Light-driven diurnal zonation in the filamentous fungus *Fusarium solani*

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ABSTRACT   Zonation in growing mycelia of *Fusarium solani* was induced by diurnal light/ dark cycles. Only those parts of the hyphae that grew in darkness for less than 20 hours developed a zone of conidia after illumination. In continuous darkness, in continuous illumination, or after a transition from light to darkness, a conidiation zone failed to appear. Only light periods exceeding a few seconds but lasting less than 21 hours during a 24 hour light/dark cycle induced zonation. This zonation was not caused by periodic staling of the growth medium.

KEY WORDS: *zonation, diurnal, photomorphogenesis, Fusarium solani*

On an agar plate, an inoculum of spores or conidia of a filamentous fungus expands from the center until the agar surface is covered by the mycelium. When the culture is kept in continuous darkness or in continuous light, a homogeneous layer of hyphae is produced. Alternating darkness and light (e.g. in a diurnal (= daily) rhythm) can generate concentric rings of sporulation or conidiation around the point of inoculation. This phenomenon, which is called zonation (see Fig. 1) has been found in several fungal organisms (Jerebzoff, 1965). In some fungi, an endogenous zonation occurs. Examples are *Neurospora crassa* with circadian rhythms (Edmunds, 1984) and *Nectria cinnabarina* with a noncircadian one (Bourett *et al.*, 1969, 1971). The endogenous zonation may be used to study oscillatory growth processes (Winfree, 1973) whereas the exogenous zonation may be used as an overall macroscopic indicator of the influence of environmental stimuli, e.g. light, on the biochemical processes that control conidia- or sporogenesis.

This contribution deals with the influence of different light regimens on the light-driven zonation of the filamentous mold *Fusarium solani* (Mart). Sacch. 1881, teleomorph: *Nectria haematococca* var. *brevicona* (Wollenw.) Gerlach 1980 (Domsch *et al.*, 1980). The influence of light/dark transitions of hyphae of an undetermined *Fusarium* species have been studied on the microscopic level by Brown (1925).

Growth pattern in different light regimens

A bundle of macroconidia of *Fusarium solani* on a fresh agar medium germinated within four hours of seeding. At 20°C, the sprouting hyphae grew radially 1 to 2 mm on the first day and about 3 mm on each of the following days until the surface of the agar was completely covered by the mold. Under a light/dark cycle of LD= 12h:12h, the fungus showed concentric rings of dense and sparse growth around the center of inoculation. This pattern is called "zonation" (Fig. 1). It began on the third day after inoculation. During each day, an additional ring appeared. The dense parts consisted of many aerial hyphae which one day later differentiated to banana-shaped macroconidiae. In the sparse region, sparse aerial hyphae and very few macroconidia could be detected microscopically. The pattern did not change when the concentration of agar was increased from 1.5% to 8%. The visibility of the ring pattern could be enhanced by staining of the agar surface with the protein stain Coomassie Blue. All conidia stained blue, whereas the mold itself remained colorless. Exceptions were the hyphae younger than 3 to 5 days, which were stained by the dye. Therefore, in old mycelia, only the rings of dense growth, containing the macroconidia, were colored, whereas the rings of sparse growth, composed mainly of hyphae, remained colorless (Fig. 1).

F. solani grown either in continuous light or in continuous darkness, displayed no zonation and covered the surface homogeneously. When the light regimen of a fungal colony was changed, characteristic patterns appeared (Table 1). Only those hyphae which during the last 20 hours before illumination had been kept in darkness, developed the typical dense zone upon illumination. The dense zone could be assigned to the darkness phase using the set of glass needles (see experimental procedures) and it could be inferred that the subsequent light phase induced the dense zone. One very dense zone (2.6 mm wide) developed in mycelia that had grown in constant darkness before the onset of a constant light phase. The transition from constant darkness to a LD= 12h:12h induced a single dense zone after the first light phase, and during the following days, zones of regular density (Fig. 2).

Growing mycelia were illuminated in diurnal regimens with light phases of different lengths (Fig. 3) to ascertain to what extent the

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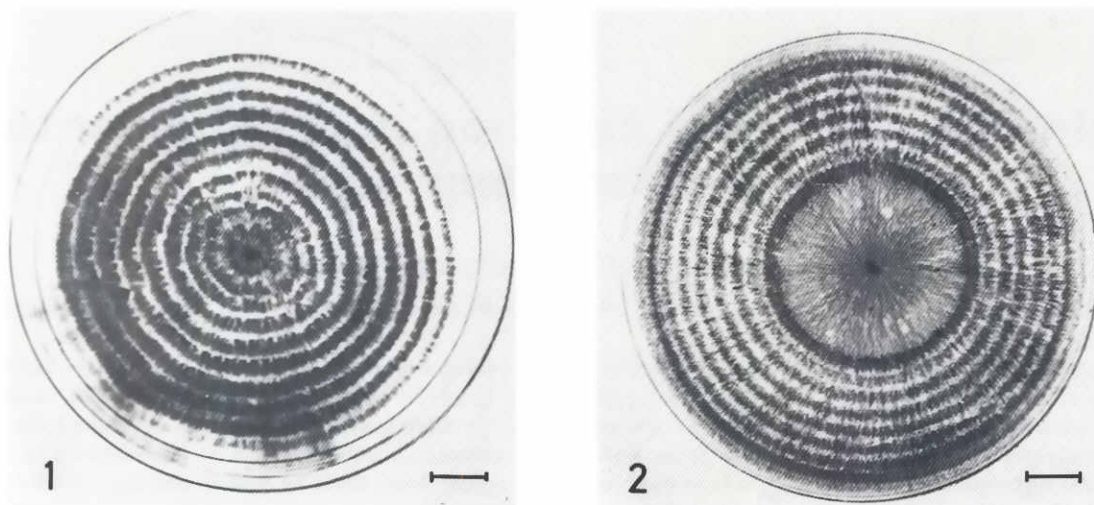
Fig. 1. Concentric ring pattern of conidation (zonation) around the point of inoculation (center of petri dish) with the filamentous fungus Fusarium solani grown on an agar plate at 20°C. The light-dark regimen was 12h light followed by 12h darkness. The conidia were stained with the protein dye Coomasie Blue. The bar represents 1 cm.

Fig. 2. Transition from continuous darkness to a light regimen of 12h light to 12h darkness. Darkness during the initial seven days of growth leads to the homogeneous inner layer of hyphae. After the onset of the light-dark regimen one very dense zone appears. It originates in the mycelium grown in darkness for the last few hours before the first light phase. During the subsequent light phases, the usual zonation is generated. Conidia are stained with Coomassie Blue. The bar represents 1 cm. F. solani was grown at 20°C on an agar plate.

length of the light period during a 24h cycle influenced the zonation pattern. The zonation became less pronounced at light phases shorter than 1 min. or longer than 20 h. No zones were visible when the lengths of the light phase were shorter than a few seconds or longer than 21 h. The width of the zone was dependent upon the length of the dark phase. Mycelia exposed to long periods of darkness developed broader zones than those which were exposed to short dark phases. In a cycle of LD= 12h:12h, the width of the zone was 1.5 mm as expected corresponding to half of the distance traversed by daily growth. The width of zones reached 2.6 mm at dark phases of 20 h. Longer dark phases did not further increase the width of a zone. Only those parts of the mycelia that were not older than 20 h seemed to be able to develop a zone.

Growth on micro-filters

In another series of experiments, F. solani was inoculated on micro-filters placed on agar plates. The small pores (0.2 μm) of the filters prevented the fungus (thickness of hyphae: 5-10 μm) from growing through the filter into the agar medium. The dissolved nutrients, however, could penetrate the pores. Under such conditions, the mold showed the usual growth pattern and zonation in a cycle of LD= 12h:12h. The growth rate decreased by 0.5 mm/d from 3.0 to 2.5 mm.

When the filters were floating on the surface of the growth medium without agar, similar patterns were observed (Fig. 4), i.e. a zonation occurred at an LD= 12h:12h and homogeneous growth appeared in continuous darkness and continuous light. The liquid medium layer was stirred by a magnetic bar. Contrary to the pattern obtained on filter disks placed on agar plates, the zones were less distinguishable and were visible only in those parts of the mycelium grown within the last three to four days. The center of the mycelium was blurred by drops of liquid of different sizes which may have been

formed by the fungus. The liquid droplets are accompanied by a dark pigmentation of the mycelium in this area.

Zonation, i.e. the development of concentric zones of sporulation or conidiation around the point of inoculation during the growth of filamentous fungi on solid medium, has been found in a number of fungal organisms (Jerebzoff, 1965). The rhythms underlying these periodicities may be endogenous (Bourett et al., 1969, 1971; Winfree, 1973; Edmunds, 1984). In most cases an external signal, (e.g. a light/dark transition) was required for the zonation (Brown,

TABLE 1

EFFECTS OF LIGHT REGIMENS AND THEIR TRANSITIONS ON THE GROWTH PATTERN OF CULTURES OF *F. SOLANI* ON AGAR PLATES

Light regimen	Growth pattern
LL	no zonation
DD	no zonation
LD	zonation
DD→LL	1 broad ring formed by hyphae grown in darkness during the last 20 h before illumination
DD→LD	as above; followed by zonation
LL→DD	no ring; no zonation
LL→LD	zonation; 1st ring after 1st dark phase
LD→LL	no further rings
LD→DD	no further rings

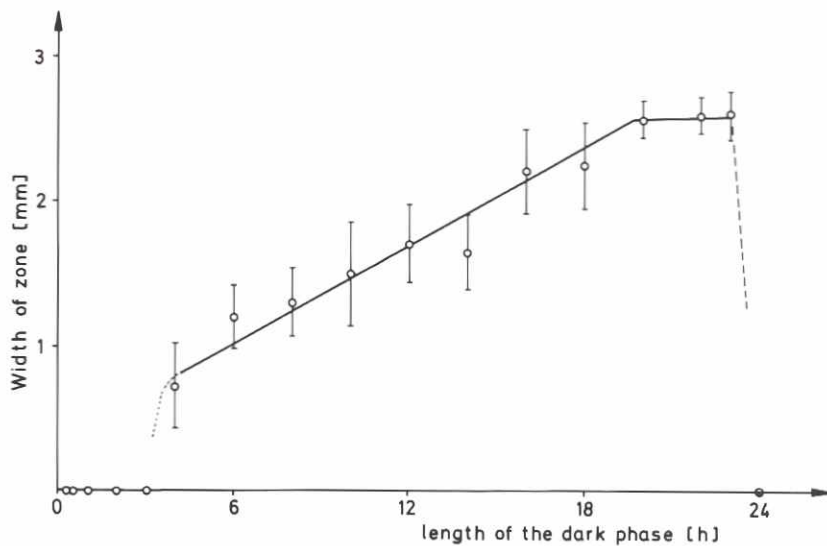|	
DD :	continuous darkness
LL :	continuous illumination
LD :	12h light:12h dark cycles
→ :	change between two light regimes

Fig. 3. Width of the zones of conidation as a function of the length of the dark phase during 24h LD cycles. *The width of the zones reached its maximal limit in a light regimen of 4h light followed by 20h darkness. Dark periods shorter than 4h during a 24h cycle permitted no zonation. The vertical bars in the graph indicate the standard deviation. A line connecting the measured values are given for orientation (F. solani was grown on agar plates at 20°C).*

1925; Hall, 1933; Gressel and Rau, 1983). To date, endogenous zonation rhythms could not be induced in *Fusarium solani*. A light phase of a duration between less than 1 min. and 20 h within a 24 h dark/light cycle was necessary for the expression of a zonation pattern. Also with *Sclerotina (Monilia) fructigena* (Hall, 1933) and *Trichoderma* (Gressel and Rau, 1983) as with *F. solani*, the hyphae grown in darkness were capable of forming a dense zone after exposure to light. Hyphae of *F. solani*, less than 20 h old, displayed such a zone.

The LD transition may trigger biochemical processes, since in *Fusarium aquaeductum* a short pulse of light influenced the carotenoid pathway in the hyphae. This effect was probably mediated by a photoreceptor which responded to blue light (Rau, 1980). It is still unknown whether a photoreceptor (possibly cryptochrome or mycochrome [Gressel and Rau, 1983]) is involved in the process of zonation. Action spectra for the zonation process may reveal its nature.

The zonation on agar plates has been interpreted as periodic staling of the growth medium (Gressel and Rau, 1983) or by periodic replacement of superficial hyphae by the ones submerged in agar. The latter explanation assumes a reduced growth rate of hyphae on the surface of the agar compared to the ones below the surface in the agar medium (Lysek, 1972). However, the fact that patterns are formed with mycelia of *F. solani* growing on microfilter disks contradicts this explanation for *F. solani*, since the pore size of the filters prevented the hyphae from growing through the filter, thus excluding the hyphae from the agar phase below. The reduced growth rate of the hyphae on filters may result from a hindered diffusive nutrient supply through pores of the filter. The influence of periodic staling of the medium was studied by cultivating mycelia on filter disks floating on a liquid medium. The large volume of a stirred liquid medium provides a homogeneous nutrient supply to the mold rather than a periodic one.

Whereas zonation on filter disks on agar was very similar to that on the surface of agar itself, it was disturbed in mycelia on filter disks floating on a liquid medium. In the latter case, liquid droplets, probably mainly consisting of water, accumulated on the filter in parts of the mycelia that were older than three to four days. The area

of hyphae covered with the liquid displayed a dark pigmentation. The origin of the pigment has not been further elucidated. We assume that the accumulation of liquid might be caused by osmotic forces. Since older hyphae are different in their protein content (see staining of proteins in Fig. 1) from those formed recently, they may also differ in their properties. The transport of cytoplasm from older hyphae to the growing tip (Robinson, 1969; Moss, 1984) may be a cause of their aging. In cultures of *F. solani*, this process would explain the staining of newly formed hyphae by the protein dye Coomassie Blue. According to the stain, only the young hyphae and the macroconidia, which are preparing for germination, are rich in protein. However, the definite identification of the cause of the liquid accumulation needs further investigation.
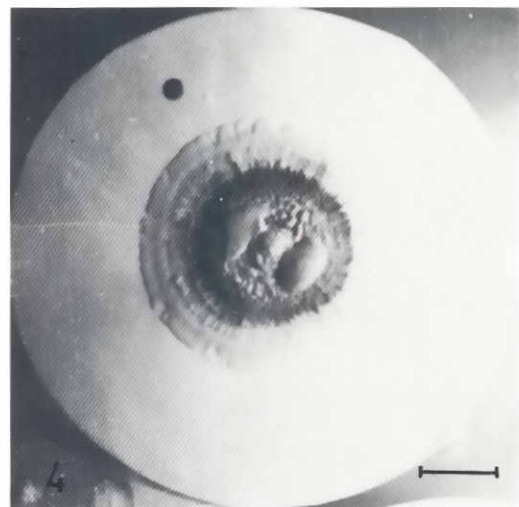

Fig. 4. Zonation pattern on a micro-filter disk floating on the surface of liquid nutrient broth. *The pattern of zonation is clearly seen in the peripheral parts of the hyphae. The dark center of the mycelium is blurred by drops of liquid formed by the mold. The bar represents 1 cm.*

Experimental Procedures

Organism

Fusarium solani Strain 62416 was obtained from the German Collection of Microorganisms (DSM), Göttingen, FRG.

Growth Medium

Agar plates were prepared by a modified version of the method of McCurdy (1963): 5 g of soluble starch, 2.5 g of casitone (Difco, Detroit, USA), 0.5 g of $MgSO_4$ x 7 H_2O, 0.328 g of K_2HPO_4 x 3 H_2O, and 15 g of agar were dissolved in 1 l of water. All the reagents were of highest quality and, if not stated otherwise, purchased from Merck, Darmstadt, FRG. The medium was autoclaved for 30 min. at 120°C. The liquified medium was poured into plastic petri dishes (ø= 9 cm) to a height of 3 mm.

Growth of the fungi

Usually a bundle of conidia was centrally inoculated on the surface of an agar plate. Cultures of *F. solani* were kept in a cabinet (manufactured by our workshop) in which the temperature was controlled at 20°C ± 0.2°C and the light could be switched on and off. During the light phase, the culture dishes were illuminated by six fluorescent tubes (Philips TLD 15 W/25) placed 5 cm above the petri dishes. The cyclic light/dark phases were controlled by a timer. Small glass needles were pricked vertically into the agar at the tips of the growing hyphae to mark the front of the mycelia during light/dark transitions. The velocity of growth was determined by measuring the distances between needles. Later, the surface of the agar was stained (see staining) and the widths of the stained zones were measured with a micrometer mounted in the ocular of a microscope.

The mold was also cultured on micro filter disks (cellulose nitrate; ø= 7 cm, pore size= 0.2 μm; Sartorius, Göttingen, FRG; type: 11307) which either were placed on agar plates or left floating on agarless growth medium in glass vessels (ø= 19 cm, height: 4 cm) (Winfree, 1973). The vessels were filled with medium to a depth of 3 cm. The experiments were repeated at least six times.

Staining

The cultures were stained with Coomassie Brillant Blue R-250 (Serva, Heidelberg, FRG) according to a procedure used for the staining of proteins in gels (Klose and Feller, 1981).

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