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COREMIUM AND RHIZOMORPH DIFFERENTIATION IN *SPHAEROSTILBE REPENS*. I. – INTERACTIONS BETWEEN AGGREGATED ORGANS DURING MORPHOGENESIS OF THE THALLUS[1]

by B. BOTTON* and M. KHOURY**

ABSTRACT — The Ascomycete *Sphaerostilbe repens* Berk. & Br. grown on agar medium gave rise to coremia and rhizomorphs distributed in two series or waves separated by vegetative mycelium. The first group appeared at the centre of the colony, then as the mycelium grew, the second group differentiated at the periphery of the colony. During morphogenesis of the thallus, inhibitory correlations occurred between aggregated organs in the course of differentiation. By using transplanting, or diffusion barriers created by inserting obstacles or cutting trenches in the agar medium, and by triggering aggregation at different sites on the colony, the ensuing distribution of coremia and rhizomorphs showed that these organs inhibited primordia development in their vicinity. The inhibition occurred between aggregated organs produced within a wave as well as between waves of organs. When the distance between the mycelial margin and aggregated organs increased, the mycelium gradually and temporarily lost its aggregating capability which was recovered when hyphal apices were sufficiently distant from the aggregating centers. The inhibition ascribable to a wave was directed both centrifugally and centripetally. These numerous correlations obviously regulate the observed pattern of coremium and rhizomorph development in the colony and some possible interpretative hypotheses are proposed.

RÉSUMÉ — L'ascomycète *Sphaerostilbe repens* Berk. & Br. cultivé sur milieu gélosé différencie des corémies et des rhizomorphes qui se répartissent en deux vagues séparées par du mycélium végétatif; l'une est localisée au centre du thalle et l'autre à sa périphérie. Lors de la morphogenèse, le thalle est le siège de corrélations d'inhibition entre organes agrégés. En utilisant les techniques de bouturage, de barrières de diffusion (obstacles, fossés creusés dans la gélose) et en provoquant la différenciation des organes à différents emplacements sur le thalle, il est possible de montrer que la différenciation des organes agrégés inhibe à proximité la formation de nouvelles structures. Cette inhibition se manifeste entre les organes agrégés formés au sein d'une même vague et entre les vagues d'organes agrégés elles-même. Les extrémités mycéliennes qui s'éloignent d'une vague au cours de la croissance, perdent temporairement leur capacité à s'agréger puis la retrouvent lorsqu'elles sont suffisamment éloignées des centres d'agrégation. L'inhibition imputable à une vague peut s'exercer aussi bien en direction centrifuge qu'en direction centripète. Ces corrélations multiples régulent manifestement la distribution des corémies et des rhizomorphes sur le thalle et quelques hypothèses explicatives possibles sont évoquées.

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KEY WORDS : aggregated organ, Ascomycete, coremium, differentiation, morphogenesis, pattern control, rhizomorph, *Sphaerostilbe repens*.

INTRODUCTION

Several factors combine to decide the time and place of origin of aggregated organs in a fungal colony. External parameters such as nutritional and physical factors play significant roles in the initiation and development of aggregated structures.

The successive phases of morphogenesis are also controlled by internal correlations which can very often be interpreted as a trophic intercellular competition or an internal circulation of hormonal substances or excretions. The soundness of both has been verified in fungi and seem capable of regulating developmental phenomena of numerous parts or organs within a mycelium.

According to BUTLER (1961) the dominance of the axis over the ramifications might be controlled by internal competition. The experiments carried out by LARPENT (1966) and FEVRE (1972) confirmed this and showed that the apices of the axes were powerful enough to convert a significant amount of metabolites for their own use. In the genus *Doratomyces*, there also exists a trophic competition between vegetative hyphae, ascendant filaments of coremia and rhizoids which grow in the opposite direction (BRETON, 1974).

The intervention of morphogenetic substances or excretions controlling morphogenesis is also suggested. Indeed, the development of excised coremia of *Doratomyces purpureofuscus* required the presence of mycelium which seemed able to produce molecules provided to the reproductive organs (BRETON, 1978). It has been shown experimentally that there exist branching factors in *Neurospora crassa* (ROBERTSON, 1959), *Ascobolus immersus* (CHEVAUGEON, 1959) and *Podospora anserina* (NGUYEN VAN HUONG, 1962; CHEVAUGEON & NGUYEN VAN HUONG, 1969).

According to GOUJON (1967) the initiation of sclerotia buds in *Corticium rolfsii* depends both on the synthesis of inductors and on the trophic competition produced by the filament apices located at the margin of the colony. The same interpretations have been put forward to explain localizations and rhythmic appearances of basidiocarps on thalli of Basidiomycetes where a competition occurs between primordia of fruit bodies during the process of differentiation (SINDEN & al., 1962; INGOLD & NAWAZ, 1967; MANACHERE, 1971). However, in *Coprinus congregatus*, the carpophores successively exert an inhibitory and a stimulatory effect on the producing mycelium according to whether they are at the juvenile or mature stage (MANACHERE, 1971, 1977; ROBERT, 1978).

It seems obvious that the importance of the competitions for nutrients and the interventions of morphogenetic factors are common characteristics involved in initiation and development of differentiated organs in fungi.

The Ascomycete *Sphaerostilbe repens* gives rise to aerial coremia and rhizomorphs immersed in the culture medium. These two types of structures are anatomically continuous and their ensemble has been named «aggregated units» (GUILLAUMIN, 1970; BOTTON, 1983a-b).

During growth of the fungus, a first series or wave of aggregated organs is formed in the centre of the colony. After 3 1/2 days, rhizomorphs and coremia stop differentiating and the colony extends radially exclusively as undifferentiated mycelium. A second series of organs differentiates from the seventh day of culture on. This second group is composed of several circles of aggregated units formed progressively as the hyphae elongate (Fig. 1).

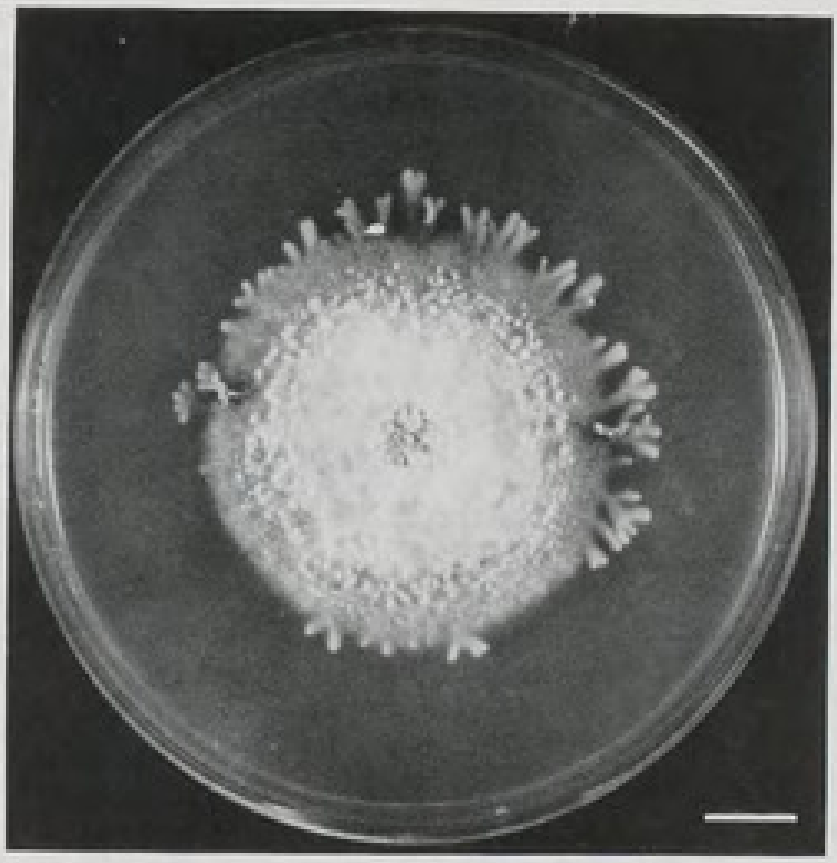

Fig. 1 — Top view on agar medium of a 13-day-old thallus of *Sphaerostilbe repens*. A first series of aggregated units has been formed in the centre of the culture, then separated by about 10 mm of undifferentiated mycelium, a second series of organs is being formed at the periphery of the colony as filament elongation proceeds. Rhizomorphs which elongate more rapidly than mycelium are being extended beyond the hyphal margin. (Scale = 10 mm).

Fig. 1 — Vue de dessus d'un thalle de *Sphaerostilbe repens* âgé de 13 jours cultivé sur milieu gélosé. Une première série d'unités agrégées s'est différenciée au centre de la culture, puis séparée par environ 10 mm de mycélium végétatif, une deuxième série d'organes se forme à la périphérie de la colonie au fur et à mesure que les hyphes s'allongent. Les rhizomorphes qui s'accroissent plus vite que les hyphes dépassent le mycélium à sa périphérie. (Échelle = 10 mm).

The aim of the present work was to study the interactions that could arise between aggregated organs and subsequently between the different series of aggregated organs during morphogenesis of the fungus.

MATERIALS AND METHODS

Organism and culture methods

Sphaerostilbe repens Berkeley and Broome (strain C.B.S. 275.60), supplied by the Centraalbureau voor Schimmelcultures of Baarn (Holland), was kept on a 2 % malt agar medium. The fungus was cultivated in Petri dishes on a synthetic agar medium whose composition in g/l was as follows : sucrose, 45; tartaric acid, 2.64; NH_4NO_3, 3; $(NH_4)_2SO_4$, 0.2; KH_2PO_4, 0.6; K_2CO_3, 0.4; $MgSO_4.7\ H_2O$, 0.85; $FeSO_4.7\ H_2O$, 0.086; $ZnSO_4.7\ H_2O$, 0.04; $MnSO_4.H_2O$, 0.008; $CaCl_2.2\ H_2O$, 0.014, Agar (Difco), 20. The pH was adjusted to 5.4 with NaOH before autoclaving at 120°C for 20 min. After autoclaving the pH was 5.3. Cultures were inoculated by spores harvested from coremia. The inoculum contained 10^3 spores in suspension in 10 µl of distilled water. *Sphaerostilbe repens* was grown in the dark at 28°C.

Experimental procedures used to study the pattern of aggregated organ development

Interactions between primordia of aggregated units were analyzed by removing small areas of the colony which were examined as concerns their capability of giving rise to coremia and rhizomorphs. The central zone of the thallus where the aggregated organs normally form was divided into cubic portions of 1 x 1 x 1 mm which were deposited on a new agar medium. The mean lag period for differentiating coremia was determined by periodically examining the explants under a binocular lens. This technique was also used to estimate the aggregative capacity of the vegetative mycelium.

Interactions between series of organs were studied by interrupting the continuity of the hyphae. Obstacles such as coverglasses were inserted in the developing colonies and the number of coremia formed at obstacles was determined. Similarly, diffusion barriers were created by removing circular strips of agar with the overlying hyphae. Each trench extended to the plate bottom. Aggregation was estimated by the number of coremia differentiated along the trench edges.

Differentiation of the central wave was inhibited by placing 35 mm diameter plastic discs on 32-hour-old thalli. In these conditions, hyphae turned into «immersed mycelium» unable to aggregate (EL-KHOURI & BOTTON, 1982). Once apices reached the edge, they differentiated coremia and rhizomorphs along the discs which were removed at several developmental stages in order to induce organ differentiation in the central part of the colonies.

Simultaneous differentiation of the central organs and of an experimental wave at the periphery of the colony was obtained by placing a glass plate (50 x 50 x 3 mm) on a 32-hour-old thallus. Aggregation was triggered by removing glass plates at different times before the hyphae had reached the edges of the plates.

Techniques of measurement

Production of aggregated units was determined as the average number of organs per unit area of an agar culture by using the following technique : cylinders of the colony were punched out wiht a cork borer of cross section 78 mm^2. With a hand microtome the samples were cut horizontally by a razor blade in order to separate agar and rhizomorphs from overlying mycelium and coremia. The aggregated units were then distinguishable by their rhizomorph cross sections which were counted under a binocular lens.

Production of coremia was simply determined by counting them under a binocular lens from a cylinder of the colony punched out with a cork borer of cross section 28 mm^2.

RESULTS

Interactions between aggregated organs during the process of differentiation

In the central area of the thallus, numerous aggregated units (up to one hundred or so) differentiate simultaneously over approximately 28 mm^2. It seems obvious that the distribution of these organs does not occur at random but results from interactions between primordia.

The central zone was cut into cubes of 1 mm^3, the organ regeneration of which was observed after transfer into a new medium. The time required for aggregation of the first coremium (aggregate of mycelium clearly erected) was determined on each transplant by observation every two hours under a binocular lens.

In control cultures, aggregation occurred, on the average, after 42 hours of incubation. When the transplants were transferred after 20 hours of incubation, the greatest number differentiated at the 47th hour of incubation with a continuous distribution on both sides of this modal class (Fig. 2). When the transplants were transferred after 30 hours of incubation, the distribution curve levelled off from 47 to 51 hours and when the transfer was performed at the 38th hour of incubation the curve became clearly bimodal with one peak of aggregation at the 47th hour and the other at the 51st hour of incubation (Fig. 2).

Although no sign of aggregation was distinguishable at any time of transplantation, a difference of behaviour progressively appeared and by the 38th hour two populations of transplants could be separated on the basis of their capacity to differentiate aggregated structures.

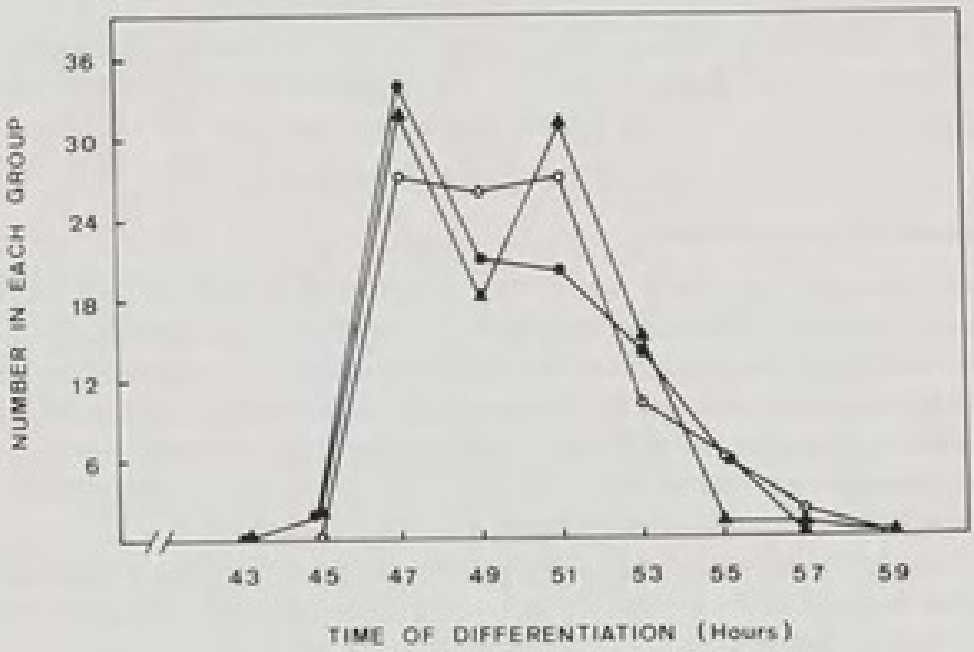

Fig. 2 — Frequency curves of the times of aggregation of the transplants taken from thalli at 3 different ages : 20 hours (●), 30 hours (○) and 38 hours (▲).
Transplants consisted of cubic mycelial parts cut from central regions of cultures where aggregation is expected. Coremium differentiation was observed every 2 hours with a sample of 100 transplants cut at each time from four colonies. The number of differentiated transplants at each time is indicated on the ordinate. Times on the abscissa include the growth period of the fungus prior to the transplanting.

Fig. 2 — Courbes de fréquences des délais d'agrégation des boutures prélevées sur des thalles âgés de 20 heures (●), 30 heures (○) et 38 heures (▲).
Les boutures cubiques sont prélevées à partir de la région centrale des cultures où l'agrégation doit se situer. La différenciation des corémies est observée toutes les 2 heures sur une population de 100 boutures prélevées à chaque temps à partir de quatre colonies. Le nombre de boutures différenciées à chacune des périodes est porté en ordonnée. Les délais d'agrégation mentionnés en abcisse sont déterminés depuis l'ensemencement des cultures fournissant les boutures.

These results suggest that the aggregated units in the process of differentiation inhibit the surrounding regions of the thallus; consequently the former group gave rise to coremia after an incubation period of 47 hours, while the latter group coming from the inhibited areas differentiated 4 hours later.

Influence of a series of aggregated organs on subsequent differentiation of the thallus

As coremia and rhizomorphs are formed first in the central region of the thallus, they may inhibit production of new organs in their vicinity and it seemed of importance to study the capability of hyphae to give rise to aggregated structures from the colony margin to the central aggregating zone.

– Aggregating capability of excised transplants of vegetative mycelium

With young cultures, the best capability of the transplants to regenerate coremia was found near the central aggregated units (Fig. 3a). Then, as hyphae elongated, the zone next to the central area progressively lost its high morphogenetic potential (Fig. 3b-c) and development of coremia gradually became restricted to a peripheral zone located at several mm from the central wave (Fig. 3c-d). Inocula taken from the apices always exhibited a weak regenerating capacity.

It appears that the aggregating capacity of the hyphae shifted from the central old region to the subapical cells of the filaments to constitute the area where the second wave of aggregated structures normally forms.

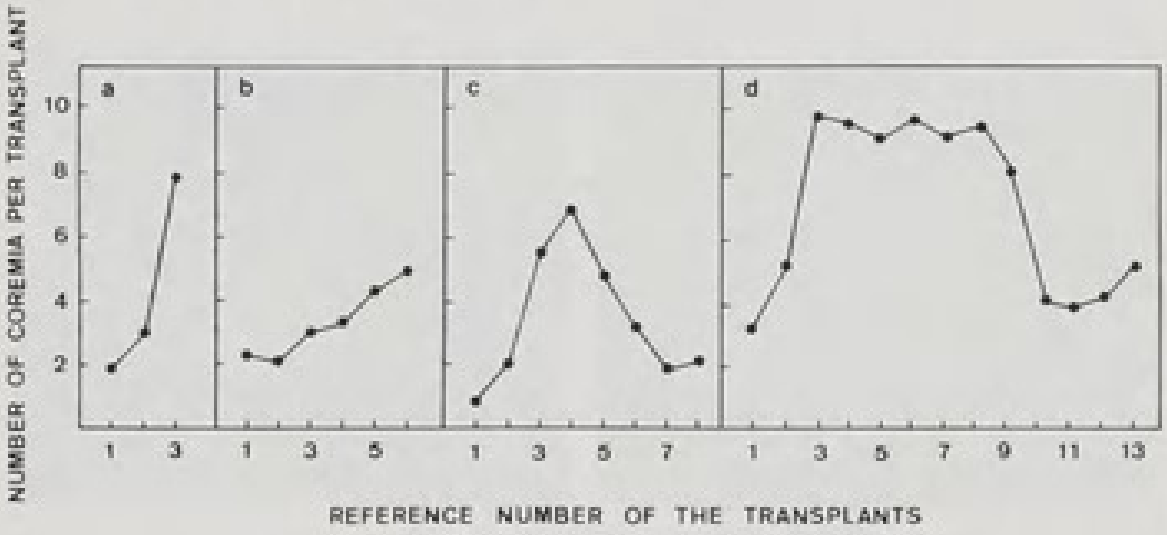

Fig. 3 – Number of coremia differentiated on transplants, depending on their origin on the thallus. Transfers were done from 4-day-old thalli (a), 6-day-old thalli (b), 8-day-old thalli (c) and 12-day-old thalli (d). Mycelial samples of uniform size (1x1x1 mm) were cut along radii and transferred to a new culture medium. Reference numbers correspond to number one which represents fragments taken from hyphal apices to the last number which represents fragments located near central waves. Coremium aggregation was determined on 6-day-old subcultures. Results are the mean of 30 transplants taken at each site.

Fig. 3 – Nombre de corémies différenciées sur les boutures selon leur emplacement d'origine sur le thalle. Le bouturage est réalisé à partir de cultures âgées de 4 jours (a), 6 jours (b), 8 jours (c) et 12 jours (d). Les boutures de taille uniforme (1x1x1 mm) sont prélevées selon plusieurs rayons de la colonie et repiquées sur un milieu de culture neuf. Les boutures sont numérotées, le numéro un constituant l'apex des hyphes et le dernier numéro étant la bouture juxtaposée à la vague centrale. La formation des corémies a été enregistrée 6 jours après le bouturage. Les résultats sont la moyenne de 30 boutures par emplacement.

– Effect of a physical barrier on differentiation

When a transverse barrier such as a coverglass was placed in the culture between the two waves of aggregated units, coremia and rhizomorphs were

formed preferentially at the side of the barrier exterior to the centre of the mycelium (Fig. 4a, Fig. 5). A coverglass placed along one radius of the thallus did not allow differentiating organs at either side (Fig. 4b). It appears thus, that the central wave inhibits production of new organs in its proximity. Maximal inhibition was found between 6 and 9 mm from the central series of differentiating organs. Indeed, when the obstacles were near the centre of the colony or at some 12 mm or more from the central aggregated units, coremium differentiation increased (Fig. 5). However, when barriers were placed at the margin of a 15-day-old colony (at about 25 mm from the colony centre), differentiation was the same at both sides of the barrier whatever its disposition with regard to the hyphae. The influence of the central group of organs obviously no longer extends to this distance.

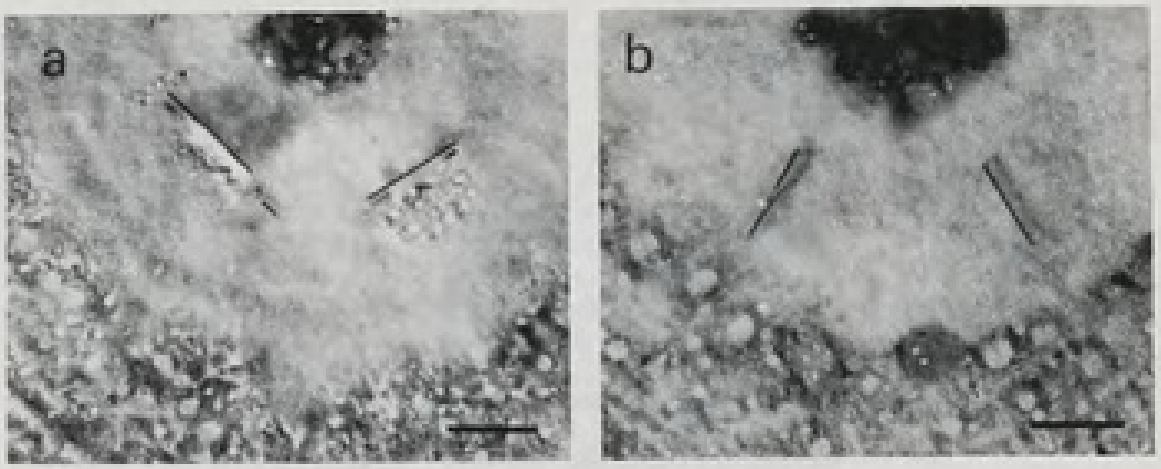

Fig. 4 — Pattern of coremium development at barriers inserted transversally (a) and radially (b) in a developing colony. Top view of 18-day-old thalli.
In this experiment, sterile cover glasses (5 mm width) were inserted down to the plate bottom on 8-day-old thalli, in the vegetative mycelium which lies between central and peripheral waves. A transverse orientation of the barrier led to coremium production located on the external side of the obstacle (a) while a radial orientation of the barrier was without any effect (b). (Scale = 5 mm).

Fig. 4 — Localisation des corémies à proximité des barrières placées transversalement (a) ou radialement (b) dans un thalle en croissance. Vue de dessus des thalles âgés de 18 jours.
Dans cette expérience, des lamelles stériles de 5 mm de largeur, sont insérées jusqu'au fond de la boîte sur des thalles de 8 jours, au niveau du mycélium végétatif localisé entre les deux vagues d'organes agrégés. Le barrage transversal engendre une production de corémies du côté externe (a) alors qu'un barrage dans le sens radial de la culture demeure sans effet (b). (Échelle = 5 mm).

When a barrier was created by cutting a trench in cultures of different ages at 4 mm behind the mycelium margin, aggregated structures differentiated exclusively along the outer edge within 48 hours after the strips were removed (not shown). As the diameter of the circular channels increased, the experimental

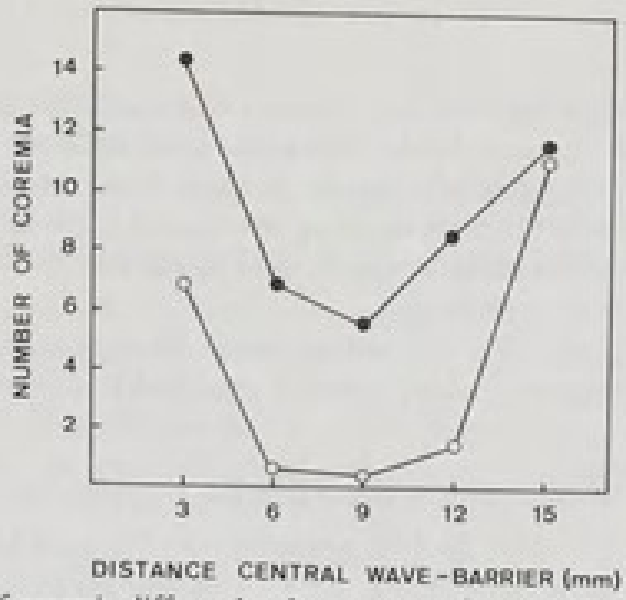

Fig. 5 — Number of coremia differentiated at transversal barriers inserted at different positions between the central wave and the mycelium margin. Diffusion barriers were created by inserting coverglasses in 10-day-old colonies. Coremia were numbered along the outer side (●) and along the inner side (○) of the obstacle after 6 days of incubation.

Fig. 5 — Nombre de corémies formées au niveau des barrages transversaux mis en place en différents endroits entre la vague centrale et l'apex des hyphes. Les barrières de diffusion sont créées en insérant des lamelles dans des thalles âgés de 10 jours. Les corémies sont dénombrées de long de la lamelle, sur la face externe (●) et sur la face interne (○) après 6 jours d'incubation.

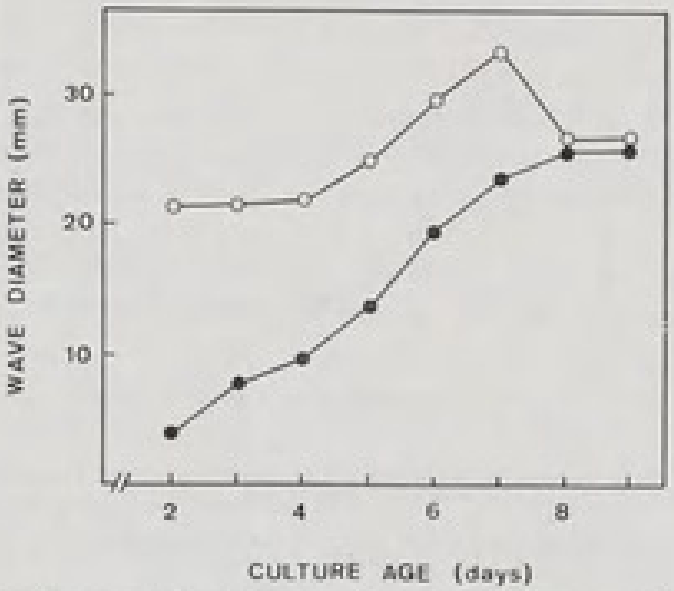

Fig. 6 — Influence of an experimental wave of aggregated organs on the subsequent differentiation of the thallus. A circular trench of increasing diameter stimulated aggregation along the outer edge and resulted in rejecting the subsequent second wave provided that thalli were less than 8-day-old. Diameter of the experimental wave along the outer edge of the trench (●). Internal diameter of the second wave (○).

Fig. 6 — Influence d'une vague expérimentale d'organes agrégés sur la différenciation consécutive du thalle. Un fossé circulaire de diamètre croissant provoque l'agrégation sur la lèvre externe située du côté des apex et a pour conséquence de repousser dans l'espace la différenciation de la deuxième vague pourvu que les thalles soient âgés de moins de 8 jours. Diamètre de la vague expérimentale sur la lèvre externe du fossé (●). Diamètre interne de la seconde vague (○).

induced series of organs increased in diameter too, and consequently the second wave of organs differentiated further out at an approximately constant distance from the experimental series (Fig. 6). From the 8th day of culture on, making a trench did not hinder differentiation of the second wave in the proximity of the trench suggesting that between the 7th and 8th day of culture, an irreversible change must occur in the apices.

These results indicate that the central series of organs, as well as the series induced by wounding the colony, inhibit production of new organs in their vicinity.

– Bidirectional translocation of the inhibition generated by aggregated organs

The previous experiments lead to believing that the central wave inhibits the experimental wave, this latter being in turn able to inhibit the second wave of aggregated organs. This inhibition exerted outwards the colony, is actually due to the successive production of organs as the elongation of filaments proceeds

Fig. 7 – Influence of a peripheral wave on differentiation of the central region of the thallus. Plastic discs were placed on 32-hour-old thalli and were removed at several different ages of the cultures indicated on the abscissa; 9 days later, aggregated units were counted following the procedure described in the experimental part.
Number of aggregated units along the disc (▲); number of aggregated units in the central region of the thallus (●); time of differentiation of the aggregated units in the central region of the thallus after removal of the disc (○).

Fig. 7 – Influence d'une vague périphérique sur la différenciation de la partie centrale du thalle. Des disques de plastique sont placés sur les thalles âgés de 32 heures puis sont enlevés à différents âges de la culture indiqués en abscisse; 9 jours plus tard les unités agrégées sont dénombrées selon le procédé décrit dans la partie expérimentale.
Nombre d'unités agrégées autour du disque (▲); nombre d'unités agrégées dans la région centrale du thalle (●); délai d'apparition des unités agrégées dans la région centrale du thalle après l'enlèvement du disque (○).

and one might question what the influence of the external wave is on the development of the central series of organs.

In order to respond to this, an experimental series of coremia and rhizomorphs was at first induced at the periphery of a disc and in order to allow differentiating of central organs, discs were removed as a function of time.

As shown in figure 7, the number of aggregated organs on the experimental wave increased with time of removing the disc and a maximum of organs was differentiated from the 13th day of incubation on. Moreover, the later the disc was removed, the fewer were the central aggregated units and the longer was the time required for their differentiation. Beyond 13 days of incubation, no organ was formed in the central region of the thallus.

The inhibitory effect of a differentiating wave can obviously be directed towards the centre of the colony.

Simultaneous regeneration of central and peripheral waves and influence on the differentiation of the intercalary region of the thallus

Glass plates deposited on young mycelium were removed before apices had reached the edge and organ differentiation occurred preferentially, both in the central area and at the margin of the colony, this latter group of aggregated units being qualified as an experimental wave. As this procedure has been shown to considerably stimulate organogenesis (EL-KHOURY & BOTTON, 1982) regeneration of coremia could also be more or less induced everywhere in the space separating the two waves, depending on the distance between them.

In control cultures, the coremium density of the central wave was high, greater than that of the second wave (Fig. 8a). Removing the glass plates after 5 days was accompanied only by a regeneration of the central and experimental waves with no coremia formed in the interval (Fig. 8b). When plates were removed later, distances between the waves increased and coremium regeneration occurred everywhere, although organs were fewer near the central wave (Fig. 8c).

This technique clearly shows that aggregation can occur between differentiated organs provided that organogenesis centers are distant enough from each other.

Suppression of a series of aggregated organs and incidence on regeneration of the other regions of the thallus

If early differentiated organs inhibit the potential aggregation sites, the elimination of such organs should promote differentiation in the inhibited areas.

By using the same device as in the previous experiment, the elimination of the future central wave at the same time as the glass plate was removed, stimulated coremium formation in the proximity of the ablation (Fig. 8d). The elimination of the mycelium margin led to an increase of the density of the coremia in the median zone and particularly in the central region of the colony (Fig. 8e).

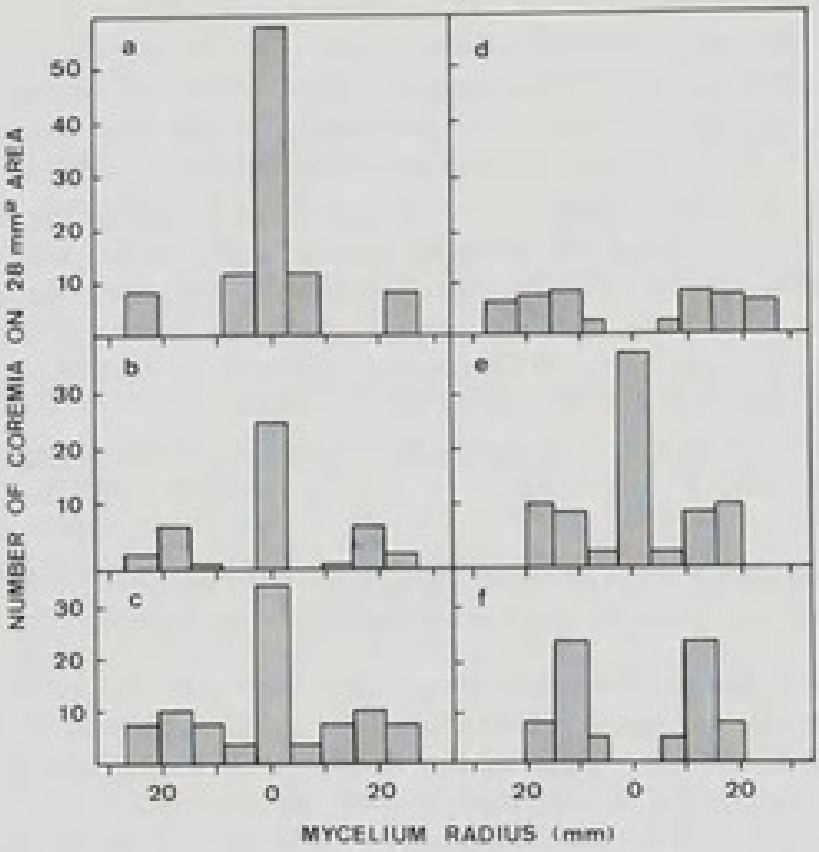

Fig. 8 — Coremium distribution following simultaneous triggering of aggregation on the whole colony. Vegetative mycelium developed for several different periods under a glass plate. After its removal which triggered aggregation, regenerated coremia were counted 8 days later on 28 mm^2 areas taken along two perpendicular diameters for each culture. Figures are the mean of 20 determinations at each site.

a) Control culture. b) Aggregation triggered on a 5-day-old thallus. c) Aggregation triggered on a 9-day-old thallus. d) Aggregation triggered on a 9-day-old thallus with simultaneous elimination of the central region. e) Aggregation triggered on a 9-day-old thallus with simultaneous elimination of the mycelium margin. f) Aggregation triggered on a 9-day-old thallus with simultaneous elimination of central and peripheral regions.

Fig. 8 — Distribution des corémies à la suite d'un déclenchement simultané de l'agrégation sur tout le thalle. Le mycélium végétatif se développe pendant différentes périodes sous une plaque de verre. Le retrait de la plaque déclenche l'aggrégation et les corémies formées sont dénombrées 8 jours plus tard sur des surfaces de 28 mm^2 prises suivant deux diamètres perpendiculaires pour chaque culture. Les chiffres sont la moyenne de 20 déterminations à chaque emplacement.

a) Culture témoin. b) Agrégation déclenchée sur un thalle âgé de 5 jours. c) Agrégation déclenchée sur un thalle âgé de 9 jours. d) Agrégation déclenchée sur un thalle âgé de 9 jours avec élimination simultanée de la région centrale. e) Agrégation déclenchée sur un thalle âgé de 9 jours avec élimination simultanée de la périphérie du mycélium. f) Agrégation déclenchée sur un thalle âgé de 9 jours avec élimination simultanée des régions centrale et périphérique.

The inhibitory effect of the peripheral aggregated units thus influenced all the thallus. The elimination of both potential waves promoted coremium differentiation in the median zone (Fig. 8f).

DISCUSSION

The results of this study demonstrate that in *Sphaerostilbe repens* aggregated organs are not distributed sporadically but that correlations by competition between structures in the process of differentiation regulate their distribution on the mycelium.

Differentiation of an aggregated unit inhibits formation of new organs in the vicinity. The inhibition occurs between organs formed within a group as well as between waves of aggregated units themselves and is translocated centrifugally and centripetally.

Morphogenesis in *Sphaerostilbe repens* and especially the characteristic concentric series of coremia and rhizomorphs produced when a colony grows from a point inoculum can also be regulated by various other factors such as external, chemical and physical factors (BOTTON, 1977; BOTTON, 1980), but these may be overriden by the internal correlations. In addition to the competition between aggregated organs, several other mutual relations have been observed, e.g. between mycelium and rhizomorphs (BOTTON & CASALIS-GOUGEROT, 1979) and between immersed and aerial mycelia (EL-KHOURI & BOTTON, 1982).

Several questions have been raised by these results, especially what is the inhibition ascribed to and how is the inhibition translocated ? The mechanism of inhibition so far remains unknown; it could result either from nutrient deprivation by the early formed organs or from the production of inhibitory substances. In the central part of the thallus, inhibition due to the differentiating organs is effective only over short distances (usually there exists less than one millimiter between primordia) and it is likely that the inhibition is translocated by filaments rather than by the external medium. Series of aggregated organs are inhibitory over much longer distances; this was especially true for peripheral waves which modified coremium regeneration on the whole colony including the central wave. The removing of plastic discs during the time peripheral aggregated units were developing, suggests that immersed mycelium might translocate the inhibition as it was the sole type of filaments existing at that time. This experiment in which central organs were all the more inhibited as peripheral aggregated units were more fully developed, indicates that inhibition was not temporary but lasted both during initiation and growth of aggregated units. In addition, experiments using transplants lead to believing that the inhibitory effect is persistent and efficient long after the building of series of aggregated organs; indeed vegetative zones gave rise to weak regenerations, once excised.

The pattern of aggregated organ development in *Sphaerostilbe repens* has some similarities with *Sordaria fimicola* where perithecium production was restricted along the outer edge of a trench and a diffusible central inhibitor has been put forward to explain this (POLLOCK, 1975). However, in many fungi including *Sordaria*, reproductive organs usually occur when the mycelium reaches obstacles such as the edge of a dish and its linear growth is thus restric-

ted (BAHN & HOCK, 1973; LYSEK, 1976). Two major hypotheses have been put forward : the first is that the colony margin is stimulated when it encounters an increase in concentration of secreted metabolites as it approaches a barrier (BUSTON & RICKARD, 1956); the second is that stimulation is a consequence of inactivation of an inhibitory by an increased concentration of metabolites in the apices following cessation of growth at a barrier (CHET & HENIS, 1968). However, in *Sclerotium rolfsii*, inhibition of linear mycelial growth by itself is not a major simple cause for sclerotium induction (OKON & al., 1972). In *Sphaerostilbe repens*, additional experiments have not led to the conclusion that the ceasing of hyphal elongation is a prerequisite of the formation of coremia and rhizomorphs (BOTTON, 1980) and further investigations are needed to explain the formation and distribution of the aggregated organs on the colony. Studies concerning the translocation and the nature of the inhibitory effect are being published in separate papers.

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