RELATIVE AERATION REQUIREMENTS BY SOFT ROT AND BASIDIOMYCETE WOOD-DESTROYING FUNGI

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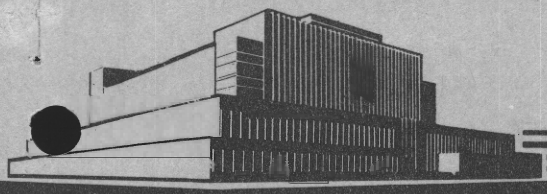

FOREST PRODUCTS LABORATORY
MADISON 5, WISCONSIN

UNITED STATES DEPARTMENT OF AGRICULTURE
FOREST SERVICE
In Cooperation with the University of Wisconsin

RELATIVE AERATION REQUIREMENTS BY SOFT ROT AND BASIDIOMYCETE WOOD-DESTROYING FUNGI

By

CATHERINE G. DUNCAN, Pathologist

Forest Products Laboratory,[1] Forest Service
U.S. Department of Agriculture

Summary

Isolates of 50 soft-rot fungi and 41 Basidiomycete decay fungi were studied for growth responses in sealed and partially closed culture tubes. All isolates maintained a uniform rate of linear growth until oxygen was exhausted in the tubes, which suggests that respiration was mediated through the cytochrome system. Linear growth was suppressed more by lack of aeration with the soft rotters than with the Basidiomycetes, but the reserve was true as regards many soft rotters isolated from cooling towers, when growth was measured by the weight of mycelium produced. Taking total growth (weight of mycelium) as the preferable index of relative tolerance of restricted aeration, it is concluded that the prevalence of soft rot in the wet wood of cooling towers, and the near absence of Basidiomycetes, is attributable at least in part to a superior tolerance of poor aeration on the part of the soft-rot fungi concerned. No significant difference in growth responses was noted between white rotters and brown rotters in the Basidiomycete group.

Introduction

Certain Ascomycetes and imperfect fungi are capable of causing rot in very wet wood where decay by Basidiomycete fungi cannot take place although temperature and conditions of nutrition are quite adequate. One probable explanation for this difference in decay capacity is that the soft rotters may require less oxygen, since much of the air in the wood cells is replaced by water. Another conceivable explanation is that the soft rotters may have a greater tolerance for the increase in carbon dioxide, the appearance of exudate materials--the so-called staling products--or a change in pH of the surroundings. Both reproductive processes and the vegetative growth of fungi are known to be affected by a severely limited supply of oxygen or increased quantities of carbon dioxide, conditions which might be present in a poorly aerated environment.

The present study was planned to obtain information on the rates of growth of representative fungi causing the two types of decay, when the fungi were grown on malt agar, over which the exchange of air either was limited by requiring it to take place through water or else was entirely prevented. Quantitative measurements of oxygen depletion and carbon dioxide build-up were not made. However, it was hoped that differences in growth might be found between the fungus groups when subjected to uniformly restricted aeration. Such differences would indicate physiological characteristics that might supply a clue as to why some Ascomycetes and imperfect fungi, such as those causing soft rot, are capable of attacking wood under conditions of wetness seldom tolerated by the Basidiomycetes.

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[1] Maintained at Madison, Wis., in cooperation with the University of Wisconsin.

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Methods

Growth of the fungi was determined by (1) linear rates of growth and (2) total dry weight of mycelium produced on malt agar in modified test tubes (6)[2] whose openings were closed with either cotton, rubber stoppers, or water of a given depth.

The test tubes were 20 centimeters long by 2 centimeters in diameter at the mouth. They had been modified by a deep indentation of the wall on one side near the mouth, so as to form a dam on the lower side of the tube to prevent escape of the liquid when the sterilized medium was cooled and solidified with the tube in a horizontal position. This resulted in a uniform, narrow strip of substrate along one side of the tube, approximately 15 centimeters long, 2 centimeters wide, and about 1 centimeter deep along the middle. In order to keep the substrate in place when the tubes were later placed in a vertical position, the tubes were not disturbed until the strips had been allowed to harden for 24 hours.

The malt agar medium contained 2 percent each of Difco malt and agar in distilled water, with the pH adjusted to 6 before sterilization. This nutrient was not necessarily optimum for all the fungi to be used but was known to permit good, uniform growth. Fifteen cubic centimeters of the melted agar were put into each test tube, which was then plugged firmly with cotton and autoclaved at 15 pounds of pressure (121° C.) for 15 minutes.

The inoculum (approximately a 3-millimeter cube) was cut with a two-pronged blade from the growing margin of a Petri-dish culture so as to provide mycelium 2 to 7 days old. The inoculum was placed at the forward end of the agar strip, mycelial surface downward, so that new growth started directly on the medium and extended linearly toward the closed end of the tube. For most fungi, the advancing margin of new growth was nearly straight and at right angles to the long axis of a tube. Six tubes were inoculated with each fungus isolate.

The inoculated tubes were placed in an incubation room on racks that held them vertically, with the rounded ends uppermost. This position of the tube was necessary in order to keep the malt-agar surface free of condensed water, which prevented an even growth margin.

The incubation room was maintained at 80° F. (26.7° C.) and 70 percent relative humidity. Although this temperature was optimum for most of the Basidiomycetes, it was somewhat below that for many of the imperfect fungi studied (3).

Measuring Linear Growth

As soon as the fungus started to grow from the inoculum onto the agar strip, the measurements of linear growth were begun by marking on the culture tube the position of the advancing mycelial margin, using for the purpose a ground-glass marking line made on the same side of the tube as the substrate. This mark was considered the starting point for the growth record, and was made at the same time for all six tubes inoculated with a particular isolate.

Immediately after the initial marking, the cotton plugs were removed from 4 of the 6 tubes containing a given fungus and replaced with two different types of closure, each intended to limit aeration of the cultures. A rubber stopper was inserted tightly into two of the four tubes (subsequently referred to as "sealed" tubes) and the connection covered with wax to prevent air exchange. Water was placed in the open necks of the other two tubes (subsequently referred to as "water-closed tubes") to a distance of about 1 inch with the purpose of limiting but not preventing air exchange. This type of closure was accomplished by placing the tubes upside down in wooden racks so that the mouths extended 1 inch below the water surface. The water was maintained at a depth of 2 inches in a large pan. Observations of growth were made without removing the tubes from the water. The remaining two tubes inoculated with a given isolate (socalled "normal" tubes) were left, with the cotton plugs, through which exchange of air could take place relatively freely. All the tubes were incubated for the duration of the growth studies in a room maintained at 80° F. (26.7° C.) and 70 percent relative humidity.

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[2] Underlined numbers in parentheses refer to the literature cited at the end of this report.

Growth of the fungi was measured every 24 hours by viewing the margin of the mycelium through the substrate and marking it on the ground glass strip. Observations were made of the two sealed and corresponding two normal tubes until 48 hours after linear growth had ceased in the former. This meant that the record of linear growth for the normal tubes covered a period 48 hours longer than that for the sealed. Linear growth in the two water-closed tubes likewise was recorded until it ceased.

At the completion of the linear growth observations, the distances between the markings on the tubes, representing the daily growth rates, were measured to the nearest millimeter, and the average daily rates with the respective tube closures were computed from these measurements.

Measuring Growth by Dry Weights

After the completion of the linear growth studies, the dry weight of the mycelium produced in each sealed and normal tube was determined. The growth tubes were opened, filled to within 1 inch of the top with water, and steamed for 30 minutes to separate the mycelial mat from the agar. The melted contents of a tube were then poured through a tared filter paper, and the mycelial residue was washed by passing approximately 250 cubic centimeters of boiling water through the filter system. The filter paper and mycelium were then oven-dried and weighed, and the weight of the mycelium was computed.

Selection of Fungi

A list of the 91 fungi used in these growth studies is found in table 1. Of this number, 41 were Basidiomycetes, including 21 white and 20 brown rotters. None of these Basidiomycetes, except Poria nigrescens and Peniophora mollis, are known to cause frequent decay in wood that is consistently very wet. These two white-rot exceptions cause considerable decay in the structural members, and occasionally in slats of water cooling towers.

Fifty of the 91 isolates were soft-rot fungi, 47 being identified as Fungi Imperfecti and three as Ascomycetes. All had been isolated either from wood in cooling towers or from wood in contact with soil. Four genera--Acremonium, Cephalosporium, Phoma, and Phialophora--were represented by more than one isolate, since they are most frequently isolated from cooling tower slats. Likewise, members of these four genera have shown considerable capacity to produce soft rot under laboratory conditions.

Results

Before examining the differences in growth results among the fungi, it is of interest to note a characteristic of their growth under reduced aeration which indicated a particular type of respiration. From plottings of the amount of growth against length of time (fig. 1), it was evident that the rate of linear growth by a given fungus did not vary significantly from the starting point until growth ceased because of lack of oxygen. This straight-line relation of growth to time over the entire period of development was maintained, without observed exception, by both soft-rot and Basidiomycete fungi grown in either the sealed or water-closed tubes.

In plants and animals, the type of respiration that proceeds at a regular rate until the oxygen supply is essentially exhausted is commonly associated with the cytochrome system. According to Cochrane (2), the cytochrome system appears to be general in fungi as judged by spectroscopic observations, although such measurements cannot indicate with precision whether the components of a new system are the same as those of the more familiar yeast and animal system. To check this general assumption, a soft rotter (Acremonium sp., R48B) and a brown rotter (Poria monticola, Mad., 698) were examined visually with a microspectroscope[2] after the heavy mycelial suspensions had been treated with sodium hydrosulfite ($Na_2S_2O_4$) to reduce the cytochromes. The appearance of bands near 552, 562, and 605 millimicrons indicated the presence of cytochromes c, b, and a.

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[2]The possibility that the cytochrome system might be involved here was suggested by R. H. Burris of the University of Wisconsin. Dr. Burris also made the microspectroscope observations.

Linear Growth Studies in the Sealed and Water-Closed Tubes

A summary of the linear growth rates relative to the normal, and of the total number of days over which growth could take place in the sealed and water-closed tubes, is given in table 2 for the 50 soft-rot fungi and the 41 brown- and white-rot Basidiomycetes. These data indicate that, for the majority of fungi, differences in linear growth between cultures in the water-closed and sealed tubes were small, being for all types slightly greater in the water-closed than in the sealed. Similar small differences were exhibited in the number of days through which the fungi were able to grow in the two types of tubes. These small differences were disappointing, since it had been hoped that the water-closed tubes would be less restrictive of growth and, therefore, would provide comparisons among the fungi at growth-retarding but not inhibiting levels of aeration. It seems noteworthy in this respect only that the slight superiority of development in the water-closed tubes was consistently greater in the case of the Basidiomycetes than with the soft rotters.

One striking difference in linear growth unrelated to the type of culture tube is brought out by table 2. The percentage of normal linear growth by the Basidiomycetes (95 percent) was much greater than that by the soft rotters (67 percent). There was no important difference in relative growth rates between the white rotters and brown rotters.

The marked difference in relative growth rates between the soft-rot and Basidiomycete fungi in sealed tubes is additionally apparent from a comparison of the frequency distribution of the fungi in various growth rate groups. Such a comparison is provided by the bar graphs in figure 2. Included in the figure are separate data for four genera of soft rotters that have been most frequently isolated from cooling towers. It may be noted that all the brown rotters and most of the white rot isolates maintained a linear growth rate that was at least 80 percent of normal. By contrast, only about one-fourth of the 50 soft rot isolates attained as high a relative growth rate. Without additional data, it cannot be said how typical may be the moderate differences shown in figure 2 between all the soft rot isolates and the four selected soft rot genera, or between the white rot and brown rot species. In any case, the respective differences are not indicated to be large enough to be of practical importance.

It was expected, of course, that fungi which normally inhabit the wettest and most poorly areated wood would be more capable than others of approaching normal growth rates in the sealed tubes. However, the growth rate of the cooling tower isolates in the genera, Acremonium, Cephalosporium, Phialophora, and Phoma, were no less affected by the closed tube condition than the soft rotters in general. Thus, neither in this comparison within the group of soft-rot fungi, nor in the comparison of relative growth rates of the soft rotters and the Basidiomycetes in sealed tubes, is there a clue as to why soft rotters appear to be more tolerant of very wet, poorly aerated wood than are brown- or white-rot fungi. Such a clue was provided, however, by comparisons of total production of mycelium--as contrasted with amounts evidenced only by linear growth.

Comparison of Growth Capacities Measured by the Dry Weight of Mycelium Produced

The dry weight of the mycelium of each fungus isolate in the sealed and normal tubes was obtained as soon as linear growth ceased in the sealed tubes. The mean dry weights of mycelium, as percentages of normal weight, are shown in table 3. The mean mycelial weights of the brown-, white-, and soft-rot fungi in the sealed tubes were 78, 81, and 87 percent, respectively, of the normal weights. The four genera of soft rotters most frequently isolated from cooling towers exhibited on the average no decrease in rate of mycelium production (mean growth percent equals 104). Thus, although these figures indicate no marked difference in mycelium production under restricted aeration between the Basidiomycetes and the soft rotters as entire groups, the difference is the opposite of that observed for linear growth. It seems particularly significant, moreover, that the four principal cooling-tower isolates were able to maintain fully their normal rate of growth until the oxygen supply was exhausted.

A superior capacity of these cooling-tower fungi to develop with limited oxygen is more strikingly evidenced in the growth-frequency diagrams of figure 3. Approximately 80 percent of the soft rot isolates, representing Acremonium, Cephalosporium, Phialophora, and Phoma species, produced a weight of mycelium amounting to not less than 90 percent of normal, in contrast to no more than about 50 percent of the Basidiomycete species.

No significant difference between the white- and brown-rot fungi in capacity to maintain production of mycelial mass is indicated.

Discussion and Conclusions

Measurement of growth by the linear development of the mycelium on malt-agar indicated that the brown- and white-rot fungi (Basidiomycetes) were capable of greater growth than the soft-rot fungi where air was excluded from the culture tube. Measurement of growth by the dry weight of the mycelium produced indicated that the soft-rot fungi most frequently isolated from the wet wood of cooling towers were capable of greatest growth under such conditions.

Since dry weight reflects the total rather than a part of the development of a fungus, and for other reasons that will follow, weight rather than linear growth of mycelium is believed to be the better index of the tolerance of restricted aeration by these fungi. From this standpoint, the isolates in the four genera of soft-rot fungi most frequently isolated from cooling towers exhibited superior tolerance. This furnishes at least a partial explanation of why soft rot, but seldom brown or white rot, is found in the wetter wood of cooling towers. It seems pertinent to add that Basidiomycete decay fungi typically do not invade wood that is wet enough to materially limit the ingress of oxygen.

The reason for the differences between linear and weight growth of the fungi under the conditions of poor aeration is not known. Other investigators have found, however, that these two measurements of growth can differ considerably, and it has been concluded by some that mycelial extension sometimes is a poor criterion of development. In comparing the diameters and dry weights of similarly aged Petri-dish cultures of Ceratostomella ulmi in the presence of varying amounts of pyridoxine, Fries (4) found that the average size of 5-day-old colonies without pyridoxine was 16.3 millimeters, whereas that of colonies receiving pyridoxine was 12.3 millimeters. The weights of mycelium produced under these two conditions were in the reverse order--5.2 and 18.1 milligrams, respectively. Similar differences between diameters of growth and dry weights of C. fimbriata also have been shown by Lilly and Barnett (5).

Brancato and Golding (1), on the other hand, came to the conclusion that the diameter of a fungus colony is sufficiently reliable for determining growth rates because there is no acceleration of growth rate with time under a given set of conditions. They did not, however, directly compare colony diameters with weight except when determining the effects of depths of media. In their comparison, Aspergillus niger, A. flavus, and Penicillum notatum produced the same diameter of colonies on media 1 or 4 millimeters in depth, but the weight of mycelium was 2 to 30 milligrams greater on media with the greater depth.

In the present study, maintenance of a normal weight production of mycelium by the four genera of cooling-tower fungi despite a reduced rate of linear growth probably has a simple explanation. It is assumed--with limited confirmatory observations--that the mycelium was developed by these fungi at a greater than usual depth in the agar substrate, in amounts sufficient to offset the lesser surface development. It would follow, of course, that those Basidiomycetes which were able to maintain a normal rate of linear growth, did so at the expense of their usual depth of subsurface development.

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1935. A Tube for Culturing Fungi. Science 82:467-468.

Table 1.--Soft rot and Basidiomycete fungi whose relative aeration requirements were assessed in normal, sealed, and water-closed tubes

Generic name	Isolate No.	Generic name	Isolate No.
SOFT ROT FUNGI		BASIDIOMYCETE FUNGI	
ASCOMYCETES		WHITE ROTTERS	
Chaetomium cochliodes	P 7A	Corticium B	MD 5096-56
Chaetomium globosum	P 10A	Corticium galactinus	MD 223
Orbicula sp.	P 35	Fomes applanatus	Mad. 708
FUNGI IMPERFECTI		Fomes rimosus	Mad. 696
Acremonium sp.	R 11	Merulius tremellosus	MD 103
Do.	R 18	Mycoacia stenodon	ML 32
Do.	R 35	Odontia bicolor	Wash. Flo. 126A
Do.	R 39	Peniophora mollis	ML 22
Do.	R 48B	Peniophora sp. No. 1	MD 192
Do.	R 53B	Polyporus adustus	MD 194
Do.	R 55F	Polyporus gilvus	Wash. 71316
Do.	P 19	Polyporus tulipiferae	Mad. 517
Do.	P 36	Polyporus versicolor	Mad. 697
Alternaria sp.	R 4	Poria abietinus	Wash. 97099
Do.	P 2A	Poria nigrescens	Mad. 4856
Bispora sp.	P 12F	Poria subacida	Mad. 4844
Do.	P 26B	Schizophyllum commune	Mad. 619
Bispora effusa	CBS	Unknown E	Mad. 5096-23
Cephalosporium sp. No. 1	R 47C	Unknown F	Mad. 5134
Do. No. 4	R 75	Unknown	MD 195
Do. No. 5	P 12I	Unknown	MD 322
Do. No. 7	P 13		
Cladosporium sp.	P 18	BROWN ROTTERS	
Corethropsis sp.	R 58E	Coniophora avida	MD 323
Diplodia sp.	P 9C	Coniophora olivaceae	MD 361
Fusarium sp. No. 1	R 27	Coniophora puteana	Mad. 515
Gliocladium sp.	R 58B	Daedalia quercina	Wash. 59058
Graphium sp.	R 47F	Fomes roseus	Mad. 701
Haplochalara sp.	P 6B	Lentinus lepideus	Mad. 534
Do.	G 1A	Lenzites saepiaria	Mad. 604
Monosporium olivaceum	R 38	Lenzites striata	Wash. 103133
Pestalozzia sp.	P 16	Lenzites trabea	Mad. 617
Do.	P 17	Polyporus schweinitzii	MD 132
Pestalozzia funerea	P 40	Pleurotus ostreatus	MD 19
Do.	Re 1B	Poria cocos	MD 276
Phialophora richardsiae	R 1	Poria incrassata	Mad. 563
Do.	R 7	Poria monticola	Mad. 698
Do.	R 12	Poria oleraceae	Mad. 4907
Do.	R 30	Poria vaillantii	Wash. 90877
Phoma sp. No. 1	R 2	Poria xantha	Mad. 5096-35
Do.	R 57B	Unknown	MD 31
Phoma sp. No. 2	R 69	Unknown	ML 23
Pullularia sp.	R 15	Unknown	ML 19
Sclerotium sp.	G 4		
Stysanus sp.	G 3		
Do.	S 91		
Unknown sp.	R 59A		
Do.	R 59B		
Do.	P 5A		
Do.	P 15		
Do.	P 29		

Table 2.--Relative rates of linear growth and the number of days until stoppage of growth by the different types of fungi in sealed and water-closed tubes

Type of fungus and number of isolates	Growth rate relative to normal			Duration of growth		
	In sealed tubes	In water-closed tubes	Difference	In sealed tubes	In water-closed tubes	Difference
	Percent	Percent	Percent	Days	Days	Days
Soft rotters (50)	66	68	2	10.2	11.0	0.8
Mean	67			10.6		
Basidiomycetes						
White rotters (21)	90	96	6	8.4	12.3	3.9
Brown rotters (20)	95	99	4	8.5	10.8	2.3
Mean	95			10.0		

Table 3.--Relative amounts of mycelium produced by the different types of fungi in sealed tubes

Type of fungus and number of isolates	Dry weight of mycelium produced relative to normal
	Percent
Soft rotters	
All isolates (50)	87
Isolates most frequent from cooling towers (20)[1]	104
Basidiomycetes	
White rotters (21)	81
Brown rotters (20)	78

[1] Isolates in genera Acremonium, Cephalosporium, Phialophora, and Phoma.

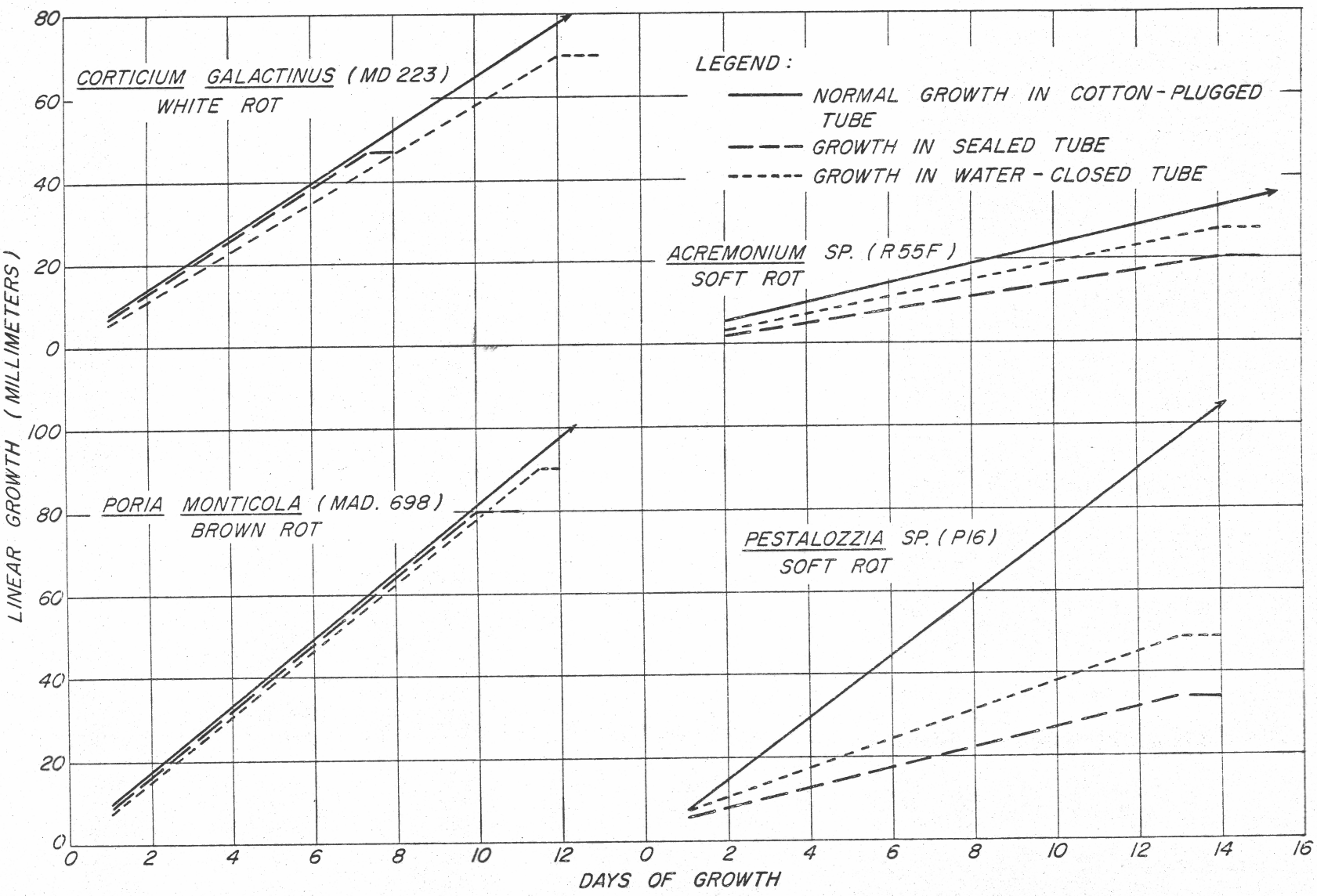

Figure 1.--Examples of linear growth-time relations observed for white-, brown-, and soft-rot fungi growing on malt agar in normal, sealed and water-closed tubes.

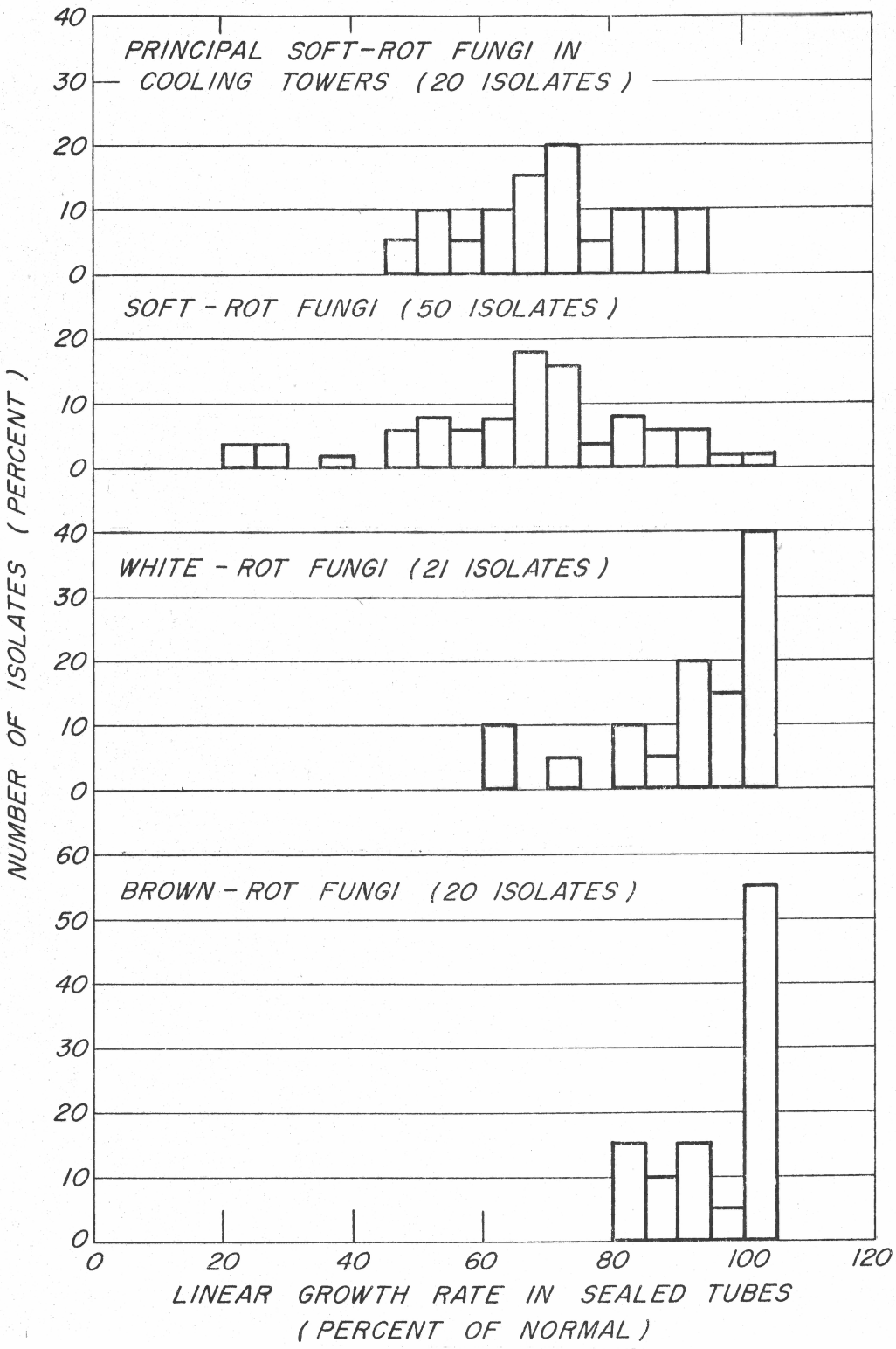

Figure 2.--Frequency distribution of the fungi according to indicated relative capacities for linear growth in sealed tubes.

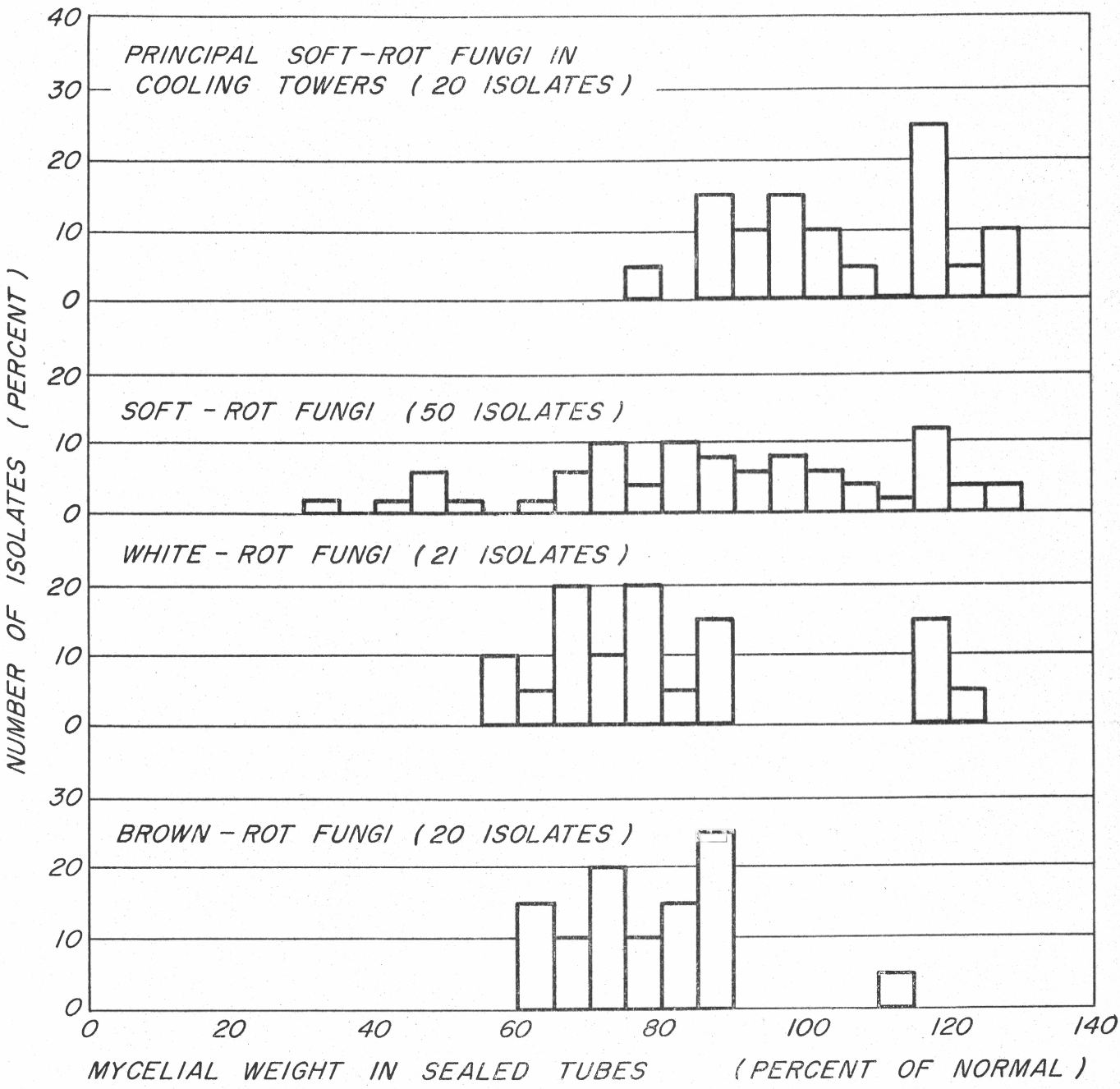

Figure 3.--Frequency distribution of the fungi according to indicated relative capacities for increasing mycelial weight in the sealed tubes.

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