Influence of laser irradiation on state of chlorophyll-containing tissues of apple trees infected with pathogenic bacterium *Pseudomonas syringae*

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**Abstract.** The study of the laser radiation impact on the functional state of the tissues of the apple-tree of the Antonovka ordinary variety showed that the use of coherent light enables to increase the level of photosynthetic activity. It is worth noting that the greatest effect of laser stimulation was observed in variants with artificial infection with the bacterium *Pseudomonas syringae* Van Hall (the causative agent of bacterial necrosis of fruit trees). This fact testifies to the activation of plant defense reactions against the pathogen. In this regard, the use of coherent light to increase plant resistance to pathogens is a promising direction for the development of organic farming.

1. **Introduction**

Plants respond to a particular external influence including a pathogenic factor by changes in physiological processes. Many phytopathogens do not cause the rapid death of the trees but they cause disturbances in the structure of their photosynthetic apparatus. The degree of this process depends on the phase of disease development and lesion strength. Infection of plants leads to a decrease in the efficiency of accumulating light energy by the photosynthetic leaves apparatus, which occurs in the early stages due to a reduction in the electron acceptors pool. Later, the pathological process is aggravated, which impairs the photosynthetic activity of the leaves by reducing the concentration of chlorophyll, the number of chloroplasts and the death of a part of the leaf blade. [1].

*Pseudomonas syringae* Van Hall being a causative agent of bacterial necrosis of fruit trees is a harmful disease and is widespread in various countries of the world (Belarus, Ukraine, New Zealand, Australia, South Africa, the United States and in a number of Western European countries) [2–7].

*P. syringae* causes a wide range of symptoms including bark tissue death and leaf spots. Two different reactions of the infected tree cells to the penetration of the bacteria are possible. One possible outcome is a susceptible interaction, which is characterized by the pathogen proliferation and the progressive development of characteristic symptoms. Another pattern is a hypersensitive reaction of the infected tree’s resistant cells [8]. The main signs of this disease in apple trees are the lesion of flowers and shoots, which acquire a brown color, die off in spring; necrotic spots appear on the shoots, after which thickened wounds form, the branches of the tree die off. Bacterial necrosis from infected buds spreads directly to the branches. Then the disease affects the trunk leading to bark necrosis. With
a different form of lesion, brown spots are formed on the leaves of the apple tree, which appear first along the edges and then gradually move into the depths of the plate, on the branches, petioles, fruits and flowers, where the pathogen enters from the leaves through the vascular system.

Many toxins produced by *P. syringae* are well-studied. Tabtoxin, phaseolotoxin and coronatin have different influencing mechanisms but ultimately lead to changes in the photosynthetic apparatus and chlorosis of plant tissues. Tabtoxin causes irreversible inhibition of glutamine synthetase activity. This enzyme is the only way to effectively detoxify ammonia, which causes various disorders of the chloroplast membrane. Phaseolotoxin is a reversible inhibitor of ornithinecarbamoyltransferase. It causes accumulation of ornithine as well as arginine deficiency, which leads to chlorosis. Coronatin simulates the signaling system of jasmonic acid, promotes the chlorophyll degradation and inhibits the biosynthesis of salicylic acid in the chloroplast. The sensitivity of plant cells to toxins determines the degree of their susceptibility to disease [8, 9].

Chloroplasts play an important role in the integration of signals from the pathogen and the infected tree, regulating photosynthesis, plant defense reactions, and other biological processes. Stimulation of the resistance of the photosynthetic apparatus to the effectors of the causative agent of the disease and its toxins can be a strategy for increasing the activity of plant defense reactions to a wide range of pathogens [9].

It has been established that the effect of coherent light is nonspecific in relation to any particular type of plant defense reaction. Laser radiation having a phytostimulating effect contributes to an increase in the functional activity of plants, their resistance to various damaging factors including pathogens and toxins [10; 11].

The state of the photosynthetic apparatus of plants can be assessed using fluorescence analysis, which is non-destructive and allows the registration of dynamic processes. Such express diagnostics carried out in a short time reveals the biological effect of the influence of one or another external factor on the object or process under study. The fluorescent monitoring method provides control over the state of plants [12-14].

The aim of this work was to study the effect of laser radiation on the photosynthetic activity of apple leaf tissues infected with the pathogenic bacterium *P. syringae*.

2. Materials and methods

The work was carried out in the research problem laboratory “Biophotonics” of the Federal State Budgetary Educational Institution of Higher Education Michurinsk State Agrarian University.

To obtain toxic metabolites and artificial infection, a bacterial strain *Pseudomonas syringae pv. syringae* ICMP 9067 from the collection of microorganisms of the All-Russian Plant Quarantine Center was used.

Metabolites were obtained after cultivating the pathogen in liquid potato-glucose medium for a month followed by sterilization by passing through a membrane filter (Millipore 0.22 μm, France).

To determine the nature of the bacterial metabolites impact on the leaves of the infected plant, they were placed in vessels with a solution of the culture filtrate. Variants with a concentration of toxic metabolites of 5%, 10% and 15% were taken as experimental ones. Leaves placed in water served as a control. The damage to plant tissues was assessed and varieties were ranked according to the degree of resistance to bacterial toxins with regards to a six-point scale:

0 - no lesion;
1 - very weak lesion (chlorous or necrotic spots are rare);
2 - weak lesion (less than 10% of the leaf surface is occupied by necrosis or up to 25% – by chlorosis);
3 - medium lesion (from 11 to 25% necrosis or from 26 to 50% chlorosis);
4 - severe damage (from 26 to 50% necrosis, more than 50% chlorosis);
5 - very severe damage (more than 50% necrosis).

The nature of the effect of the bacterium on the infected plant was determined using the pathogen metabolites on various apple genotypes (Antonovka ordinary, Bogatyr, 62-396, 14-1).
A bacterial suspension for artificial infection was prepared by washing off the biomass from the surface of the colonies with sterile distilled water. The concentration of the inoculum was adjusted to 50-60 cells in the field of vision of the microscope at ×640 magnification.

The research was carried out on the cuttings of the leaves of the apple tree Antonovka ordinary. The leaves were cut with a drill with a diameter of 2 cm. They were placed in humid chambers (Petri dishes with moistened filter paper).

Some of the leaf cuttings were treated with a suspension of \( P. \text{syringae} \) bacteria (Fig. 1), after which they were irradiated on a rotating platform with a semiconductor laser having a wavelength of 660 nm, a power density of 2.5 W/m\(^2\) for 120 and 480 seconds (Fig. 2). Leaves not exposed to coherent light served as a control.

The photosynthetic activity of apple leaf cuttings was assessed on the 7th day after the experiment was laid on a LPT chlorophyllfluorimeter (Russia) according to the Kf indicator (specific photosynthetic activity), whose value characterizes the amount of light energy used for the photosynthesis process.

Statistical processing of the obtained data was carried out using standard computer programs Microsoft Office Excel 2007.

![Figure 1. Leaf cuttings of Antonovka apple tree in humid chamber.](image1)

![Figure 2. Sheet die cuts irradiation on rotating platform.](image2)

3. Results

To study the effect of laser radiation on the functional state of the tissues of the apple tree leaf after preliminary infection with the causative agent of bacterial necrosis, the pathogenic properties of the strain of \( P. \text{syringae} \) under study and the selection of a susceptible apple form were preliminarily carried out. Testing the properties of phytopathogens in laboratory conditions enabled to control the development of the pathogen, neutralize it in a timely manner and avoid its spread on infected plants in natural growing conditions.

Evaluation of the findings on the effect of bacterial metabolites on the host plant revealed that the culture liquid filtrate with 5.0% concentration had a stimulating effect on the leaves of the 62-396 form and the Bogatyr variety. The average value of the degree of their necrotization was 1.32 and 1.54 points, while in the control this indicator was equal to 1.45 and 1.84 points, respectively. In the Antonovka cultivar, the ordinary leaf infestation was estimated at 2.67 points in a 5% solution of bacterial metabolites, with 1.56 points in the control variant.
When using the culture liquid filtrate at 10.0% concentration, the differentiation of apple genotypes by the degree of resistance to bacterial toxins was noted. In the variants with the mentioned content of metabolites, the leaves differed in the degree of necrotization depending on the genotype. It was 1.76, 2.76 and 3.57 points for form 62-396 and varieties Bogatyr, Antonovka ordinary, respectively. Form 14-1 proved to be resistant to the studied metabolites. Therefore, no symptoms of damage were found in this variant of the experiment. In a 15% culture filtrate, the studied apple genotypes were distributed according to the degree of resistance, as well as in the variant with 10% content of bacterial metabolites. However, the degree of tissue necrosis was slightly higher (by 0.56 points on average) (Fig. 3).

The experiment has showed that the metabolites of the *P. syringae* bacterial strain have a phytotoxic effect causing necrotization of apple leaf tissues. At the same time, Antonovka ordinary is the most susceptible variety characterized by the highest level of lesion symptoms manifestation. In this regard, this variety was taken as a model for research on the effect of coherent light on the functional state of chlorophyll-containing apple tissues infected with the causative agent of bacterial necrosis.

![Figure 3](image-url)

**Figure 3.** Influence of *P. syringae* metabolites on degree of necrotization of leaves of different forms and varieties of apple trees

The study of the pathogenic properties of infecting agents and the response of a plant organism to infection by the method of preliminary cutting is popular due to providing a representative result [15 - 16]. When using leaf cuts, the cut is located along the edge of the investigated area of the leaf.

In the course of laboratory experiments on the effect of laser irradiation on the state of chlorophyll-containing tissues of leaf cuttings of the Antonovka apple tree, it was found that irradiation contributed to an increase in the specific photosynthetic activity (Kf) index both in the experiment with preliminary infection with *P. syringae* bacteria and without it.
On the 7th day after the experiment was set up, no visible changes had yet been observed, and in the variant with the use of inoculation, a decrease in the intensity of photosynthesis in comparison with this indicator in leaf cuttings that were not treated with a bacterial suspension, i.e. from 0.427 c.u. up to 0.406 c.u. was found.

Specific photosynthetic activity of uninfected chlorophyll-containing tissues in variants with the use of coherent light was 0.502 c.u. and 0.546 c.u. at exposure times of 120 s and 480 s, respectively. This was 17.6% and 27.9% more than the value of the studied indicator in the control (0.427 c.u.) (Fig. 4, a).

As is indicated by the Kf criterion, the laser stimulation effect was also noted on inoculated leaf cuts. Coherent light treatment promoted chlorophyll-containing tissues activation. With irradiation for 120 s and 480 s, this figure was 0.539 c.u. and 0.494 c.u. respectively, and in the control – 0.406 c.u. Thus, laser stimulation was 32.8% and 21.7%, respectively (Fig. 4, b).

**Figure 4.** Influence of laser irradiation on the photosynthetic activity of leaf tissues of apple tree Antonovka: a) irradiation without inoculation; b) irradiation after inoculation with *P. syringae* bacteria.

**4. Conclusion**

The studies have shown that irradiation with coherent light enables to increase the functional activity of the tissues of leaf cuttings of the apple tree Antonovka. At the same time, the greatest effect of laser stimulation was noted in the variants with preliminary infection with the *P. syringae* bacterium. This indicates an increase in the immune status of plants as a result of the activation of the infected tree defense reactions against the pathogen. The data obtained indicate the effectiveness of the use of coherent light to increase the resistance of plants to phytopathogens and their toxins, which is a promising direction contributing to the development of organic farming.
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