COLLETOTRICHUM CIRCINANS AS A SEMI-QUANTITATIVE TEST UNIT FOR THE GROWTH SUBSTANCE PRODUCED BY RHIZOPUS SUINUS

WHILE testing the filtrate of *Rhizopus suinus* Nielson on the growth of various species of fungi, it was noted that *Colletotrichum circinans* (Berk.) Vogl. gave consistent increments in yield over a limited range of concentrations. This characteristic response of the organism mentioned suggested its use as a means of measuring the relative growth substance concentration. Subsequent work has shown that this fungus will serve not only as a qualitative but also as a semi-quantitative test unit for the growth accelerator concerned. Nielson and Hartelius (1932) showed that the extract from *Rhizopus* exerted an influence on the dry weight yield of *Aspergillus niger* Van Tiegh., but gave negative results when tested with the oat coleoptile. Although its chemical structure is still unknown, it has properties which render it insoluble in common organic solvents, and it is stable to both oxidation and heat. Their results, which were duplicated in this laboratory, prove that it is not a nutrient and that it is not identical with heteroauxin.

In order to measure this substance, investigators have relied, heretofore, upon *Aspergillus niger*. The *Aspergillus* technique does present certain distinctive features, *viz.*, (1) the time involved is short and a large number of runs can be completed in a given time; (2) the effect is an initial one, and (3) the comparisons are based on dry weight yields, and thus an unevenness of growth does not invalidate the results. The *Colletotrichum* technique, on the other hand, has several definite advantages, *viz.*, (1) the change in yield is greater per increment of growth substance added; (2) the results of replicates show a lesser variation; (3) temperature fluctuations over a few degrees (standard 25° C.) are of little significance; and (4) successive daily changes in the growth rate for a given culture can be recorded and studied.

The method is essentially as follows: The *Rhizopus* mat is filtered from its liquid substrate and the resultant filtrate is concentrated, by partial evaporation, to one third or less of the original volume. This constitutes the stock solution. A 1 cc aliquot of the stock solution or a dilution thereof is placed in the bottom of a sterile 100 mm petri dish. To this is added 30 cc of a nutrient-agar solution containing M/400 $MgSO_4$, M/220 KH_2PO_4, M/16 NH_4NO_3, M/5.5 sucrose, 1.7 per cent. agar and a trace of ammonium tartrate.

Plates made in this manner are cooled and are inoculated with circular blocks (1 mm diam.) of non-fruiting hyphae of *Colletotrichum circinans* growing on an agar substrate. Diameter measurements are made every few days with the aid of a mimeoscope. These figures are compared directly or comparisons are made of the slopes of the plotted curves. Diameter comparisons, and not area measurements, are used since they more nearly approach the true values. This point will be presented in a subsequent paper.

The example below, which has been repeated several times, will serve to illustrate this technique. The actual diameters are measured every two or three days. The values for the third day are used as the starting point, since the transfer of the inoculum affects the initial growth, in an unpredictable manner, invalidating the use of the size of the planting as the criterion. Values for subsequent days are measured as increases over the values for the third day. These last are then corrected so that each test will have the same base line as the control.

These corrected diameters (in mm's) become:

	Days					
	3	5	7	9	12	15
Control	0	7.2	11.0	14.8	19.0	23.0
plus 1 cc	0	9.8	17.2	21.8	28.2	32.2
" 2 cc	0	11.2	18.2	24.2	32.8	38.6
" 3 cc* ...	0	10.4	18.6	26.0	35.4	44.4

* The value 3 cc refers to 1 cc of a stock solution three times as concentrated as the original filtrate.

The values for the fifteen-day period are most profitably used. When an unknown is run, it is necessary to interpolate for those which fall intermediate to the values listed above. This method, therefore, becomes of special interest for small changes in the amount of growth substance and can be used also for larger changes by concentrating or diluting the stock solution so that the test values will fall within this range.

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