Plant Physiol. (1966) 41, 303–306

On Autotropism in Botrytis: Measurement Technique and Control by CO_2[1]

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Received June 21, 1965.

Summary. The mutual orientation of the germination of nearby pairs of *Botrytis* spores growing in a simple, dilute medium was studied. If the medium is equilibrated with room air, they show a very strong tendency to germinate both toward each other and in a *cis* arrangement, i.e., toward the same side of the line joining their centers. If the medium is equilibrated with air enriched with 0.3 % or 3 % CO_2, (i.e., 10 or 100 times the normal CO_2 concentration) then the cells show an equally strong tendency to germinate away from each other. The interaction shows little dependence upon pH.

The origin of growth foci in plant cells may be considered to be a prototype of the central developmental problem of localization, and, in many lower forms at least, the origin and maintenance of such foci is clearly a major factor in morphogenesis.

An interesting approach toward understanding these questions is furnished by the widespread autotropic reactions of fungal hyphae, i.e., by their tendencies to grow either toward or away from each other. Sometimes, they grow toward each other in a path which indicates that both developing and established growth foci stimulate the origin and extension of other such foci up to several cell diameters away (1, 2, 4, 6). These observations suggest the action of diffusible, locally effective, growth stimulators somehow localized just outside these foci. This view received some further support from a recent study of rheotropism (7). Sometimes, however, hyphae grow away from each other, apparently in response to gradients of self-made inhibitors (8, 10), though there is no evidence that these inhibitors are concentrated near the growth foci.

To pursue these clues, it would seem important to measure the degree of autotropism under controlled conditions. Such measurement is most difficult to do in the mycelial tangles often observed, but as will be shown here it is easy if one considers a very simple case: the interaction of nearby pairs of spores.

Materials and Methods

Most of these have been detailed before (7). The wet spores are ovoid cells, about 8 × 11 μ. When dry they stick tightly to hydrophobic surfaces (particularly if these have an electrostatic charge) and remain stuck when later covered with water. Hence, dry spores were suspended in portions of the inert fluorochemical liquid, FC-75 and electrostatically precipitated in containers made of or coated with polystyrene, at concentrations between 10 and 40 cells per mm^2. Then they were covered with a 0.07 % Czapek-Dox broth and grown in the dark at 23°. This medium consists of 0.06 % sucrose and salts (3).

Orientation was observed when almost all of the cells had developed 1 outgrowth, but few, if any, had formed 2; this stage appeared 8 to 14 hours after wetting. Sufficient areas were scanned for all suitably close pairs of cells, each of which bore 1 outgrowth and was separated by at least 3 cell diameters from any third cell. Sometimes, touching pairs were observed; sometimes, nontouching. The latter are defined here as pairs separated by a gap of 1 to 10 μ.

Each outgrowth in each semi-isolated pair considered was scored plus or minus if it began more nearly toward or away from the vicinal cell respectively. A pair was scored ++ if both outgrowths were +. Moreover, a pair was scored *cis* or *trans* if its outgrowths began on the same or opposite sides of the line joining the cell centers respectively. Thus all pairs were grouped into 6 categories as diagrammed in figure 1.

Results and Analysis

Positive Autotropism. Table I shows measures of mutual outgrowth orientation obtained from a large number of semi-isolated pairs which were categorized as in figure 1. The most obvious aspect of these data is a strong tendency for outgrowths to begin toward the neighboring cell: among cells in touching pairs,

[1] Supported by grants from the National Institutes of Health and the National Science Foundation.

92 % were scored plus; among nontouching, 72 %.

Nearly as evident is the tendency of the outgrowths to begin in a *cis* arrangement. Thus, among touching + + pairs, 96 % are *cis*. As table II shows, *cisness* is inversely related to the distance between outgrowth origins. It falls from 96 ± 1 % for touching + + pairs (with origins about 0–10 μ apart) to 62 ± 7 % for nontouching − − pairs (with origins about 10–30 μ apart). Of course, if the origin directions in a pair were not interdependent, then about 50 % of the pairs would be *cis*.

Finally, outgrowth directions are positively correlated not only as indicated by their *cis* tendency, but also by the increased likelihood of one outgrowth being + if the other is +; − if the other is −. Table III shows this last, independent aspect of the data. In it, I compare the actual number of + +, + −, and − − cells observed with the numbers that would have been found if the outgrowth direction of one cell were independent of that in the other, on the one hand, and completely linked with it on the other.[2] There is in fact some linkage. The degree of linkage is most easily expressed by the fractional deficiency of actual + −, or mixed pairs as compared to the independently expected case. For the touching cells 75 is 26 % less than 102 so this measure of linkage is 26 %; for the nontouching cells, 17 %.

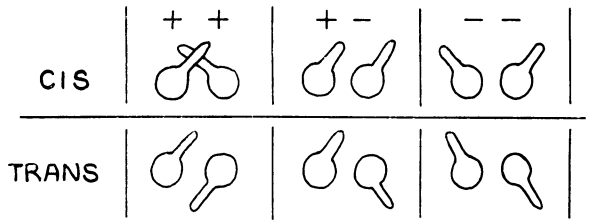

FIG. 1. Method of categorizing vicinal cell pairs.

The last 2 aspects can be described as a tendency of outgrowths on nearby cells to begin near each other. Presumably this tendency arises from a mutual stimulation which is greater the closer the interacting regions are and which is partly responsible for the tendency of cells to grow toward each other too.

These cells' rheotropic behavior indicates that they emit a diffusible, unstable, locally effective, macromolecular growth stimulator (7). It is the simplest and hence the best hypothesis that this stimulator is responsible for the observed interactions of cell pairs. On this interpretation, then, it can be inferred that this stimulator, while initially emitted uniformly by each spore, comes to be emitted most rapidly by the very presumptive growing points that it favors. In other words, it is inferred that the stimulator's emission and effect both come to be centered on the growth point anlage; thus it acts as a link in a chain of self-augmenting causes serving to initiate growth at a particular point.

[2] Let p and m be the fraction of all cells which are plus and minus respectively. Then for cells independent of each others outgrowth directions, the expected fractions of + +, + −, and − − pairs is given by p^2, $2pm$, and m^2 respectively; for fully linked cells, by p, 0, and m. Actual numbers of pairs rather than fractions of the total are listed in order to facilitate the intuitive assessment of statistical significance.

Table I. *Categorization of All Vicinal Cell Pairs Observed under Standard Conditions*

Gap between cells	No. of expts	No. of cells	+ +		+ −		− −		Total cells +	Total pairs *cis*
			cis	*trans*	*cis*	*trans*	*cis*	*trans*		
None	10	1336	85 %	4	8	3	2	1	92	92
1–10 μ	7	994	45	11	22	11	7	4	72	84

Table II. *Data of Table I Arranged to Relate Cisness to Distance Apart of Outgrowth Origins*

Errors are standard errors based upon sample size only.

Condition	+ + Touch	± Nontouch	± Touch	± Nontouch	= Touch	= Nontouch
Gap between origins	0–10 μ	0–20	5–15	5–25	10–20	10–30
% *Cis*	96 ± 1 %	80 ± 2	72 ± 5	68 ± 4	72 ± 10	62 ± 7

Table III. *Data of Table I Analyzed to Show Linkage between Tendencies of Each Cell in a Pair to Grow Toward (or Away) from the Other*

	No. of touching pairs			No. of nontouching pairs		
	+ +	+ −	− −	+ +	+ −	− −
Actual	575	75	18	276	166	55
If independent	562	102	5	259	200	38
If fully linked	612	0	56	359	0	138

Table IV. *Categorization of All Vicinal Cell Pairs Observed under Conditions Which were Standard Except that Medium was Equilibrated with Stale Culture Air Instead of Room Air.*

Gap between cells	No. of expts	No. of cel's	++		+−		−−		Total cells +	Total pairs *cis*
			cis	*trans*	*cis*	*trans*	*cis*	*trans*		
None	3	664	2 %	0.6	7	7	56	28	9	64
1–10 μ	1	222	3	5	26	7	32	18	29	60

Table V. *Comparison of the Effects of Standard Medium Equilibrated with Stale Culture Air with One Equilibrated with Room Air Enriched with a Similar Fraction of CO_2*

All observations were upon touching cells in the same series of experiments.

% Room air	% Culture air	% Extra CO_2	No. of cells	++		+−		−−		Total cells +	Total pairs *cis*
				cis	*trans*	*cis*	*trans*	*cis*	*trans*		
0	100	0	560	3 %	3	26	14	43	12	26	72
97	0	3	772	2	2	24	6	56	11	19	81
90	10	0	244	7	4	20	6	43	22	23	69
99.7	0	0.3	224	6	4	23	11	48	8	27	78
99	1	0	262	87	3	4	3	5	0	92	94

Reversal by CO_2. The dependence of this interaction upon several variables was explored. The most striking affect found was that of using a culture medium equilibrated with stale culture air instead of room air. (By stale culture air I mean that in a sealed 2-liter container which had held dishes with 100 cm^2 of close-packed fruiting *Botrytis* mycelia for a week.) As table IV shows, this gas effects a striking reversal of the group effect: among touching pairs, only 9 % of the test cells grew toward their neighbors as contrasted with 92 % in the control cells; among nontouching pairs, 29 % vs. 64 % were found.

In the presence of culture air, the degree of *cisness* is small for −− pairs (as it is under room air), otherwise small and statistically insignificant or unevaluable (table II). The degree of linkage for touching cells is calculated as 16 %; for nontouching cells it is statistically insignificant.

Altogether, my tentative interpretation is this: culture air somehow causes pair interaction to be dominated by a locally effective but uniformly emitted inhibitor, though the locally emitted stimulator continues to have some effect.

A gross chromatographic analysis of the stale culture air revealed 78 % N_2, 17 and one-half % O_2 and 4 and one-half % CO_2. This result suggested that the effective difference between room and culture air is the high CO_2 concentration in the latter. Hence the effects of culture air were compared to those of room air containing similar concentrations of added CO_2. These results (table V) indicate that the effective component in culture air is, in fact, CO_2. They also indicate that while an increase in CO_2 to 0.3 %, or 10 times the normal level, effects a sharp reversal of the autotropic effect, a further 10-fold increase, to 3 % effects little further change.

Ineffective Variables. A number of variables were found to have little or no effect upon these interactions. A) By using appropriate citrate and Tris buffers, the interactions were found to be nearly independent of pH in the range from 3 through 8. From this it can be inferred that CO_2 reversal of the group effect is not mediated through a change in extracellular pH. B) A small but persistent bubble is seen to be trapped in the space between and under each cell pair grown in our usual manner. These may be avoided by wetting the dry spores (stuck to the dishes) with degassed medium, which is then immediately replaced with the standard air-equilibrated medium. In this way no difference was found between the growth interactions of pairs grown with and without bubbles. It is also possible, though relatively troublesome, to sow spores by precipitation from either air or an aqueous medium; pairs obtained in these ways, interacted just as those precipitated from the usually employed fluorochemical carrier. The ineffectiveness of these latter 3 variations, gives some further reason for inferring that the interactions are chemically rather than mechanically brought about.

Discussion

While this is the first report of the dependence of autotropism on the carbon dioxide level, it may well be a quite widespread phenomenon. The more general question of developmental control by CO_2 has been most recently reviewed and discussed by Loomis (5).

A recent discussion of CO_2 as a developmental control in fungi is found in a paper Niederpruem (9).

Acknowledgment

The author acknowledges the capable assistance of Mr. Nicholas Yamodis and Mrs. Claire Brunton in executing this work.

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