rhizoctonia cultures except those of R 20. There was evidently little if any correlation between the degree of zonation and the H-ion concentration of the medium.

The sclerotia in the R 1, R 6, and R 19 cultures were all normal except for aggregates occurring in the R 19 cultures on a medium at pH 8.4. Normal and mat-forming sclerotia were present in all of the R 3 and R 40 plate cultures. Normal and weft-like sclerotia were

present in all of the R 15 plates. Only small white weft-like sclerotia were produced by the R 33 cultures.

The largest individual sclerotia, 8 mm. in diameter, were found occurring in the R 14 strain on the pH 8.4 and 8.6 media. The next largest sclerotia occurred in the R 19 cultures on media from pH 3.7 to 8.4 inclusive. Sclerotia from 3 to 4 mm. in diameter occurred in the R 19 cultures on the two most alkaline media. Sclerotia 3 mm. in diameter also occurred in the R 40 culture grown on the medium at pH 4.9. All of the remaining sclerotia were two or less millimeters in diameter. Although there are indications that in a few of the cultures sclerotial development is retarded on this medium when its H-ion concentration is pH 3.2, the acidity of the medium appears to have little effect upon the size and type of sclerotia that develop.

Rhizoctonia Cultures on Cooked Plant Tissue. Tests were made to compare the cultural characteristics of the ten pure-lined Rhizoctonias when growing on four kinds of cooked vegetable tissue.

Twenty-four plugs measuring 64 mm. long and 13 mm. in diameter were cut each from fresh beets, Irish potatoes, carrots, and sweet potatoes. A slanting surface was cut on each of the plugs, after which they were rinsed in tap water and in distilled water and were then placed in test tubes. The plugs were sterilized in flowing steam for one-hour periods on three successive days, after which inoculations were made with the Rhizoctonia cultures.

Culture R 20, the slowest growing Rhizoctonia, was transferred to the vegetable tissues 12 hours before cultures R 14, R 2, R 6 and R 33 were transferred and 24 hours before cultures R 15, R 3, R 1, R 40, and R 19, the fastest growing cultures, were planted. The tubes containing the inoculated plugs were then kept in an incubator. During the first of the two tests made, the range of temperature of the incubator was between 22.5° to 27.5° C., with an average of 23.5° C., according to a recording thermograph. In the second test the range of temperature was 23.5° to 27.5° C., with an average of 25° C.

The cultures were examined at the end of ten days and the cultural characteristics recorded. All the Rhizoctonias grew much more slowly on the sweet potato tissue than on the other three vegetable tissues. Cultures R 1, R 19, and R 15, when grown on potato plugs, always produced aerial mycelium that formed collars at least 1 cm. above the substratum on the sides of the tubes. Mycelial collars also consistently occurred in the R 15 culture and occasionally in the R 3, R 33, and R 1 cultures when grown on beet tissue.

The mycelium of R 33 remained white on all of the four vegetable media in both tests. At the end of the ten-day period, the mycelium of R 20 was reddish brown on the beet, potato, and carrot plugs, but was gray on the sweet potato tissue. The remaining eight cultures were all quite similar in color when grown on the same vegetable tissue, being mostly brownish gray on beet and potato and lighter in color on the carrot and sweet potato plugs. In the second test most of the cultures were slightly darker than they were in the first test.

Some variation was noted in the size of the sclerotia produced in the Rhizoctonias compared in this test. The R 2 and R 19 cultures produced large sclerotia, some of which were 10 mm. in diameter. The dimensions of the individual sclerotia of R 40, R 3, R 1, R 15, and R 14 were difficult to determine because the sclerotia of these cultures frequently coalesced to form large aggregates. The maximum diameter of the R 20 sclerotia was approximately 1 mm. and the maximum diameter of the R 1 sclerotia was approximately 2 mm. when grown on these vegetable plugs. Culture R 33 produced no sclerotia.

Rhizoctonia Cultures in Living Plant Tissue. An attempt was made to determine if any of the ten pure-lined cultures of Rhizoctonia, including R 1, R 2, R 3, R 6, R 14, R 15, R 19, R 20, R 33 and R 40 selected for use in this investigation, can produce infection of living tissues of apple fruits, potato tubers, and the roots of table beets, sweet potatoes, and carrots.

Eleven each of apples (Winesap), potatoes (Irish Cobbler), table beets, sweet potatoes, and table carrots were used in this test. The fruits and vegetables were submerged for five minutes in 1-1000 $HgCl_2$ solution and then for three minutes each in two separate changes of steril distilled water. A group consisting of one apple and one each of the vegetables was inoculated with each Rhizoctonia and this group was then placed in a steril moist chamber. A separate moist chamber was used for the inoculations with each different Rhizoctonia. The surfaces of the apples and vegetables, where the incisions were to be

made, were swabbed with alcohol and then flamed. Two triangular cuts 1 to 1½ cm. deep were then made into the vegetables and apples with a steril scalpel and inoculum taken from seven to ten-day-old cultures was inserted into the cavities. The triangular pies of tissue were held in place with steril toothpicks. For controls, incisions without inoculations were made into one apple and one each of the vegetables. All the apples and vegetables were left at laboratory temperature in the moist chambers where high humidity was maintained during the ten to 24-day incubation periods.

The above experiment was repeated, but in a third trial the carrots were not used as no sign of decay had been noticed from any of the inoculations. Results on the reactions of cultures R 6, R 40, R 1, and R 2 were inconclusive in the above three tests and additional inoculations were made with these cultures that confirmed the tendencies noted in the first three tests.

The presence or absence of rot was determined by slicing the tissue away with a knife at the points of inoculation. When decaying tissue was found near a point of inoculation, reisolation of the fungus was always attempted by transferring a small portion of the decayed tissue to a plate of potato dextrose agar. These reisolations were made as far from the points of inoculation as was possible.

In none of the five plant tissues, did decay result from inoculations of R 20, R 14, and R 33. One of the five potatoes inoculated

with R 2 developed a dry rot 1 to 4 mm. in depth at the point of inoculation. In one of six inoculations, R 15, as proved by reisolations, was associated with bacteria in a small area of decayed tissue of an apple. (Table 5.)

The cultures R 3 and R 19 each produced a very definite soft rot of apple which extended into the surrounding healthy tissue for 2 to 8 mm. in each one of the six apples inoculated. Culture R 40 produced a soft rot of apples and penetrated the healthy tissue from 2 to 12 mm. The inoculations with R 6 produced a soft rot of potato tubers, a soft rot of apples and a spongy dry rot of beets.

Table 5.--Effect of inoculation of ten Rhizoctonias into living tissue of apple fruits, potato tubers, and roots of beets, sweet potatoes, and carrots. Manhattan, Kans., 1932-1933.

	10-day period Oct. 22 and 23 to Nov. 1 and 2.					17-day period Nov. 4 and 5 to Nov. 21 and 22.					15-16-day period Dec. 6 and 7 to Dec. 22.					24-day period Jan. 23 to Feb. 16				24-day period Jan. 23 to Feb. 16			
Culture	Apple	Beet	Potato	Sweet potato	Carrot	Apple	Beet	Potato	Sweet potato	Carrot	Apple	Beet	Potato	Sweet potato		Apple	Potato	Sweet potato		Apple	Potato	Sweet potato	
R 20	0	0	0	0	0	0	0	0	0	0	0	0	0	0									
	0	0	0	0	0	0	0	0	0	0	0	0	0	0									
R 14	0	0	0	0	0	0	0	0	0	0	0	0	0	0									
	0	0	0	0	0	0	0	0	0	0	0	0	0	0									
R 2	0	0	0	0	0	0	0	0	0	0	0	0	R	0			0				0		
	0	0	0	0	0	0	0	0	0	0	0	0	R	0			0				0		
R 6	0	R	0	0	0	0	R	?	0	0	R	R	?	0		R	R		0		R		0
	0	R	0	0	0	R	R	?	R	0	R	R	?	0		R	R		0		R		0
R 33	0	0	0	0	0	0	0	0	0	0	0	0	0	0									
	0	0	0	0	0	0	0	0	0	0	0	0	0	0									
R 15	0	0	0	0	0	R	0	0	0	0	0	0	0	0									
	0	0	0	0	0	0	0	0	0	0	0	0	0	0									
R 3	R	0	0	0	0	R	0	0	0	0	R	0	0	0									
	R	0	0	0	0	R	0	0	0	0	R	0	0	0									
R 19	R	0	0	0	0	R	0	0	0	0	R	0	0	0									
	R	0	0	0	0	R	0	0	0	0	R	0	0	0									
R 40	0	0	0	0	0	R	0	0	0	0	?	?	0			R	0			R			
	R	0	0	0	0	0	0	0	0	0	?	0	0			R	0			R			
R 1	0	0	0	0	0	0	0	0	0	0	R	0	0	0	R								
	0	0	0	0	0	0	0	0	0	0	R	0	0	0	R								

0 = no rot. R = rot.

DISCUSSION

It is known that some fungi may show certain degrees of degeneration when cultured on artificial media over long periods (Gilmore, 1926, and Brierley, 1926). In this investigation cultures of Rhizoctonia which had been grown for several years on artificial media were compared with recently reisolated cultures of the same Rhizoctonias but no indication of loss in vigor was noted.

A large number of investigators have studied the effect of temperatures on the growth rates of Rhizoctonias and the optimum temperatures for mycelial growth are apparently from 22° to 33° C. In this investigation it was found that the optimum temperature for mycelial growth is close to 24° C. for four of the six cultures tested and close to 27° C. for the other two.

A number of the pure-lined cultures of Rhizoctonia were grown on potato dextrose agar in sealed test tubes to determine the effect a limited supply of oxygen has on these fungi. Very scanty growth occurred in all of the sealed tubes. After 41 and 91 days certain of the tubes were opened and mycelial plantings were transferred to potato dextrose agar in Petri plates. Mycelial growth developed from all of these plantings, indicating that while the cultures were unable to grow in a very limited supply of free oxygen, they were able in this environment to remain alive for 91 days.

Ten pure-lined Rhizoctonia cultures from widely different parts of the United States, isolated from a number of different host plants and from soil, were grown in the laboratory on various nutrient media and their cultural characteristics studied. This was done to find which of them are similar and which, by dissimilarities, can be demonstrated to belong to different strains.

It was noted that no two of the ten cultures are exactly alike in all their cultural characteristics (Table 6). It was, however, found that certain ones are quite similar and that certain of them are strikingly different. Based on similarities and on rather wide differences, it is believed that these ten cultures can be placed into several distinct groups.

Table 6.--Cultural characteristics of ten Rhizoctonias on various media. Manhattan, Kans., 1932-1933.

	Rhizoctonia cultures									
	R 1	R 15	R 19	R 6	R 40	R 3	R 2	R 14	R 20	R 33
Hourly rate of growth (microns) on										
Potato dextrose agar	832	778	1003	569	801	764	604	584	275	592
Carrot agar	825	749	826	469	745	740	613	547	327	514
Richard's agar	655	531	450	370	430	535	418	403	128	304
Color mycelium on										
Potato dextrose agar	Light gray	Light gray	Brownish gray	Brownish gray	Brownish gray	Brownish gray	Brownish gray	Brown	Light reddish brown	White
Richard's and carrot agar	Light gray	Light gray	Brownish gray	Gray	Gray	Gray	Gray	Brown	Light reddish brown	White
Maximum size (mm.) of sclerotia on										
Potato dextrose, carrot, or Richard's agar	3	1	4	3	2	3	12	4	4	0
Acidified potato dextrose agar	1	2	6	2	3	1	2	2	1	0
Alkaline potato dextrose agar	1	1	6	8	2	1	2	1	0	0
Maximum size (mm.) on cooked vegetable tissue	4	3	5	10	4	2	10	4	1	0
Sclerotia coalesced	S.C.	S.C.	--	S.C.	S.C.	S.C.	S.C.	S.C.	--	--
Decayed apples	--	--	D	D	D	D	--	--	--	--
Decayed potatoes	--	--	--	D	--	--	--	--	--	--
Decayed beets	--	--	--	D	--	--	--	--	--	--
Optimum H-ion concentration of Potato dextrose agar	5.6-6.8	5.6	6.8	6.8	5.6-6.8	6.8	5.6	6.8	4.9	5.6-6.8
Mycelial collar in test tube cultures on										
Potato tissue	M.C.	M.C.	--	M.C.	--	--	--	--	--	--
Beet tissue	Occasionally	M.C.	--	--	--	Occasionally	--	--	--	Occasionally

S.C. = sclerotia coalesced. M.C. = mycelial collar. D = decayed.

Rhizoctonia culture R 15 was isolated from soil at Manhattan, Kansas. The mycelium of this Rhizoctonia is light gray in color in ten-day-old cultures on potato dextrose, carrot, and Richard's agar. This culture is a comparatively rapid grower on nutrient media. Its hourly rate of growth was found to be 778 microns on potato dextrose agar, 749 microns on carrot agar, and 431 microns on Richard's agar. The sclerotia produced are usually small, being about 1 mm. in diameter, but larger sclerotia ranging from 2 to 3 mm. in diameter were occasionally found on the media used. Several sclerotia sometimes coalesced to form larger aggregates. Inoculations with R 15 caused no decay in the living tissue of apple, potato, or beet tissue. An acidity of pH 5.6 to about 6.8 was found to be optimum for mycelial growth when growing on potato dextrose agar. At pH 3.2 or 9.8 the growth rate of the mycelium was reduced to nearly half of that which occurred when the H-ion concentration was optimum. When the acidity of this medium in potato dextrose agar was either pH 3.2 or 9.8, the growth rate of the mycelium was reduced to nearly one half of that which occurred when the H-ion concentration was optimum. This culture consistently produced mycelial collars when grown on cooked potato and beet tissue in test tubes.

Rhizoctonia culture R 1 was isolated from a stem lesion on a potato grown at Manhattan, Kansas. The mycelium of this Rhizoctonia in ten-day-old cultures on potato dextrose, carrot, and Richard's agar is light gray in color. This culture is a comparatively rapid grower

on nutrient agar media, its hourly rate of growth being 832 microns on potato dextrose agar, 825 microns on carrot agar, and 655 microns on Richard's agar. The sclerotia produced are usually small, being about 1 mm. in diameter. Larger sclerotia ranging from 2 to 4 mm. in diameter were noted occasionally. Several sclerotia sometimes coalesced to form large aggregates. Inoculations with R 1 caused no decay in the living tissue of apple, potato, or beets. An H-ion concentration of pH 5.6 to 6.8 was found to be optimum for mycelial growth when growing on potato dextrose agar. When the H-ion concentration of the medium is either pH 3.2 or 9.8 the growth rate of the mycelium is only about one half as fast as at the optimum. This culture consistently produced mycelial collars when grown on cooked potato tissue and usually when grown on beet tissue.

Cultures R 1 and R 15 are quite similar in general appearance, size of sclerotia, growth rates, and reactions to different acidities. Because of these similarities, it appears that they belong to the same strain of Rhizoctonia. These two cultures are different from the other eight cultures that were compared in such characteristics as mycelial growth rate, color of mycelium, size of sclerotia, and pathogenicity on living plant tissues. Because of these differences, it appears that the other 8 cultures investigated do not belong to the same strain as do cultures R 1 and R 15.

Culture R 14 was isolated from a sclerotium on a potato grown at Tribune, Kansas. The mycelium of this Rhizoctonia on potato dextrose

agar is brown in color in 10-day-old cultures. This culture is a moderately slow grower on nutrient media, its hourly rate of growth being 583 microns on potato dextrose agar, 507 microns on carrot agar, and 403 microns on Richard's agar. Sclerotia 2 to 4 mm. are common. Coalescing of sclerotia frequently occurs. When inoculated into the living tissue of apples, potatoes, or beets, this Rhizoctonia caused no decay. An H-ion concentration of pH 6.8 was found to be optimum for mycelial growth when growing on potato dextrose agar. When the H-ion concentration of the medium is either pH 3.2 or 9.8 the growth rate of the mycelium is only about one half as fast as at the optimum. This culture did not produce mycelial collars on cooked potato or beet tissue in test tubes.

Rhizoctonia culture R 2 was isolated from a potato stem lesion in the Delta region of California. The mycelium of this Rhizoctonia in ten-day-old cultures on potato dextrose agar is brownish gray in color. This culture grows at a moderate rate, its hourly rate of growth being 604 microns on potato dextrose agar, 613 microns on carrot agar, and 418 microns on Richard's agar. The sclerotia produced by this culture are usually 1 or 2 mm. in diameter, but sclerotia 10 and 12 mm. in diameter occur occasionally. Several sclerotia sometimes coalesce to form large aggregates. Inoculations with R 2 caused no decay in the living tissue of apple, potatoes and beets. An H-ion concentration of 5.6 was found to be optimum for mycelial growth when

growing on potato dextrose agar. When the H-ion concentration of the medium is either pH 3.2 or 9.8 the growth rate of the mycelium is only about half as fast as when the H-ion concentration is optimum. This culture did not produce a mycelial collar when cultured on cooked potato and beet tissue in test tubes.

Cultures R 14 and R 2 respond in a like manner when grown on similar media. General characteristics of the mycelium, growth rates, and pathogenicity are so similar that they apparently belong to the same strain of Rhizoctonia. These two cultures differ sufficiently, however, from the other eight in such characteristics as growth rate, color of mycelium, size of sclerotia, and pathogenicity that they evidently represent a distinctive strain of Rhizoctonia.

Rhizoctonia culture R 3 was isolated from a stem lesion of bean at Terminus, California. The mycelium of this Rhizoctonia, when growing on potato dextrose agar is brownish gray in color in 10-day-old cultures but is gray in color when grown on Richard's or carrot agar. This culture is a moderately rapid grower on nutrient media, its hourly rate of growth being 764 microns on potato dextrose agar, 740 microns on carrot agar, and 535 microns on Richard's agar. Sclerotia 1 to 2 mm. in diameter are common and sclerotia 3 mm. in diameter occur occasionally, while coalescing of sclerotia frequently occurs. When inoculated into living tissue of apples, this fungus caused infection and decay and such inoculations into living tissues of potatoes and beets did not produce infections. An acidity value of pH 6.8 in

potato dextrose agar was found to be optimum for mycelial growth. At pH 3.2 and pH 9.8 the mycelial growth rate was reduced approximately one third below the growth rate at an optimum H-ion concentration. This culture produced no mycelial collars when grown on potato tissue in test tubes, but occasionally produced collars when grown on beet tissue.

Rhizoctonia culture R 40 was isolated from cotton at Hummok, Arkansas. The mycelium of this Rhizoctonia in ten-day-old cultures is brownish gray in color. This culture is a moderately rapid grower on nutrient media, its hourly rate of growth being 801 microns on potato dextrose agar, 745 microns on carrot agar, and 430 microns on Richard's agar. Sclerotia from 1 to 2 mm. in diameter are common in cultures of this Rhizoctonia and sclerotia 3 to 4 mm. in diameter occasionally occur. In many cases several sclerotia coalesce to form large aggregates. Culture R 40 produced infection and caused decay when inoculated into the living tissue of apple fruits, but similar inoculations produced no infection in potato tubers or in beet roots. This Rhizoctonia grew rapidly on potato dextrose agar, having a wide variation in acidity. The growth rate was practically the same on the medium at pH 3.7, 4.9, 3.6, 6.8, and 8.4. When the acidity was at pH 3.2 and pH 9.8 the growth rate was approximately one third slower than when growth occurred at an optimum acidity. No mycelial collars were produced on cooked potato and beet tissue.

Cultures R 40 and R 3 respond similarly when cultured on the same kind of medium. General characteristics of the mycelium, size of

sclerotia, growth rates and pathogenicity are so similar that they apparently belong to the same strain of Rhizoctonia. These two cultures differ sufficiently from the other eight cultures in such characteristics as growth rate, color of mycelium, size of sclerotia, and pathogenicity that they evidently represent a different strain than those to which the others belong.

Rhizoctonia culture R 20 was isolated from a pansy plant at St. Paul, Minnesota. The mycelium of this Rhizoctonia in ten-day-old cultures on potato dextrose, carrot, and Richard's agar is light reddish brown. This culture is a very slow grower on nutrient agar, its hourly rate of growth being 280 microns on potato dextrose agar, 327 microns on carrot agar, and 128 microns on Richard's agar. Sclerotia commonly occur in cultures of this Rhizoctonia and vary in size from 1 to 4 mm. in diameter. Sclerotia were never found to coalesce on any of the media used. Inoculations with R 20 caused no decay in the living tissue of apple, potato, or beet. An H-ion concentration of pH 8.4 was found to be optimum for mycelial development on potato dextrose agar. When the acidity of the medium was pH 3.2, the mycelial growth rate was only one fourth of that on a similar medium where the H-ion concentration was optimum.

Culture R 20 is the slowest growing individual of the ten cultures that were compared. It is unlike any of the other cultures in that its growth rate is markedly greater on carrot agar than on potato dextrose agar. This is distinctly different from any of the other nine cultures

that were compared. The mature mycelium of this culture has a distinctive reddish color that is not found in the mycelium of any of the other nine cultures. Because of these differences, culture R 20 is considered as belonging to a distinct strain of Rhizoctonia.

Rhizoctonia culture R 33 was isolated from a flax plant at Fargo, North Dakota. The mycelium of this Rhizoctonia in ten-day-old cultures on any of the media used, was always white. This culture is a moderately slow grower on nutrient media with an hourly rate of growth of 592 microns on potato dextrose agar, 514 microns on carrot agar, and 304 microns on Richard's agar. This culture did not produce normal sclerotia. Inoculation with culture R 33 caused no decay in the living tissue of apples, potatoes, or beets. Acidities of pH 5.6 to 6.8 were found to be optimum for mycelial growth on potato dextrose agar. At pH 9.8 and pH 3.2 the mycelial growth rate is only about one half and one sixth respectively of that occurring at the optimum H-ion concentration. This culture occasionally produced mycelial collars when grown on beet tissue but never produced this aerial growth on potato tissue.

Culture R 33, because of its nearly pure white mycelium and because of absence of sclerotia, is probably the most distinctive Rhizoctonia of the ten cultures that were compared. The sharp contrasting characteristics of this culture seem to justify considering it as representing a strain separate from the others.

Rhizoctonia culture R 6 was isolated from sugar beet in Colorado. The mycelium of this Rhizoctonia in ten-day-old cultures on potato dextrose agar is brownish gray in color. It is a comparatively slow grower on nutrient media with an hourly rate of growth of 569 microns on potato dextrose agar, 469 microns on carrot agar, and 370 microns on Richard's agar. The sclerotia produced by this culture usually are approximately 1 mm. in diameter in ten-day-old cultures, but sclerotia 8 to 10 mm. in diameter occasionally are found, as are larger aggregates which are caused by the coalescing of two or more sclerotia. Inoculations with R 6 caused decay in the living tissue of apple fruits, potato tubers, and beet roots. An acidity of pH 6.8 was found optimum for mycelial growth when growing on potato dextrose agar. When the acidity of this medium was pH 9.8 and 3.2 the growth rate was reduced approximately one third and one half respectively, as compared to the optimum. This culture did not produce mycelial collars when grown in test tubes on cooked potato or beet tissues.

Rhizoctonia culture R 6 has been found to differ from all of the other nine cultures that were compared in one or more cultural characteristics such as size of sclerotia, color of mycelium, growth rate, and presence or absence of mycelial collars. It was the only culture that produced infection in the living tissue of potato tubers and beet roots. Its relationship to the other nine cultures is, however, not clearly indicated from the results that were obtained.

Rhizoctonia culture R 19 was isolated from brown patch of turf at Madison, Wisconsin. The mycelium of this Rhizoctonia in ten-day-old cultures on potato dextrose agar is brownish gray in color. The culture is a very rapid grower on nutrient agar media, its hourly rate of growth being 1003 microns on potato dextrose agar, 826 microns on carrot agar, and 450 microns on Richard's agar. The sclerotia produced by this culture range in size from 1 to 6 mm. in diameter, although sclerotia 1 mm. in diameter were the most common. Large sclerotial aggregates are sometimes formed because of the coalescing of two or more sclerotia. Inoculations with R 19 into the living tissue of apple fruits caused infection and decay, but such infection did not follow inoculations into living potato and beet tissue. Acidities of pH 5.6 to 6.8 in potato dextrose agar were found optimum for mycelial growth. When the H-ion concentration of this medium was pH 9.8 and 3.2 the growth rate was reduced to one third and one fourth respectively, when compared to the growth rate at an optimum H-ion concentration. This culture did not produce mycelial collars on cooked potato or beet tissue in test tubes.

Culture R 19 is the most rapid growing Rhizoctonia used in these comparisons, but its various characteristics are not sufficiently similar or distinctive from the other nine cultures to clearly indicate relationships.

SUMMARY

Ten cultures of Rhizoctonia isolated from a number of substrata from various places in the United States were compared while growing on a number of different media. Cultures R 1 and R 15, isolated respectively from a potato plant and from soil at Manhattan, Kansas, both appear to belong to the same strain. Culture R 40, isolated from a cotton plant in Arkansas, and culture R 3, isolated from a bean plant in California, seem to represent a second strain. Culture R 14 and R 2, isolated respectively from Kansas grown and from California grown potatoes, appear to belong to a third strain. Culture R 33 from flax in North Dakota is a distinctly different culture from all others compared, as is also R 20, an isolation from pansy in Minnesota. Culture R 6 from sugar beet from Colorado and culture R 19 from turf at Madison, Wisconsin, are not sufficiently alike or different so that their relationship to the others is clear.

Six Rhizoctonias were cultured at various temperatures and the optimum for mycelial growth found to be close to 24° C. for cultures R 4, R 5, R 7, and R 14, and close to 27° C. for cultures R 1 and R 15.

There was found to be no indication of degeneration or loss of vigor in Rhizoctonias cultured continually on artificial media for 2 to 4 years.

Rhizoctonia cultures were found to remain alive for 91 days on artificial media in sealed test tubes.

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