STUDIES IN THE PHYSIOLOGY OF THE FUNGI

V. The Growth of Certain Fungi in Plant Decoctions

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In a previous paper attention has been drawn by the writers[1] to the growth relations of certain fungi in a few plant decoctions with and without additional nutrients. In the continuation of this work much the same methods have been followed, but the number of fungi employed has been reduced to two, the one, *Aspergillus niger,* being taken as a representative of saprophytic species, and the other, *Gloeosporium* (*Glomerella*) *Gossypii* as a representative of parasitic forms.

Besides the decoctions previously employed, namely, bean, sugar beet, prune, potato, turnip, and corn meal, there have been used also decoctions of apple, mangold (mangel-wurzel), celery, carrot, and salmon. Basing the decoctions as before on 50 grams of the dry weight of the material used per liter of water, the fresh weight quantities of the additional materials involved were as follows: apple, 294.1 gm.; carrot, 438.2 gm.; mangold, 546.4 gm.; and celery, 333.3 gm. In the case of the salmon it was not practicable to determine the amount of material required on a dry-weight basis, so that one can, 15½ oz. (439.4 gm.) net, was used for a liter of water. As far as possible the oil or fat was eliminated. In the case of the celery untrimmed bunches were employed. In each instance the product was cut into small pieces, a liter of water added to the required green weight of product, and this was steamed for one hour at 15 pounds in an autoclave, then filtered hot through Canton flannel, and finally made up to the proper volume. After stock flasks were arranged the solutions were autoclaved for 20 minutes at 15 pounds pressure. Titrations of these decoctions showed the following reaction in terms of Fuller's scale: apple, +14.5; carrot, +14.5; celery, +10.5; mangold, +15; and salmon, +43. As mentioned later, however, little weight is attached to these values.

[1] Duggar, B. M., Severy, J. W., and Schmitz, H. Studies in the physiology of the fungi. IV. The growth of certain fungi in plant decoctions. Ann. Mo. Bot. Gard. 4: 165–173. *f. 1–4.* 1917.

In combining other nutrients with the standard decoctions it is impracticable to make the various solutions in a series entirely comparable. It has seemed that the greater error would result from any attempt to prepare a double-strength decoction, to be diluted with a solution of the nutrients introduced; so it was determined to add to the decoction the various nutrients in solid form. This necessarily increased slightly the volume of the solution. The slight excess over the correct volume was, however, discarded, since any increase of volume would diminish the area of the flask. Some few tests, as indicated in tables I and II, required the dilution of the decoction to one-half. In every case the volume of culture medium in each Erlenmeyer flask was 25 cc., the cultures were made in duplicate, and in certain instances parts of a series

TABLE I

GROWTH OF ASPERGILLUS NIGER ON VARIOUS DECOCTIONS WITH AND WITHOUT OTHER NUTRIENTS

Culture medium	Dry weight in grams				
	Apple	Mangold	Carrot	Celery	Salmon
1 Natural decoction	.019	.178	.052	.085	.094
1a Standardized dec't. (to + 15 Fuller's scale)	....	....	....	.075	.066
2 Nat'l. dec't. + 13.68% sugar	.038	.259	.077	.489	.863
3 Nat'l. dec't. + 13.68% sugar +1% KNO_3	.311	.319	.679	.946	1.063
4 Nat'l. dec't. + 1% KNO_3	.145	.232	.144	.080	.075
5 Nat'l. dec't. +13.68% sugar + .5% KH_2PO_4	.046	.309	.119	.489	.768
6 Nat'l. dec't. + .5% KH_2PO_4	.021	.233	.051	.076	.117
6a Nat'l. dec't. + 1% KH_2PO_4	.022	.240	....	....	....
7 Nat'l. dec't. + 13.68% sugar +1% KNO_3+.5% KH_2PO_4	.462	1.016	.922	1.257	1.028
8 Nat'l. dec't. +1% KNO_3+.5% KH_2PO_4	.221	.304	.151	.079	.085
9 ½ nat'l. dec't. and ½ Richards' sol.*	.445	.547	.537	.699	.738
10 ½ nat'l. dec't. and ½ distilled water	.016	.104	.028	.036	.037
½ nat'l. dec't. and ½ Richards' sol	.472	.476	.455	....	.469
½ nat'l. dec't. and ½ ash sol	.025	.137	.034	....	.057
Nat'l. dec't. + .25% $MgSO_4$ + 13.68% sugar	.034	....	....	....	....
Nat'l. dec't. brought to 10^{-6} with NaOH	....	.235	....	....	....
Nat'l. dec't. brought to 10^{-6} with K_2HPO_4	....	.239	....	....	....

* The nutrient solution designated Richards' solution in these tables is made up as follows: KNO_3 1 gm., KH_2PO_4 .5 gm., $MgSO_4$ .25 gm., sugar 5 gm., distilled water 100 cc.

were repeated. The manipulation of the cultures, dry-weight determinations, etc., were exactly as described in our previous paper.

The complete results of these series are given in tables I, II, and III, and most of these data are shown graphically in figs. 1–5. These curves are based primarily on the relations of

TABLE II

GROWTH OF GLOEOSPORIUM GOSSYPII ON VARIOUS DECOCTIONS WITH AND WITHOUT OTHER NUTRIENTS

Culture medium	Dry weight in grams				
	Apple	Mangold	Carrot	Celery	Salmon
1 Natural decoction	.049	.241	.123	.071	.135
1a Standardized dec't. (to + 15 Fuller's scale)	....	....	....	.072	.111
2 Nat'l. dec't. + 13.68% sugar	.059	.379	.344	.817	.596
3 Nat'l. dec't. + 13.68% sugar + 1% KNO_3	.333	.330	.561	.689	.730
4 Nat'l. dec't. + 1% KNO_3	.223	.195	.153	.073	.160
5 Nat'l. dec't. + 13.68% sugar + .5% KH_2PO_4	.171	.444	.283	.912	.653
6 Nat'l. dec't. + .5% KH_2PO_4	.085	.281	.149	.104	.163
6a Nat'l. dec't. + 1% KH_2PO_4	.040	.279	....	....	....
7 Nat'l. dec't. + 13.68% sugar + 1% KNO_3 + .5% KH_2PO_4	.395	.835	.533	.748	.552
8 Nat'l. dec't. + 1% KNO_3 + .5% KH_2PO_4	.216	.348	.164	.099	.131
9 ½ nat'l. dec't. and ½ Richards' sol	.470	.581	.485	.497	.535
10 ½ nat'l. dec't. and ½ distilled water	.038	.158	.073	.047	.096
½ nat'l. dec't. and ½ Richards' sol	.551	.492	.513	....	.509
½ nat'l. dec't. and ½ ash sol	.044	.134	.032	....	.098
Nat'l. dec't. + .25% $MgSO_4$ + 13.68% sugar	.126	....	....	....	....
Nat'l. dec't. brought to 10^{-6} with NaOH	....	.209	....	....	....
Nat'l. dec't. brought to 10^{-6} with K_2HPO_4	....	.284	....	....	....

the different cultures containing the decoctions, but for comparison half-strength and full-strength Richards' solution, also Richards' solution containing 13.68 per cent sugar, are included.

In the tables the numerals at the left of the various culture solutions correspond to those in the legends of the figures, and therefore a fuller explanation of the figures is afforded by referring to the tables. The addition of sugar and other nutrients to the decoctions (or other solutions) is expressed in the tables by the plus sign, and is to be interpreted that the decoction is used as solvent and contains those amounts. Where two solutions are mixed half the quantity of each is employed, so that the resulting medium is half strength with respect to each.

TABLE III

ASPERGILLUS AND GLOEOSPORIUM ON RICHARDS' SOLUTION AND MODIFIED DECOCTIONS

	Culture medium	Dry weight in gms.	
		Aspergillus	Gloeosporium
11	½ Richards' sol. and ½ distilled water	.424	.202
12	Richards' sol	.587	.343
13	Modified Richards' sol. to contain 13.68% sugar + .5% K_2HPO_4	.842	.369
	Modified Richards' sol. to contain 13.68% sugar + .25% K_2HPO_4 + .25% KH_2PO_4	.755	.522
	½ nat'l. bean dec't. and ½ Richard's sol	.457	.542
	½ nat'l. bean dec't. and ½ bean dec't. ash sol	.043	.044
	½ nat'l. prune dec't. and ½ Richards' sol	.456	.517
	½ nat'l. prune dec't. and ½ prune dec't. ash sol	.070	.082
	½ nat'l. sugar beet dec't. and ½ Richards' sol	.437	.498
	½ nat'l. turnip dec't. and ½ Richards' sol	.455	.501
	½ nat'l. turnip dec't. and ½ turnip dec't. ash sol	.044	.051

Naming the decoctions in the order of the best growth as determined by the criterion of weight the series for *Aspergillus* is as follows: mangold, salmon, celery, carrot, and apple; while for *Gloeosporium* the same order prevails except that celery and carrot exchange places. The mangold is relatively rich in sugar, and this perhaps explains the higher growth quantities in the decoction of this product as compared with the other native decoctions mentioned. The addition of sugar alone to mangold decoction, as might be expected, gives very little more growth than the addition of one of the mineral nutrients. With the addition of sugar alone to the various decoctions *Aspergillus* shows the greatest increase in salmon decoction, about 900 per cent, the celery medium exhibiting the next greater increase; while with *Gloeosporium* the order of these two higher is reversed. The marked increase in growth in salmon decoction with the addition of sugar is a clear indication that this substratum is well provided with the other essential nutrients. The general appearance of the curves indicating the yields in celery solutions demonstrates a fairly close agreement with the curves for salmon solutions.

It is interesting to note that while *Aspergillus* makes a heavier growth in decoctions plus sugar and mineral nutrients (except in the case of apple) than in the Richards' solution

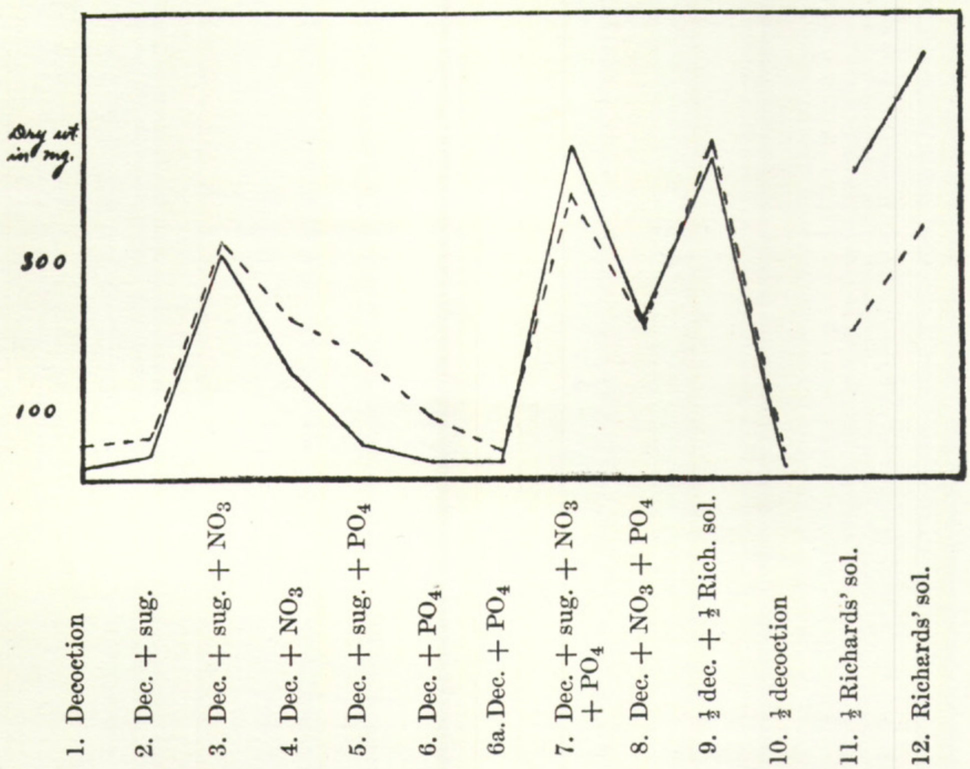

Fig. 1. Growth of *Aspergillus* and *Gloeosporium* on apple decoction and other nutrients; *Aspergillus*, solid line; *Gloeosporium*, broken line.

containing the same amount of sugar, still the differences are scarcely as great as might be expected, taking into consideration the fact that the decoction is a complex food solution. That the solution containing apple decoction, sugar, etc., yields less growth than the Richards' solution is noteworthy. With *Gloeosporium* obviously the Richards' solution is not as satisfactory as the decoction-containing solutions, but as yet there is no evidence as to the nature of the nutrients lacking in the former.

In table III ash solutions of several decoctions are mentioned. These were prepared by drying down 25 cc. (for two cultures) of each decoction, then carefully incinerating. This ash was then dissolved or diffused in 25 cc. of water and combined with the decoction. In all cases this addition of mineral constituents gave slightly increased yield.

Aspergillus fruited well in the great majority of cultures. In the mangold solutions some depression of fruiting occurred

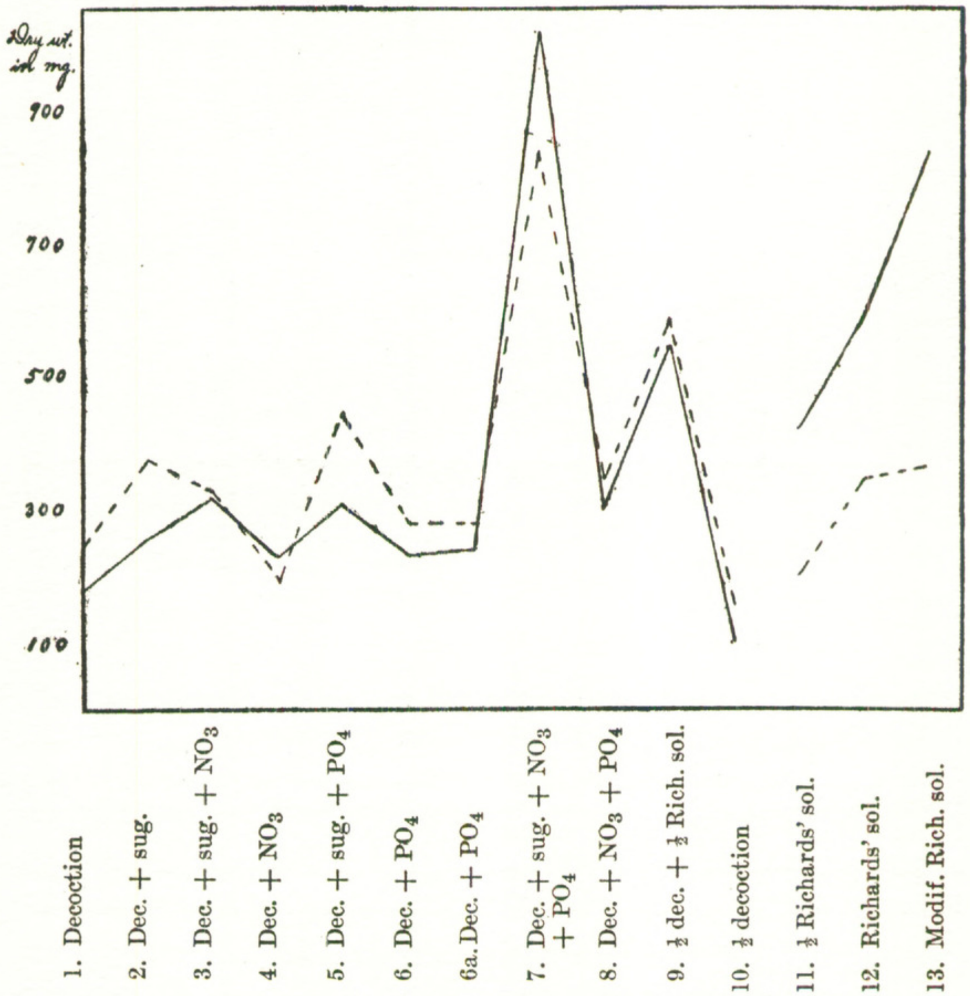

Fig. 2. Growth of *Aspergillus* and *Gloeosporium* on mangold decoction and other nutrients; *Aspergillus,* solid line; *Gloeosporium,* broken line.

where the nutrients were less well balanced, as where sugar and nitrate or sugar and phosphate alone were added. Much the same effect was noted in the carrot and celery decoctions, though here it was less pronounced. While spore production was considerable on the various salmon cultures, the general appearance of the fruiting surface was gray rather than black.

This seemed to be due not only to the smaller number of spores, but also to lesser pigmentation.

Gloeosporium yielded a heavy gelatinous growth upon most of the media containing mangold, celery, and carrot decoctions, but there were considerable differences in the amounts

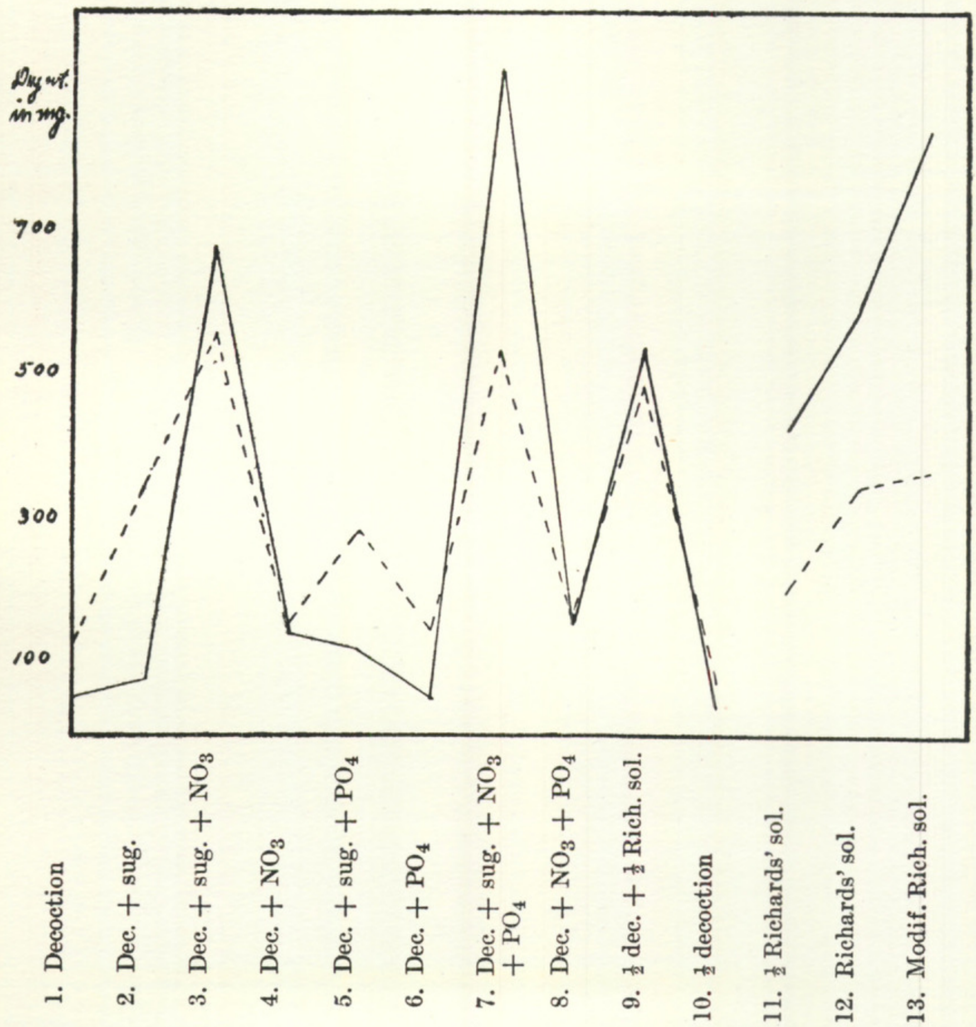

Fig. 3. Growth of *Aspergillus* and *Gloeosporium* on carrot decoction and other nutrients; *Aspergillus*, solid line; *Gloeosporium*, broken line.

of spore formation. The media containing carrot decoction gave abundant fruiting when sugar was added in the presence of nitrate, and less in all other cultures. On the celery-containing solutions there was less difference in the amount of fruiting observed, and the mangold was intermediate in this respect. This fungus also fruited scarcely at all on the salmon decoction alone or even when one or more of the mineral

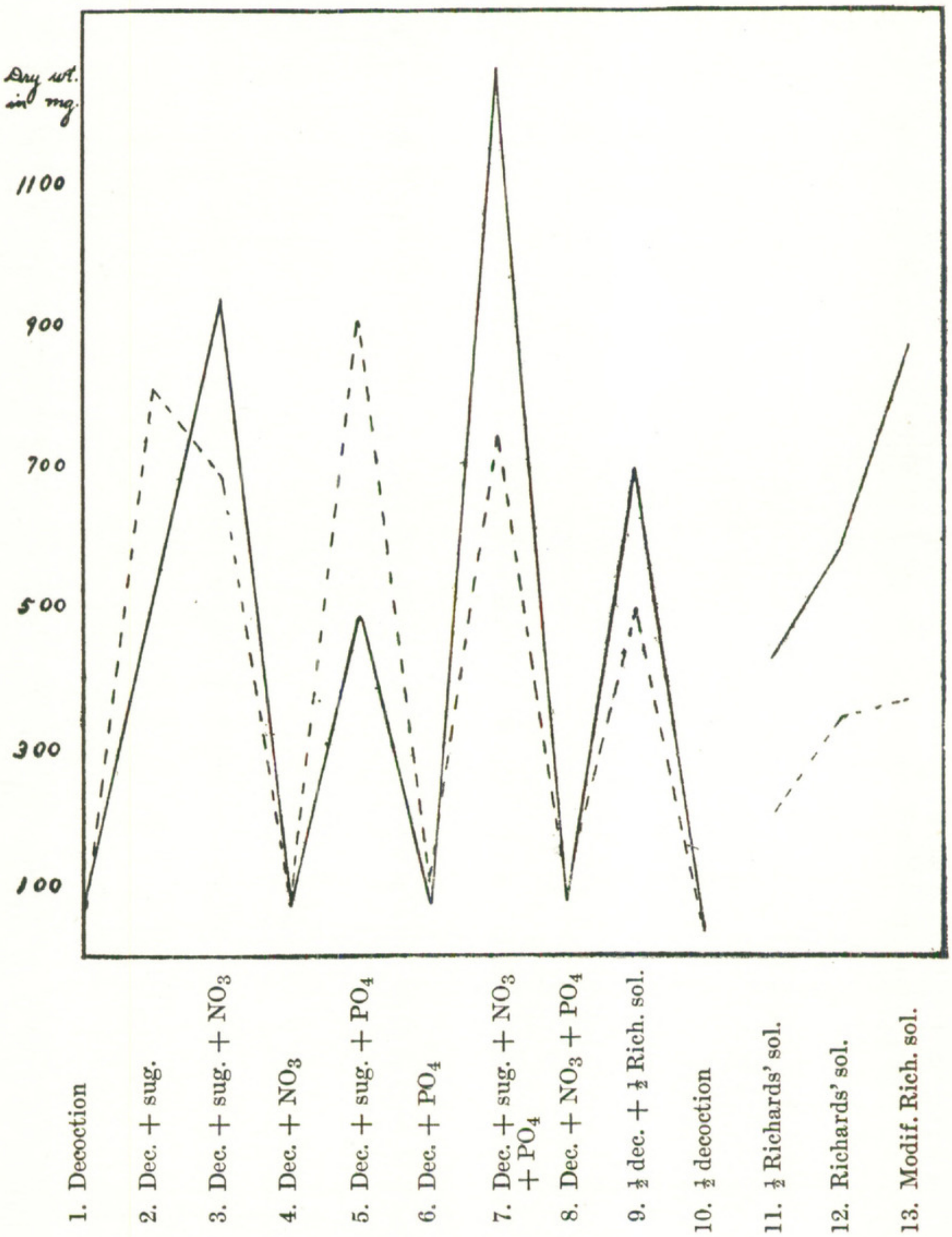

Fig. 4. Growth of *Aspergillus* and *Gloeosporium* on celery decoction and other nutrients; *Aspergillus*, solid line; *Gloeosporium*, broken line.

nutrients were added to the decoction, yet fruiting was most abundant in all the cultures to which sugar was added without nitrogen. This is of interest in connection with the indication that the addition of nitrogen to the salmon decoction does not materially influence growth.

In this paper the criterion by which the value of a solution is judged is that of the weight of mycelium produced.

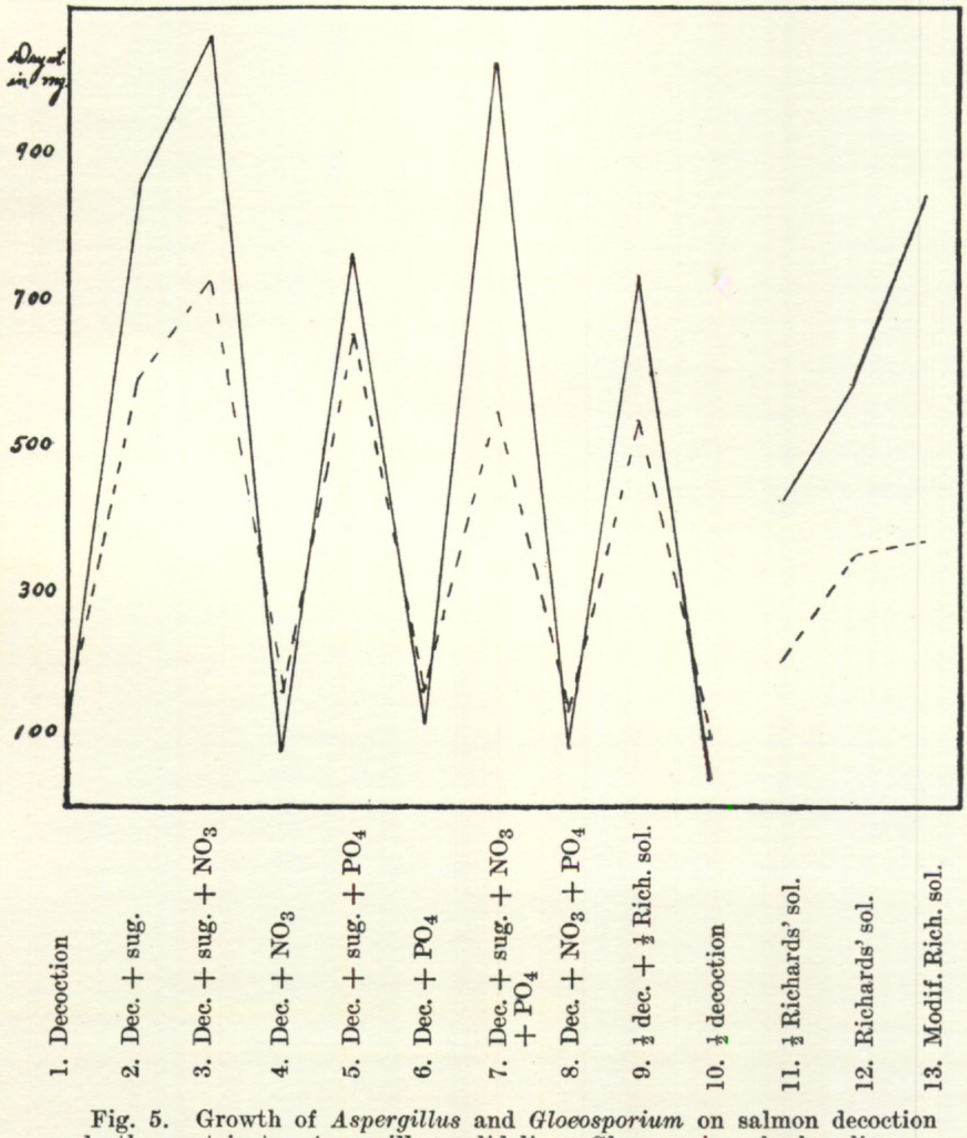

Fig. 5. Growth of *Aspergillus* and *Gloeosporium* on salmon decoction and other nutrients; *Aspergillus*, solid line; *Gloeosporium*, broken line.

This is a practical criterion for general purposes, but the pathologist is particularly interested in "normal" growth, which often means considerable growth and abundant fruiting. Any data, therefore, which bear upon the relation of nutrition to fruiting are worthy of consideration. *Aspergillus* fruits so readily that experiments with this form might seem to be of little value, yet knowledge concerning factors effecting an inhibition of fruiting is likewise important. Richards[1] and others have shown that associated with the increased growth resulting from the addition of $ZnSO_4$ and certain other metallic salts is the depression of fruiting. Numerous observations of this general nature might be collected.

In this paper it is not intended to enter upon a general discussion of nutrients affecting spore production. It has seemed, however, that the influence upon spore formation of such variations in nutrient conditions as have been studied are worthy of record, and it is intended to pursue further this line of inquiry in subsequent work, for which fungi better adapted to the purpose have been selected.

In our previous paper it was pointed out that even a crude study by the colorimetric method of the hydrogen ion concentration of the various decoctions employed leads to the conclusion that the titration of such media and the standardization of these on the basis of Fuller's scale are unsatisfactory. No adequate study of this point has been undertaken, and such determinations as were made served merely as a rough check on the conditions involved in our work. The values of P_H in the various natural decoctions employed were approximately as follows: apple, 4.3; mangold, 4.5; carrot, 5.3; and salmon, 6. It is interesting to note that although *Aspergillus* grows well in the natural mangold decoction with $P_H=4.5$, when brought to $P_H=6$, there is produced a heavier mat. As in the earlier experiments reported, the hydrogen ion concentrations of the solutions in which *Aspergillus* have grown are shifted toward the acid side, while in the contrary direction in the cultures which have supported *Gloeosporium*.

[1] Richards, H. M. Die Beeinflussung des Wachsthums einiger Pilze durch chemische Reize. Jahrb. f. wiss. Bot. **30:** 665–688. 1897.