Ground monitoring of the dynamics of the development of fungal diseases of strawberry

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Abstract. A new method of ground-based monitoring of the dynamics of the development of fungal disease of garden strawberry in the field has been substantiated using systems of tetrapolar electrodes. Research has been carried out to determine the reactive electrical resistance of strawberry leaf tissue associated with the effect of three pathogens of fungal diseases of garden strawberry.

1. Introduction
In the near future, the world's population will need to produce 70-100% more food in connection with the forecast of population growth to 10 billion in 2050 [1]. The developing countries of the world are trying to solve this problem by increasing the area followed by agricultural land. But this way of increasing food supply has great risks and is unacceptable, since it destroys wildlife [2]. The second way is aimed at increasing the yield of existing crops. For this, it is necessary to conduct targeted selection and early diagnosis of biotic and abiotic stresses of plants [3]. The loss of agricultural productivity worldwide from biotic stress caused by various pathogens, pests and weeds is 20-40% [4]. To minimize these huge losses, only a few expensive molecular and imaging techniques are used to detect diseases at an early stage [5].

2. Problem definition
The earliest responses of plant cells to the action of pathogens and the elicitors produced by them are an increase in the content of calcium ions and protons in the cytosol, a change in the parameters of the transport system of the plasma membrane (rectifying selective potassium channels, electrogenic hydrogen ATPase pump, calcium-permeable and non-selective cation channels), as well as depolarization of the plasma lemma and tonoplast [6]. The participation of local depolarization of the plasma lemma in the transmission of an electrical signal to neighboring cells and the migration of this signal through phloem cells has also been proven, as a result of which a protective reaction is induced in cells remote from the site of infection or exposure to elicitors [7].

In addition, elicitors turn on various signaling systems of plant cells, which lead to the expression of protective genes, the synthesis of the corresponding proteins (antigens), and the formation of phytoalexins [8]. Phytoalexins are secondary metabolites that are never present in a healthy plant; they belong to low molecular weight antibiotic substances; the bulk is localized around the lesion site) [9]. Phytoalexins lead to a slowdown in the synthesis of enzymes of the fungal pathogen, a slowdown in its...
growth, and sometimes its destruction. The synthesis of phytoalexins is taken over by healthy cells surrounding the necrosis. It is in them that these substances are formed, and then they are directed towards danger – into necrotic cells in which the parasite is located. Plant cells are unable to withstand high concentrations of phytoalexins, so they accumulate in toxic doses in dead cells, which have nothing to lose. Their movement from healthy cells to necrotic ones occurs, bypassing the contents of the cells, outside their cytoplasmic membrane, that is, apoplectically along the interconnected system of cell walls and intercellular spaces. There is an assumption that the permeability of cell membranes is disturbed in necrotic cells, as a result of which phytoalexins penetrate through them more easily [10]. It is also known that when the pathogens of fungal diseases are damaged, the hosts do not die quickly, but the intensity of photosynthesis of diseased plants is usually much lower than the norm. This is mainly due to a violation of the structure of the photosynthetic apparatus of cells: the number of chloroplasts per unit leaf area, the volume of chloroplasts, the concentration of chlorophyll, and the ratio of chlorophylls a and b sharply decrease [10].

The above reactions of plants to the effects of these biological stressors make it possible to formulate the following possible directions for choosing an effective method for their early diagnosis:

1) changes in the photosynthetic apparatus of plant leaves can be investigated by recording the parameters of chlorophyll fluorescence emission, which directly or indirectly relate to all stages of the light phase of the photosynthesis process: water photolysis, electron transfer, generation of a pH gradient on thylakoid membranes and ATP synthesis [11];

2) specific color spots and the configuration of their distribution on the surface of plant leaves can be investigated by the colorimetric method by counting image pixels in the space of color channels of red, green and blue colors (R, G, B) [12-13];

3) changes in the content of ions in leaf tissues, parameters of the transport system of the plasma membrane and its polarization can be investigated by the method of electrical impedance spectroscopy – EIS. Changes in the frequency dependences of the real part of the complex biological impedance will mainly reflect changes in the ion content and conductivity of the transport system, and the imaginary one – changes in the polarization of cell membranes [14].

With the fluorescence method, early diagnosis detects pathogens by probing changes in the transmission or fluorescence of various parts of plants caused by pigmentation. This method is non-invasive and has wide functionality. But it requires the use of sophisticated analytical equipment and is not effective in monitoring the development of diseases in the field, where there are no stationary sources of electrical power.

Given that fungal diseases create specific color spots, they can be easily identified in the CIE Lab colorimetric system. The practical implementation of this method does not require large expenditures and complex equipment, since it can be implemented as a software application in a smartphone based on the Android operating system. However, using the colorimetric method, it is difficult to judge the degree of damage to the leaves of garden strawberries at an early stage of diagnosis, since the symptoms of a specific disease may coincide with external influences (sunburn, frost, chemical burns, etc.), as well as with viral and bacterial diseases. In addition, the implementation of the method requires the development of a complex pattern recognition system and the use of artificial intelligence systems, for example, artificial neural networks (ANN). Moreover, the ANN training procedure is lengthy and requires significant amounts of experimental data.

The method of electrical impedance spectroscopy allows one to obtain information on the processes of transport of charge carriers in any materials and allows one to characterize systems whose electrochemical behavior is caused by several inextricably linked processes [15, 16]. Impedance spectroscopy is often indispensable for obtaining information about complex charge transfer processes in animal and plant tissues. Electrical impedance spectroscopy is a relatively new technology in which a signal with a wide bandwidth and continuous frequency bandwidth is used as an excitation source for measuring the impedance characterizing the electrical properties of the test object, studying the structure and physicochemical characteristics of inorganic and organic materials. The EIS method allows obtaining a significant amount of information on the processes of transport of charge carriers in solid
and liquid materials. It is extremely important for studying charge transfer in heterogeneous systems, including phase boundaries, electrode boundaries, and microstructure elements. A methodology has been developed for determining the physiological status of plant tissues by measuring the electrical impedance [17].

Therefore, this method is most effective for monitoring the development of fungal plant diseases. Indeed, in [18], the EIS method was implemented for the detection of fungal diseases of strawberries, in the implementation of which the reactive resistance of plant tissue of a healthy leaf $X_0$ is measured at a fixed frequency $f_0$ located in the region of the $\alpha$-dispersion of tissue bioimpedance (the polarization capacity is maximum) and this resistance value is stored $X_0$. Then, the above-described measurement procedure is repeated on a part of the leaf of strawberry plants of the selected variety with the assumed defeat of the leaf fungal disease $X_i$ at the same frequency $f_0$. Further, determining the ratio $k_i = X_i/X_0$ and if $k_i \geq 1.1$ consider that this plant is subject to the action of a biostressor.

However, the application of this method for monitoring fungal diseases in the field is not effective for the following reasons.

First, the method is based on the procedure for measuring an electrical quantity. The measuring instrument is subject to mandatory periodic verification. And for this, it is necessary, first of all, to determine the equivalent circuit of the model for biological tissues for garden strawberries (Hayden's model, model with a constant phase element, etc.) and, on the basis of experimental data, to clarify the range of values of active (intracellular and extracellular resistance) and reactive (capacity of the cell membrane) constituents.

Secondly, the method requires the use of an electrically conductive gel, which is not rational in the field. Moreover, the gel dries out over time, changes its physical properties and its conductivity decreases. This factor introduces additional errors in the reactance measurement procedure.

Thirdly, the measurement result, which carries information about the degree of damage to the plant by the fungal disease, is issued in discrete form by converting analog signals into digital form, storing, averaging and dividing procedures. To see the dynamics of the development of the disease over time, it is necessary to periodically extract data from the database and build their continuous sequence. All this complicates the process of obtaining and processing data, and also impairs the visibility of the dynamic processes of the fungal disease during the implementation of the method.

The purpose of this research is to develop a new method for determining the electrical properties of plant tissue, suitable for monitoring the dynamics of the development of fungal disease of garden strawberry in real time directly on discrete areas of the plantation of its cultivation.

3. Materials and methods

In the proposed method for determining the electrical properties of plant tissue, two identical tetrapolar electrode systems with two current and two measuring electrodes are applied to the leaf blade perpendicularly from its central vein. One electrode system is placed on the area of the leaf blade without signs of damage, and the second on the part of the leaf blade with the most probable infection by the pathogenic fungus. The measuring electrodes are connected in the opposite direction, and by the difference in the change in signals from them, the dynamics of the development of a fungal disease of the selected section of the strawberry plantation over time is judged. Indeed, in the bipolar technique, two electrodes are applied, each of which is simultaneously current and measuring electrodes. Therefore, to reduce the contact resistance between the electrode and the surface of the sheet plate, various pastes, gels and other substances are used. When using the tetrapolar technique, the study area is limited by a pair of measuring electrodes, and the voltage that has arisen in them is removed with the help of another pair of electrodes. The tetrapolar technique is more accurate, because it minimizes the effect of contact resistance and electrode polarization [17]. Therefore, the output signal (voltage), in the ideal case, if the phases coincide, will be similar to the opposite connection of two DC sources.

That is, for direct current sources with opposite connection, the poles of the same name of two sources are connected to each other, and the load is connected between two other poles of the same name. In this case, the output signal is equal to the difference between the electromotive forces of the
sources. This signal on a healthy plate will be zero. Of course, the situation when the output signal with the opposite connection of two AC sources was equal to zero practically will not occur, even if tetrapolar electrode systems are installed on identical healthy tissue sites. The fact is that phase distortions can introduce a connection between the reactive elements of electrode systems and the reactive parameters of biological tissue at the place where the measuring electrodes are installed. But even in the presence of phase distortions, the output signal of counter-connected tetrapolar electrode systems located on healthy tissue will be at a fixed level and will not change for a certain time. When a fungal disease occurs and develops, the level of this signal will change, by which it is possible to judge the extent of the disease affecting the control plant selected as an indicator of the discrete plot of the garden strawberry plantation. In addition, the proposed method does not impose strict requirements for ensuring the accuracy of the impedance measurement and additional errors from the influence of influencing quantities (temperature and humidity of the air, solar radiation, etc.). For a technical monitoring tool, it is important to ensure that there is no time drift of the signal, since the electrode systems are in the same external environmental conditions. All these essential features of the proposed method for monitoring fungal diseases will simplify the implementation of early diagnosis of fungal diseases of garden strawberry in the field.

4. Results and discussion

To confirm the method, we carried out in the period from 20.07.2020 to 21.08.2020 its practical testing on healthy and affected by brown spot leaf blades (fungus pathogen - *Marssonina potentillae* Desm) of garden strawberry varieties *Tanyusha, Daryonka* and *Festival*. The location of the study is the biological testing ground of the Siberian Federal Scientific Center of the Russian Academy of Sciences, Krasnoobsk, Novosibirsk Region. The land plot is located in the forest-steppe of the Novosibirsk Oblast region. The composition of the soil is dominated by leached medium-thick humus. The climate is continental, moderately cool, and moderately arid with an average annual rainfall of 425 mm. The choice of brown spot is due to its dominance among other fungal diseases of garden strawberry in a given area. The biological impedance of plant tissues of strawberry leaves was measured with an impedance meter 6505V (Great Britain) in the frequency range from 20 Hz to 10 kHz using 2 tetrapolar electrode systems. Figure 1 shows the characteristic differences between healthy and affected areas of strawberry leaves.

![Graph](image)

**Figure 1.** Dependence of the averaged values of the reactance of healthy (blue) and weakly affected 1% (red) sections of the plates of garden strawberries: a) *Festivalnaya* variety 08/24/2020; b) variety *Tanyusha* 08/06/2020.

Figure 1 shows that when using this method, it is possible to carry out early diagnosis of fungal diseases and its garden strawberry. The difference in reactance values between healthy and affected areas depends on the variety of garden strawberries.
This method is implemented as an experimental sample: a gadget with miniature electrodes and a smartphone (figure 2).

![Block diagram of ground-based monitoring of fungal diseases of garden strawberry.](image)

**Figure 2.** Block diagram of ground-based monitoring of fungal diseases of garden strawberry.

Tetrapolar electrodes, made in the form of non-polarizable electrodes, are fixed on the plant leaf. The conversion of the output signal from the electrodes is carried out using microcircuits AD5933, LM358 and Arduino Android [17]. The AD5933 is a 12-bit, high-speed impedance analyzer and converter. An excitation harmonic signal (LM358) is applied to the plant leaf from the generator of the impedance analyzer, which forms an electric field. The measuring signal in the form of an electrical voltage is amplified by an operational amplifier, filtered and fed to the input of an analog-to-digital converter (ADC). The output signal from the ADC is fed to the input of the digital Fourier transformer, where, by processing discrete readings, data on the reactive component of the tissue impedance are formed. Microcircuit the digital output data is transmitted via the Android Arduino controller to the smartphone (wirelessly), where it is processed and recorded. Thus, the implementation of the proposed scheme in practice will make it possible to remotely monitor the development of plant diseases on their plantations.

5. **Conclusion**

A method of ground-based monitoring of the dynamics of the development of fungal disease of garden strawberry in the field has been developed using systems of tetrapolar electrodes. It makes it possible to significantly simplify the implementation of early diagnosis of the disease and significantly reduce the influence of environmental factors on the reliability of its detection. When implementing this monitoring method, there is no need to use conductive substances (gels, pastes) when picking up an information signal and the procedures for memorizing, averaging and dividing are excluded. To implement the method, it is not necessary to use precision instruments for measuring impedance, and the result of determining the reactance, which is used to judge the development of the disease, practically does not depend on changes in external environmental factors. The method is reliable, sensitive and has wide functionality.

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