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MEETINGS OF THE ROYAL BOTANICAL SOCIETY OF THE NETHERLANDS

SYMPOSIUM "MORPHOGENESIS IN FUNGI" ON MAY 28, 1975

J. G. H. WESSELS *(Biologisch Centrum, Laboratorium voor Experimentele Fytomorfologie, Rijksuniversiteit Groningen, Haren)*

Fungi as favourable objects for studies on differentiation and morphogenesis

Developmental biologists show an increasing interest in fungi as model systems for studies on cell differentiation and morphogenesis. On the one hand, fungi can be easily handled in the laboratory and are amenable to genetic and biochemical analysis. On the other hand, they display many of the features characteristic for development in plants and animals and allow for an examination of both time-dependent and spatial differentiation of cells. Under certain conditions fungal cells respond to changes in the external milieu as do bacteria. This makes it easy to study enzyme regulation and also allows for synchronization of development. Yet, during development of multicellular structures the organism can behave as a more or less closed system in which intercellular communications occur. This can be observed e.g. during fruit-body formation in basidiomycetes. Fungi offer many cases in which cellular interactions can be conveniently studied, one example of which will be detailed by van den Ende (this Symposium).

Another area in which fungi are advantageous for study is cell morphogenesis because of the regularity and the relative simplicity of the structures formed. One example is the formation of hyphal tubes that can be studied by examining regeneration and reversion of protoplasts (cf. de Vries and van der Valk, this Symposium).

Perhaps the most important feature of fungi that makes them attractive as model systems for developmental studies is the small size of the genome. This may suggest that in fungi the genetic regulatory circuits involved in cell differentiation are less complex than in higher plants and animals. A good example of genetic regulation of morphogenesis is provided by the incompatibility system of the basidiomycetes. The incompatibility genes clearly act as regulatory genes that govern the expression of cell morphology.

R. C. STAPLES *(Boyce Thompson Institute, Yonkers, N.Y., U.S.A.)*

Factors and consequences of infection structure information in the rust and anthracnose fungi

A study was made of the requirements for DNA synthesis and nuclear division during formation of infection structures by germinating bean rust uredospores (*Uromyces phaseoli*) and conidia of the soybean anthracnose fungus (*Colletotrichum truncatum*). With bean rust uredospores, actinomycin D and hydroxyurea did not affect spore germination, but DNA synthesis, nuclear division, and infection structure development were inhibited. Cordycepin did not affect germination, DNA synthesis, formation of appressoria or initiation of vesicles, but completion of the vesicles was inhibited. Even though the spore content of polyadenylic acid increased 6.1-fold during vesicle development, cordycepin was not particularly effective in reducing synthesis of polyadenylic acid. It was concluded that nuclear division need not accompany formation of appressoria of vesicles by bean rust uredospore germlings, but synthesis of DNA was required for appearance of appressoria. Nuclear division was required for completion of the vesicles.

With conidia of *C. truncatum*, cycloheximide prevented germination only during the first 60 minutes before the germ tube emerged, and never inhibited formation of the appressorium. Fluorodeoxyuridine did not inhibit germination, but blocked appearance of the appressorium. Fluorodeoxyuridine inhibited DNA synthesis and the second nuclear division. When the conidia were transferred to drug-free media, synthesis of DNA began and nuclear division occurred normally but only vegetative mycelia developed. Actinomycin D did not block germination, DNA synthesis or nuclear division in this anthracnose fungus, but this drug also blocked formation of appressoria. Appressoria appeared when conidia were transferred from actinomycin D to normal media. We interprete these results with *C. truncatum* as meaning that the thigmotropic stimulus to differentiation somehow altered the nuclei so that after the second nuclear division, the newly formed nuclei produced the special messenger RNA (mRNA) required for construction of appressoria. Nuclei produced in the presence of actinomycin D failed to produce the putative messenger RNA but resumed its synthesis when the drug was removed as evidenced by appearance of the appressoria. Thus, we believe that the principal effect of the thigmotropic stimulus in *C. truncatum*, was to induce the development of nuclei competent to produce mRNA required for formation of appressoria. If protein was required for this change in competency, it must have been synthesized within the first hour of germination.

We believe that in the rust fungi, the nuclei which divided in the vesicle also became competent to produce mRNA required for development of infection hyphae and haustorial mother cells. While we were not able to prove this, the large increase in the content of polyadenylic acid produced in the vesicle suggests that synthesis of mRNA in the rust fungi began there. It is clear that the thigmotropic stimulus is mediated by a chemical factor in the rust fungi; but this is not known for the anthracnose fungi.

O. M. H. DE VRIES and P. VAN DERVALK *(Biologisch Centrum, Laboratorium voor Experimentele Fytomorfologie, Rijksuniversiteit Groningen, Haren)*

Chemical and ultrastructural analysis of cell wall regeneration and reversion of protoplasts from Schizophyllum commune

In the presence of $MgSO_4$ as osmotic stabilizer, nucleated protoplasts of *Schizophyllum commune* develop a large vacuole and can be isolated on the basis of their low buoyant density. All these protoplasts can regenerate a new cell wall and 50% revert to the hyphal mode of growth. The accumulation of the three main wall polymers, S-glucan (α-1,3-glucan), R-glucan (β-1,3, β-1,6-glucan) and chitin was followed biochemically and electronmicroscopically using freeze etch replicas and shadow casted isolated walls in order to assess the localization of these polymers in the wall and their significance for the initiation of hyphal morphogenesis.

Within 2 h of regeneration a network of chitin microfibrils covered by an amorphous mass of S-glucan is formed, but accumulation of R-glucan lags behind. Hyphal tubes begin to emerge after 8 h of regeneration when massive R-glucan accumulation occurs, embedding the chitin microfibrils at the inside and forming a distinct inner layer of the wall. Where the primary cell differentiates into a hyphal tube a sharp demarcation in wall structure is noticed. Removal of the outer layer of S-glucan reveals a network of chitin fibrils at the surface of the primary cell. On the other hand the hyphal surface – and even the inception of an apex on the surface of a primary cell – is relatively smooth due to the fact that the fibrils are here completely embedded in R-glucan.

Cycloheximide (0.5 μg/ml), blocking protein synthesis by 98%, inhibits both the formation of R-glucan and hyphal tubes but the formation of S-glucan and chitin remains unaffected. Polyoxin D not only inhibits the synthesis of chitin but also R-glucan formation and reversion to hyphal tubes. However, the synthesis of S-glucan and the occurrence of nuclear division are not suppressed. The results suggest that the synthesis of R-glucan is required for the initiation of hyphal morphogenesis.

H. VAN DEN ENDE *(Plantenfysiologisch Laboratorium, Universiteit van Amsterdam)*

The sexual system of the Mucorales

Three stages can be distinguished in the sexual differentiation of *Mucor mucedo*: 1) the formation of zygophores when the two mating types are in diffusion contact; 2) the oriented growth of compatible zygophores towards each other; and 3) the formation of progametangia following physical contact between these zygophores. These progametangia carry out the fusion process.

Zygophore formation is induced by trisporic acids which are produced by both strains. Evidence is reviewed indicating that both mating types act in a strictly complementary way, the *plus* mycelium producing 4-hydroxy methyltrisporate which is exclusively converted to trisporic acids by the *minus* strain. Also *vice versa*, the *minus* strain produces trisporin which is converted to trisporic acids only by the *plus* mating type. Trisporic acids stimulate the synthesis and conversion of these precursors by bringing about an enhanced synthesis of enzymes involved in the trisporic acid pathway.

It is generally believed that the mating system in heterothallic Mucorales is based on an one-locus, two allele system, which is assumed to have evolved from homothallic systems by the occurrence of non-overlapping but complementary mutations within a locus controlling an essential stage in the sexual process (K. K. JHA & L. S. OLIVE (1975): *Mycologia* **67**: 45). The fact that the *plus* and *minus* mating types of *Mucor mucedo* lack a different (set of) enzyme(s) involved in trisporic acid synthesis, and the fact that homothallic species produce trisporic acid on their own, constitute support for this contention.

H. M. DEKHUIJZEN *(Centrum voor Plantenfysiologisch Onderzoek, Wageningen)*

Differentiation of Plasmodiophora brassicae during its growth in crucifer plants and tissue culture

The life cycle of *Plasmodiophora brassicae* in turnip seedlings and infected callus tissue of *Brassica campestris* has been studied. At an early stage of infection the secondary zoospores give rise to a slime mold phase of the fungus which moves through the cortex. In contrast to the medullary rays the cortex cells are not stimulated to divide. A clear transition was observed from the relatively mobile amoeboid slime mold phase into the immobile plasmodial phase in the endodermis and medullary rays. Time lapse experiments with the interference microscope showed that the plasmodia in the medullary rays and callus tissue grow, cleave and give rise to a number of small plasmodia (5–30μ). Isolated plasmodia were not able to penetrate healthy host callus cells, instead they stimulate host cells of the medullary rays and callus tissue to produce excessive amounts of auxins and cytokinins. Consequently the host cells divide and this results in a distribution of daughter plasmodia over daughter host cells of the medullary rays and callus tissue cells.

L. DE LANGE *(Hugo de Vries-Laboratorium, Amsterdam)*

Ecological aspects of stands of macrophytes in ditches

There are several good reasons for the separate synsystematic evaluation of ditch vegetation. Its floristic composition, structure, patterns, and ecology are specific. The periodic removal of vegetation (ditch cleaning) retains a pioneer state with the characteristic course-grained pattern and limes convergens (VAN LEEUWEN 1966). The conditions in the small bodies of water are unstable and subjected to the influence of the substrate, of the use of the banks for various purposes, of seepage, of changing weather conditions, etc., whereas the ecological gradients in the horizontal and the vertical direction are of relatively little importance. An ecological differentiation between synusia and vegetation zones is unwarranted. The progressive synsystematic subdivision of aquatic vegetation (TÜXEN 1955 discerned one, DEN HARTOG & SEGAL 1964 9 classes!) is, at least for the biotope under discussion, undesirable. WESTHOFF & DEN HELD (1969), who do not go so far, still segregate the class *Lemnetea.* A synsystematic separation of rhizophytes and pleustophytes in this biotope is not well-founded. The continuum of the water surrounding an aquatic plant and the interstitial water results in relatively little differentiation in their ionic composition. The mean difference between the electric conductivity of the interstitial and the surface water at 61 sites in the broads district of N.W. Overijssel, for instance, was only 26 μS (S = 9.3) in an overall range of conductivity values between 450 μS and 910 μS. The differences in illumination, pH, and source of carbon do not seem great enough to warrant a separate treatment. The fact that lemnids are easily transported from one place to another is no sufficient ground for a segregation of this life form at the level of a phytosociological class either, the capability of these plants to maintain themselves in a new habitat rather being decisive. The co-occurrence of species of all life forms, including lemnids has been statistically ascertained in ditches and in brooks (e.g., by DE LANGE 1972), and the intracommunal relations between synusia of vegetation stands in small waters have also been established in a number of cases (compare MCLAY 1974). A synsystematic integration of the various life forms occurring in the biotope under discussion including the lemnids to a level not usually higher than that of an alliance has been proposed by, among others, STANIEWSKA-ZĄTEK (1964), KRAUSCH (1968), and DE LANGE (1972).

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MEETING OF THE SECTION FOR PLANT MORPHOLOGY AND ANATOMY, ON MAY 29, 1975

A. M. W. MENNEGA *(Projektgroep Houtanatomie, Instituut voor Systematische Plantkunde, R.U., Utrecht)*

On unusual wood structures in Scrophulariaceae*

The Scrophulariaceae, though chiefly a family of herbaceous plants, contain also a number of genera with trees (*Paulownia* and *Wightia*) and genera with species of suffruticose and fruticose habit.

My colleague Mr. A. M. Cleef, who is studying the vegetation of the high Andean parts of Colombia collected material of the small genus *Aragoa*, endemic in the paramos of the Colombian and Venezuelan Andes. Though this genus consists in the main of small broom-like shrubs, a new species, *Aragoa perez-arbelaeziana* Romero, discovered by him, is a small tree, of which the stem reaches a diameter of 8 cm. The wood of *Aragoa* of which the structure was so far unknown, proved to be noteworthy because of the complete absence of rays and parenchyma. There was no indication at all of growth rings, and the vessels can not be distinguished from the fibres as seen on a cross section, as both are of equal width. In longitudinal sections vessels become recognizable by their simple perforations, their spiral thickenings and the presence of a few simple pits; whereas the fibre tracheids lack the spiral thickenings, and possess numerous bordered pits on both the radial and tangential walls. The diameter of the vessels and fibres varies in the 5 species investigated by me between 16 and 24 μm; the length of the vessel members is on the average 260 (185–370) μm and that of the fibre tracheids 310 (220–450) μm. This unusual wood structure was found not only in the small stems with a diameter of 4–8 mm, but also in the stem of the only arboreous species.

In the literature already several genera of Scrophulariaceae have been mentioned of which the species possess wood without rays (cf. BORN 1886, SOLEREDER 1899). All these genera belong to the subfamily Rhinanthoideae. In Wettsteins's treatment of the family in Engler & Prantl these genera, among which *Veronica* and *Digitalis* may be mentioned, are referred to the tribe Digitalinae, in which is also included *Capraria*, a shrub from the coast of Florida. An investigation of the wood of *Veronica* (*Hebe*) species and of the suffruticose *Digitalis obscura* from southern Spain revealed the presence of an almost identical wood structure, though the *Digitalis* species appeared to possess a few one to two cells wide rays and vessels which were easily distinguishable from the smaller-sized fibres. In *Capraria*, on the other hand, the wood appeared to possess an entirely normal structure with rays which are two cells wide, marginal parenchyma and vessels with small bordered pits and without helical thickenings.

From the point of view of the wood anatomist the classification proposed by PENNELL (1935) for the North American Scrophulariaceae, in which the genera of the Rhinanthoideae are distributed over twelve tribes, is preferable to that of Wettstein (1895), as in this classification *Capraria* is referred to tribe I and *Digitalis* and *Veronica* respectively to tribe VII and tribe VIII. *Aragoa* is not mentioned by him, as this genus is not represented in North America. It is generally referred to the Digitalinae, but in my opinion it fits better into the tribe Veroniceae.

BARGHOORN's findings (1941) of a similar rayless type of wood in the New Zealand shrub *Alseuosmia* (Caprifoliaceae) should be recorded here too. The absence of rays is considered by him to be a very strongly specialized condition. My own findings in the species of the genus *Aragoa* seem to confirm this view.

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