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Control of cell interaction using quasi-monochromatic light with varying spatiotemporal coherence

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Abstract. **By the example of plants, fungi and bacteria, we consider the possibility of controlling the interaction of cells, being in competitive, antagonistic, or parasitic relations. For this aim we used short-time irradiation (a few seconds or minutes) with the red (633 nm) quasi-monochromatic light having different spatiotemporal coherence. It is shown that the functional activity is mostly increased in the cells whose size does not exceed the coherence length and the correlation radius of the light field. Thus, in the case of cells essentially differing in size, it is possible to increase the activity of smaller cells, avoiding the stimulation of larger ones. For example, the radiation having relatively low coherence (L_{coh}, $r_{cor} \leqslant$ 10 μm) facilitates mainly the damage of large-size plant cells by pathogen fungi, while the exposure to light with less statistical regularity (L_{coh} = 4 μm, r_{cor} = 5 μm) inhibits the growth of the *Fusarium* microcera fungus, infected by the bacterium of the *Pseudomonas* species. The quasi-monochromatic radiation with sufficiently high spatiotemporal coherence stimulated all interacting species (bacteria, fungi, plants). In the considered biocenosis, the equilibrium was shifted towards the favour of organisms having the highest rate of cell division or the ones better using their adaptation potential.**

Keywords: *quasi-monochromatic light, spatiotemporal coherence, cell interaction, competing relations, antagonistic relations, parasitic relations, dynamic equilibrium shift.*

1. Introduction

Biological organisms do not live in isolation, but evolve in established communities, i.e. biocenosis. The relations within biocenosis can be different, e.g., symbiotic, metabolic, competing, antagonistic and parasitic. In the three latter cases, the intense development of one species leads to the suppression of other ones. The equilibrium between them depends on a variety of factors, including light. In this context, the researchers usually consider the lesion effect of optical radiation, e.g., the inactivation of microorganisms in the UV range or thermal coagulation of pigmented cells by photon beams of high intensity [1–4]. Much less attention is paid to the effect of light with the parameters, compatible with normal vital activity, on the interactions within biocenosis.

In the course of evolution, the light began to be exploited by the living organisms for executing two crucial functions, i.e. photosynthesis (in phototrophs) and photocontrol [1, 2]. These functions are implemented via different mechanisms. The intensity of photosynthesis is proportional to the number of absorbed quanta, i.e., the amount of energy, utilised by chloroplasts in a wide range of wavelengths. The photocontrol processes, on the contrary, are dose-independent, possess high sensitivity and rather narrow operating spectra. These properties make it possible to correlate the physiological activity of cells with a periodic variation of the amount and quality of natural irradiation.

In biological studies, it is common to consider only the energy parameters of optical radiation, i.e., the wavelength, the power density, the duration and the periodicity of exposure. The effect of statistical (coherence) properties of light on the photocontrol processes has remained a matter of discussion for a long time. The disagreement was due to the results of experiments, carried out following similar techniques, in which the stimulating effect of quasi-monochromatic radiation from laser and thermal sources was compared. In thermal sources using spectral and spatial filters, a relatively narrow spectral line was extracted with the maximum at the laser oscillation wavelength. Such radiation was referred to as 'incoherent', while the laser radiation was treated as 'coherent'. In most experiments, the exposure with coherent and incoherent light caused a similar photostimulated effect, although in some cases the laser effect was essentially stronger (for a detailed review see [5, 6]).

To resolve the contradiction, a series of experiments were carried out, it which the speculative qualitative 'coherent–incoherent' assessment was replaced with a quantitative estimate (by determining the numerical values of the correlation radius and the coherence length in a light beam). The data obtained in different biological models (bacteria, fungi, plants) have shown that the photoinduced reaction both in procaryotes and in higher eucaryotes is determined by the degree of statistical ordering of the light, independent of its source, laser or thermal [5, 6]. The effect is most expressed when the cells fully go into the volume of the field coherence (determined by the correlation radius r_{cor} and the coherence length L_{coh}). Within the chosen biological scale, the cell size, it is proposed to refer such light fields to as highly coherent. From these positions, the above contradiction is removed. Using a thermal source with a broadband optical filter, providing a small coherence length, the stimulation effect is smaller than for a laser source. When the

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exposing field formed from the radiation of the same source acquired the coherence length exceeding the size of the irradiated cells, no difference with the laser light is observed, and in both cases, the expressed photoinduced reaction takes place. Naturally, for this aim it is necessary to satisfy other conditions, e.g., the parameters of radiation must correspond to the spectrum of activity of some photocontrol system (phytochrome, cryptochrome, etc.), and the organism itself should be competent, i.e., able to increase its functional activity.

It is interesting to apply the established regularity to the control of cenotic interactions of organisms. If their cells considerably (by 2–4 times) differ in size, then in such biosystems it becomes possible to artificially change the dynamic equilibrium using the light beams with the given spatiotemporal coherence. The experimental verification of this statement is the subject of the present paper.

2. Materials and methods

Three series of experiments were carried out with biological models, in which the interaction of organisms had different forms. The influence of highly coherent radiation of competing and parasitic relations was studied in the oat seeds (*Avena sativa* L.). The seeds of this cereal are covered with floral scales under which the spores of fungi (presumably *Mucor*) are accumulated. The scales germinated in humid environment serve as nutritive substrate. The oat seeds with saprotrophic (not damaging the plants) microbiota were irradiated with the light of a helium–neon laser (632.8 nm) during four seconds with the power density 0.75 W m^{-2}. The coherence volume of the light field essentially exceeded the size of all cells, L_{coh}, $r_{cor} \gg 1000$ μm. Half the seeds immediately after the exposure were artificially infected by the pathogen, the root rot agent *Fusarium avenaceum* (Fr.) Sacc. The seeds were couched in Petry dishes (20 seeds on a thin layer of wet cotton wool) at a temperature of 22 °C. The experiment was repeated ten times. The results of irradiation were assessed by the number of vegetating plants, formed from the seeds on the eighth day of cultivating.

The second biological model was the apple fruits *Malus domestica* Borkh. In natural conditions there are spores of pathogenic fungi *Penicillium expansum* Link, *Botrytis cinera* Pers., *Mucor racemosus* Fres., etc. If the breed is susceptible to infection, the development of pathogenic biota occurs with the lesion of epithelial and parenchymal tissues, i.e., the relation of the 'host–pathogen' type arises between the organisms. Alongside with them, the physiological diseases can take place, e.g., the sunburn and swelling, which are due to impairment of cell metabolism.

The fruits were exposed to short-time action of quasi-monochromatic light with high or low coherence. As a source of high-coherence radiation, we used a helium–neon laser. The incandescent lamp with a system of optical filters and beam-forming optics served as a source of low-coherence radiation. The statistical characteristics of the field from two radiators were essentially different. The coherence length and the correlation radius in the laser beam exceeded 100 μm, while in the formed beam of thermal radiation these parameters did not exceed 10 μm. The energy characteristics of the used beams were set identical, the error not exceeding 5%. The wavelength in the maximum of the spectral line was 633 nm, the power density was 4 W m^{-2}, the exposure time was 20 s.

The irradiation of fruits was executed at the temperature 18 °C and the background illumination 30–40 luces, created by the scattered sunlight. The control fruit samples were under the similar conditions, but carefully isolated from the quasi-monochromatic radiation. To the moment of exposure, the mean size of cells of epidermal and parenchymal tissues of the fruit amounted to 40–50 μm, and the size of pathogen cells (fungi spores) was 3–8 μm. In each repetition of the experiment with irradiation and without it we used 100–120 fruits, divided into four groups (replications). They were stored during 190 days at a temperature 4 °C and relative humidity 90%. The lesion of fruits by microbial and physiologic diseases was assessed based on visible symptoms following the standard technique [7].

In the third series of experiments the object of irradiation were the colonies of the fungus *Fusarium microcera* (the means cell size 10–12 μm), infected with the bacterium of the *Pseudomonas* species (the means cell size 3–4 μm). They were cultivated *in vitro* at 22 °C during six days on the potato-glucose agar, containing 1% of glucose and 1% of agar. Part of the prepared samples were irradiated by the quasi-monochromatic light ($\lambda_{max} \approx 634$ nm) with relatively high coherence ($L_{coh} = 135$ μm, $r_{cor} = 18$ μm). The other part was exposed to the low-coherence radiation ($L_{coh} = 4$ μm, $r_{cor} = 5$ μm) with similar energy characteristics. The above values of L_{coh} and r_{cor} were formed by changing the spectral linewidth and the angular size of the source. The source was a high-temperature incandescent lamp with a system of optical filters and aperture diaphragms. In both cases, the power density in the zone of biological object location amounted to 1 W m^{-2}, the duration of the light action being 240 s. The control samples (non-irradiated biological objects) were kept at the natural illumination of 40 luces and were optically isolated from the direct and scattered quasi-monochromatic radiation. The experiments were repeated four times. The results were assessed by the area and volume of the colonies of *Fusarium microcera*.

In the performed experiments the power and power density was recorded using a VEGA laser radiation meter (Ophir, Israel) and an IMO-2N calorimetric meter ('Etalon', Russia). The transmission spectra of the IR filter and the interference ones were measured using an Analytik Jena Specord 250 Plus spectrophotometer (Germany) with the accuracy 0.5 nm. The method of formation of quasi-monochromatic beams and the quantitative estimation of their statistical parameters are presented in Ref. [5].

3. Results and discussion

In the experiments with oat seeds, the result of laser irradiation was essentially dependent on the condition of the exogenic microbiota. In the dry air state, the spores of the fungi are inactive, and no interaction with the seeds occurs. In humid environment the spores germinate, and the mycelium develops, excreting toxic products of its metabolism. If the cultivated medium is limited, the concentration of toxins in it increases and the development of germinants is inhibited. Under the conditions of the experiment, the germinability of seeds in the presence of saprotrophic microbiota did not exceed 91% (Fig. 1, experiment S). After the short-time laser irradiation (experiment S + L), even greater reduction of viable plants number (78%) was observed. The probable reason is the stimulation of fungi and their anticipatory growth, which was seen by the accelerated growth of mycelium. The saprotrophic biota itself could not affect the plants directly,

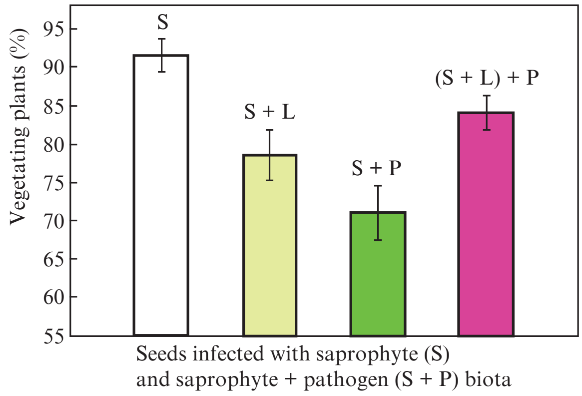

Figure 1. Number of vegetating oat plants (Gorizont breed) in the *in vitro* culture under the impact of different factors.

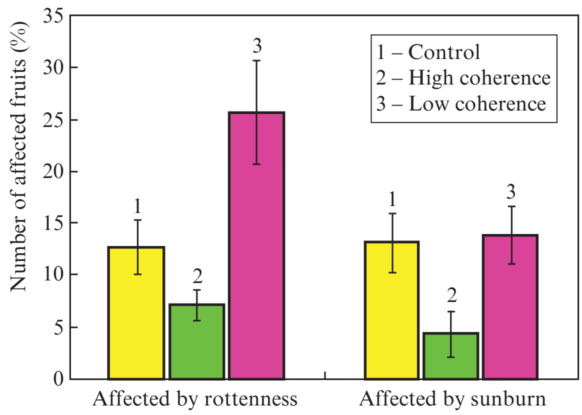

Figure 2. Influence of the quasi-monochromatic radiation coherence on the lesion of Antonovka apple fruits by the microbial (rottenness) and physiological (sunburn) diseases.

but promoted the accumulation of toxic metabolites around the seeds. Thus, in the closed cultivated medium the relations of competition for living space arose, in which under the action of high-coherence light the dynamic equilibrium point has been shifted towards the rapidly dividing cells of fungus.

Additional infection of oat seeds with the pathogen *Fusarium avenacium* significantly suppressed the development of plants. In the S + P experiment the number of non-germinated seeds or seeds with lost germinants exceeded the control indicator (experiment S) by 3.4 times with the null-hypothesis significance level $\alpha < 0.01$. The short-time action of laser radiation essentially changed the development of parasitic relations in this biocenosis. The yield of vegetating plants in the experiment (S + L) + P increased, and the number of non-functioning seeds was reduced by 1.8 times as compared to the non-irradiated seeds (S + P) ($\alpha < 0.01$). The possible explanation is that the vital activity of the pathogen via the chemical regulation chain induced the host immune reaction, enhanced under the laser irradiation. As a result of the increased antifungal activity of the irradiated seeds, manifesting itself, e.g., in the enhanced synthesis of phytoalexins, both pathogenic and saprotrophic microbiota was suppressed.

As follows from the obtained results, the high-coherence light affected the interaction between the components of the 'saprotroph–host–pathogen' dynamic system. In the case of competing relations the equilibrium was shifted towards the organisms (saprotrophic fungi) with a greater rate of cell division, than that in the germinants. In the parasitic contact, the species with more efficient protective mechanisms was the winner. In the present version of the experiment, the immune reaction of the oat germinants has limited the pathogen expansion. This type of regulation of the cenotic relations is mediate because of non-selective stimulation of both the plants and the fungi by the light with sufficiently high spatiotemporal coherence. For direct control of interaction between large and small cells, it is necessary to change the coherence volume of the field, which was implemented in the experiments with apple fruits (the second series of experiments).

The effect of low-coherence radiation on the Antonovka apple breed enhanced the lesion of fruits with fungal infection by two times as compared to the unexposed fruits, which indicates the increased activity of relatively small cells of the pathogen. In this case, the development of physiological diseases remained at the control level, and, therefore, the functional activity of large cells of the fruit did not change (Fig. 2).

On the contrary, the high-coherence irradiation reduced the microbial lesion from 12.8% to 7.1% (Fig. 2, left-hand part). One can conclude that in this case not only the pathogen cells, but also the large host cells were stimulated, which is confirmed by the reduction of the level of physiologic diseases from 13.1% in the control sample to 4.4% in the experiment (Fig. 2, the right-hand part). Thus, the photoinduced increase in the functional activity of the fruit cells caused not only the enhancement of their immune reaction, but also the general stabilisation of metabolic processes.

Similar results were obtained in the other apple breed, Sinap Severny. The short-time laser irradiation reduced the number of affected fruits by 1.7 times as compared to the intact control samples. The action of low-coherence light, on the contrary, increased this indicator by 1.9 times.

In the experiments with fruits, the direct control of the dynamic equilibrium in the 'host–pathogen' biocenosis was implemented by using the fields with different coherence volumes. The variation of light statistical properties was achieved by using two sources, the laser and the thermal one. It is important to clarify, whether the observed regularities will be conserved in the case of using the radiations of the same origin.

To answer this question, we obtained the field with relatively high or low coherence from one thermal source of radiation. The quasi-monochromatic irradiation with different statistical ordering was applied to the colonies of the fungus *Fusarium microcera*, infected with the bacterium of the *Pseudomonas* species (the third series of experiments). In such microbiocenosis, complex relations are possible. The hyphae (filament-like cellular or multicellular structures, forming the mycelium of fungi) excrete into the environment the products of their metabolism (amino acids, peptides, sugars, etc.) attractive for bacteria [8, 9]. As a result, the bacteria concentrate at the surface of hyphae and in the immediate proximity to them (Fig. 3). At this stage the metabolic relations arise between the cells of the fungus and bacterium, advantageous for the bacteria and indifferent for the fungus, and both species of organisms coexist without conflict (Fig. 4a, control).

The short-time action of low-coherence light leads to the significant (α < 0.04) suppression of the fungus colonies (Fig. 4b, experiment LC). The reason may be due to the preferred photostimulation of smaller bacterial cells that causes active colonisation of the mycelium and its lysis (decay)

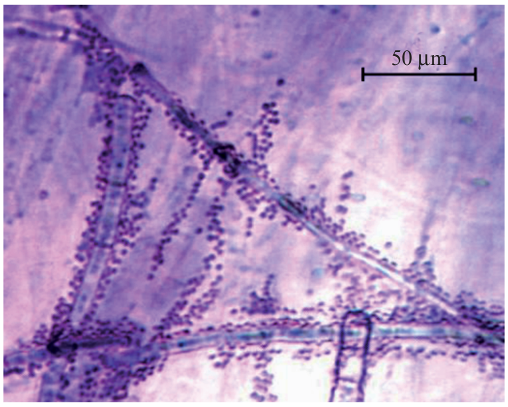

Figure 3. Localisation of cells of *Pseudomonas* bacteria in the vicinity and at the surface of hyphae of *Fusarium microcera* fungus, cultivated on artificial nutritive substrate.

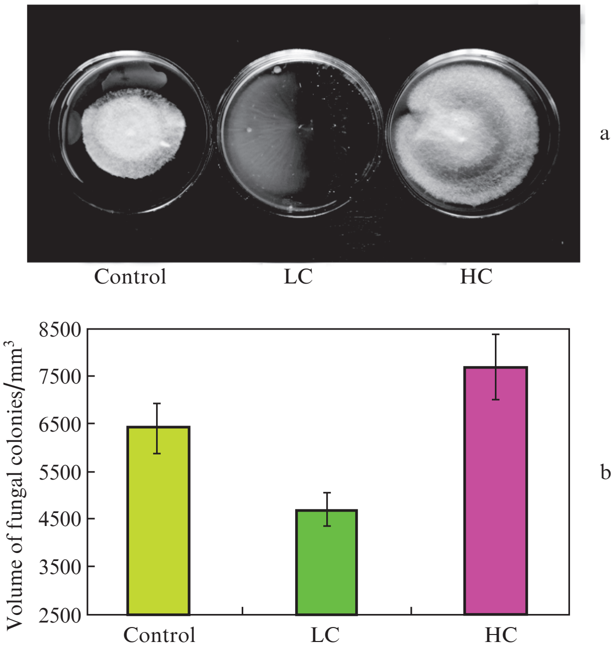

Figure 4. (a) External view of dishes with fungal colonies (a typical qualitative pattern) and (b) effect of coherence of the quasi-monochromatic light on the development of the *Fusarium microcera* fungus colonies infected with the bacterium of the *Pseudomonas* species. LC is the low coherence (L_{coh} = 4 μm, r_{cor} = 5 μm), and HC is the high coherence (L_{coh} = 135 μm, r_{cor} = 18 μm).

(Fig. 4a, experiment LC). The metabolic relations that existed between these organisms are transformed into parasitic ones as the cenotic equilibrium shifts in favour of bacteria.

The high-coherence radiation with similar energy parameters increased the viability of the fungus colonies, which began to develop more actively, than in the non-irradiated sample (Figs 4a and 4b, experiment HC). The cells of both kinds of microorganisms completely fit in the field coherence volume, and were able to increase their functional activity due to the photic stimulation. In the fungus, this manifests itself in the enhanced synthesis of protective substances (enzymes, antibiotics, lignins, etc.) [10, 11]. The increased concentration of these compounds inhibits the reproduction of bacteria and reduces the degree of their parasitism. This picture was observed in the experiment. The mycelium lysis considerably decreased as compared to the LC experiment (Fig. 4a). The relations between the considered microorganisms changed their character again and became antagonistic (with the fungus domination). After short-time irradiation of the microbiocenosis with high-coherence light, the fungus *Fusarium microcera* more efficiently used its adaptive potential than the bacteria of the *Pseudomonas* species. Thus, depending on the degree of coherence of the quasi-monochromatic radiation of the thermal source, the equilibrium in the microbiocenosis changed in a different way.

4. Conclusions

The studies carried out have shown that depending on the statistical parameters, the quasi-monochromatic radiation is capable of an essential impact on the vital activity of bacteria, fungi and plants. By setting a certain coherence volume of the field, it is possible to control selectively the interaction of cells having different sizes. The mostly stimulated cells are those, in which their size does not exceed the coherence length and the correlation radius, i.e., the cell fits in the coherence volume. This observation allows the hypothesis that the cell photoreception systems possess the properties of a phase detector and are able to react to the photons with certain phase correlation. The cooperative systems that can most probably possess such properties are chromoproteids, associated with biomembranes. If in the entire volume of the cell the spatiotemporal coherence is significant, then the structural and functional alteration will occur not in individual loci, but in the entire membrane pool, and the photoinduced reaction will be most strongly expressed. This behaviour was observed in the experiments.

To understand the possible organisation of the photosensitive cooperative system in cells the search for simpler model structure is necessary. From this position, the nematic liquid crystal polymer, doped with a small (less than 0.1 mass %) amount of dye, is particularly interesting. Such systems are very sensitive to the effect of light fields [12]. The reorientation of polymer chain fragments caused by light-induced conformation transitions in the dye molecules occurs at the power densities less than 0.1 W m^{-2}. Nearly similar power densities were used in the above experiments.

The obtained results can be used for biological protection of plants with the aim of selective enhancement of the functional activity in bacteria, antagonistic to pathogenic fungi. It is interesting to apply the low-coherence light for suppressing the proliferation of malignant tumours by selective stimulation of T-lymphocytes, which are smaller than cancer cells.

For solving practical problems, the source of quasi-monochromatic radiation with a variable coherence volume is necessary. It should operate in the wavelength range, corresponding to the activity spectrum of a certain photoregulation systems of the cells, e.g., the phytochrome one (600–690 nm). From the results of multiple experiments it follows that the power density 0.1–10 W m^{-2} will be sufficient for an expressed photoinduced response.

The range of varying the radiation statistical parameters can be determined from the estimation of the size of interacting cells. The cells of many bacteria have the size smaller than 5 μm, while in the plants it typically exceeds 20 μm. To provide the variation of the coherence length of the red (650 nm) quasi-monochromatic light within these limits, it is necessary to vary the spectral line width from ~20 to 80 nm. As shown in Ref. [13], the photoinduced reaction also strongly depends on the spatial coherence of radiation. Therefore, the correlation radius of the light beam and, correspondingly, its angular dimensions, should be also variable by a few times, with the specified power density of exposure kept unchanged.

A certain difficulty is that the cells of one species have no exactly determined size, and the geometric parameters describing them fluctuate near the most probable values. For selective stimulation, it is necessary to change the values of L_{coh} and r_{cor} in such a way that the majority of small cells could fit in the coherence volume completely, while the larger cells would exceed this volume. It is desirable to vary the statistical parameters smoothly rather than discretely in order to obtain the maximal effect of controlling the interaction of biological organisms. The creation of a quasi-monochromatic source for these purposes can be an interesting problem in the field of quantum electronics.

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