# EXPRESS ESTIMATION OF RESISTANT THE HORSE CHESTNUT TO THE INFLUENCE *CAMERARIA OHRIDELLA* DESCH. & DIM. BY USING THE METHOD OF THE INDUCTION OF CHLOROPHYLL FLUORESCENCE

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*The purpose of the present study was to determine the effectiveness of the chlorophyll fluorescence method induction for the estimation of the functional state of the photosynthetic activity of the horse chestnut and red buckeye in green areas of three ecological zones of Kiev. It was found that the representative plants in three ecological zones have a significant difference of indexes, what shows changes of the induction of chlorophyll fluorescence that reflects the processes of transformation of energy at the early stages of photosynthesis. It was stated that value of Kpl plateau in the curve of Kautsky can be used as a test for the early selection of the forms of horse chestnut, resistant to the influence of the complex environmental factors.*

***A. hippocastanum L., A. pavia L., general condition, chlorophyll, fluorescence induction, express diagnostics.***

In recent years, the state of green space in urban Ukraine has significantly deteriorated due to the adverse effects of a number of anthropogenic factors: emissions from motor vehicles, heavy metals, salinity, heat, disease and pests. A substantial number of plants is depressed critical physiological condition and does not stand anthropogenic pressure, leading to disturbances in their metabolism, their premature aging and shrinkage [1, 2].

According Kyivgreenbud in Kyiv urbolandshafts Aesculus hippocastanum is 22% tree plantations, but lately, due to high level of total anthropogenic influence, destruction of plant pests and diseases, much of it in quantity. Kiev is in critical condition [3, 4]. In recent years, moreover observed mass distribution chestnut moth minuyuchoyi Cameraria ohridella Desch. & Dim. Ascomycetes and Guignardia aesculi (Peck) Stev. [2, 4]. All this leads to the depletion of plants and reduce their decoration.

Today there are large methodological arsenal for the diagnosis of viral, bacterial and fungal infections of plants. It is, above all, biological testing for viral diseases herbaceous plant indicators, methods and immunofluorescence microscopy email, various modifications of the ELISA, immunodiffusion, serological tests [5]. However, all of the above diagnostic approaches are complex, time consuming, and mostly they require complex and expensive laboratory equipment. Moreover, the only known method currently widely used to express determination destruction plant chestnut moth in vivo in the field, is a visual examination. Of course, it does not meet modern requirements practices on the previous diagnosis of diseases.

Given the above, it is clear urgent need to develop and implement informative and express methods for diagnostically important indicators at an early stage of the pathological process in plants. In addition, methods used must be economically feasible and do not put the ravages of plant tissues. Among the methodological approaches that are currently being developed for the rapid diagnosis of extreme environmental factors on plants, pay attention, especially those based on determining the state of the photosynthetic apparatus. The process of photosynthesis is essential to plant life and is extremely sensitive to the effects of abiotic and biotic factors. Modern methods allow to obtain information about the state of the photosynthetic apparatus of plants and assess the degree of impact on his adverse environmental factors. A significant advantage of them is that they are able to register even small changes in the activity of the photosynthetic apparatus and let it do in the field and quickly and easily [5, 6]. The method of chlorophyll fluorescence induction based on the measurement of chlorophyll fluorescence in plants under intense light after a short period of dark adaptation. This method is sensitive enough to quickly install and changes in the photosynthetic apparatus under stress and regeneration potential of intact plants.

The aim of our study was to determine the efficacy of the method, based on an assessment of the intensity of induction of chlorophyll fluorescence to determine the functional state of the photosynthetic apparatus in leaves of Aesculus hippocastanum Horse Chestnut and red in three ecological zones m. Kyiv.

**Materials and methods.**

For experiments using randomly selected samples of vegetable Horse Chestnut two types: normal and red and green space m. Kyiv in three ecological zones: the Botanical Garden of the National University of Life and Environmental Sciences of Ukraine (control), parks, Holosiyivsky, Mariinsky parks and outdoor spaces near highways.

Measurements were carried out on un-chestnut leaves in ground plant growth. State of the photosynthetic apparatus of plants was determined using a prototype handheld device "Floratest" developed at the Institute of Cybernetics Glushkov National Academy of Sciences of Ukraine. The device allows you to record the fluorescence induction curve ("Kautsky curve"), the parameters of which can be analyzed as the progress of the light and dark phases of photosynthesis. Portable device "Floratest" consists of highly integrated microprocessor unit and a miniature remote optical sensor. The main characteristics of remote optical sensor is light levels and low power consumption. Optical sensor consists of a blue light-emitting diodes (type FYLS-0603UBC) and photodiode amplifier. Dimensions diodes are 6 - 3 mm. Diodes are compatible with automatic soldering equipment installation and elements that are very important in serial production. The power light emitting diodes 120 mcd, the angle of illumination - 130 °, the dominant wavelength - 470 nm. Photodiode amplifier (type TSL251R) combines a photodiode and amplifier feedback. It has low power consumption and comes in a plastic case with integrated lenses. The width of the range of photodiode amplifier is 300 nm to 1100 nm. The peak emission of chlorophyll is about 700 nm. Therefore, to narrow the range of photodiode amplifier on its surface was installed red filter. Microprocessor unit includes a controller ADuC842 low power, input amplifiers, ridynnokrystalichnyy display FDCG12864 consumption 3B, battery-powered and control buttons.

The shape of the curve of chlorophyll fluorescence induction reflects changes in every link of the process of photosynthesis caused by the influence of environmental factors and endogenous factors [6, 8].

Induction of chlorophyll fluorescence was measured in 3-time repetition of the cycle length in 3 min dark adaptation before the measurement was 10 min. For IFH curves and analysis software tool used Microsoft Office Excel.

Key indicators fotoinduktsiyi fluorescence were: Fo - background level of fluorescence; Fpl - the level of fluorescence in achieving a temporary slowdown in its signal, the so-called "plateau"; F max - the maximum fluorescence; Fst- fixed level of 3 minutes. after the lighting. All figures induction curve presented in relative fluorescence units standard (filter OC-14), with emissions in the same spectral range as the leaf chlorophyll fluorescence [9, 10].

Also defined Key = (Fmah- Fst) / Fst - factor induction fluorescence, Fv = Fmax-Fo - variable fluorescence, (Fmax-Fo) / Fmax = Fv / Fmax - depends on the efficiency of photochemical reactions of PS2, (Fpl-Fo) / Fv - if the current light intensity sufficient to reach a state of maximum vidnovlennosti QA, at the time of reaching the level of Fmax, then option (Fpl-Fo) / Fv corresponds to the relative amount QB-nevidnovlyuyuchyh PS2 complexes that do not participate in a linear electron transport, (Fmax -Fst) / Fst - value quenching of fluorescence, which affect both photochemical (CO2 fixation) and nefotohimichni processes (thermal dissipation of the energy of the excited state of chlorophyll molecules) [10-12].

**Results and discussion.**

According to the literature [13], changes in chlorophyll fluorescence of photosystem 2 (PS2) reflect the redox processes in the reaction center (RC) of the photosystem. Light quanta absorbed energy can be transferred in three ways: photochemical reactions in the form of heat dissipation and radiation of light - chlorophyll fluorescence. These processes compete with each other in a way that efficiency leads to a suppression of the other two. In normal conditions, the level of fluorescence small (1-2% of the total of absorbed light), indicating that the active cells use the energy of absorbed light. After lighting adapted to the dark leaves of plants first for a few seconds a sharp rise in the intensity of fluorescence of chlorophyll - fast phase, and then in a few minutes there is a gradual decline through certain stages - from slow to stationary phase of Fst.

To characterize the photosynthetic apparatus under stress at leaf stage for rapid phase often use the ratio of variable fluorescence Fv to the maximum level F max, which is considered an indicator of potential leaf photosynthetic activity [14, 15].

However, this option has some limitations because it applies only to the primary photochemical processes in photosystem 2 and only during the first 100-500 ms.

The action of any adverse factors (in this case may be affected minuyuchoyu chestnut moth Cameraria ohridella) reduces the ability atrahuyuchu Calvin cycle that stops the flow of electrons and reaction centers go inactive (closed) position. This absorbed light energy can not be used in photosynthesis and chlorophyll fluorescence increases as around the time of registration induction range changes [11, 13].

In plants Horse Chestnut Red resistant to minuyuchoyi moths, there was an increase in intensity Fo 23.3%, and increased the maximum fluorescence Fmax 19.0% and inpatient level Fst - at 59.1%. Such changes of fluorescence induction due to the increasing number of chlorophyll molecules in susceptible plants, which does not transmit excitation energy of the reaction center (Fo), and accompanied by the growth of "integral induction losses", ie an increase in the amount of energy that does not work on photosynthesis and also displayed in slower phases of induction, reflected on Kautsky curve (Fig. 1, 2).

Spectral fluorescence study found infected leaves Horse Chestnut increase in the ratio of intensities of short and long-wave fluorestsentsiy chlorophyll - Fst680 / Fst740, indicating that the increase in the degree of recovery carriers electron transport chain between reaction centers of photosystems 1 and 2 [16]. Note also decrease by 21% fluorescence induction coefficient Ki = (Fmax-Fst) / Fmax, which characterizes the efficiency of the flow of dark photosynthetic processes and above all rybulozo-bifosfatkarboksylazy activity - the main enzyme Calvin cycle.

In the samples of plants susceptible to destruction Fo value decreased by 25.0% compared to their stable forms that may be associated with blocking resynthesis chlorophyll degradation and destruction of chloroplast structure, reducing their number to the impact of environmental factors. This, in turn, contributes to the decline of the maximum fluorescence (Fmax) at 29.0%, compared with an increase of 15.0% in value in resistant plants.

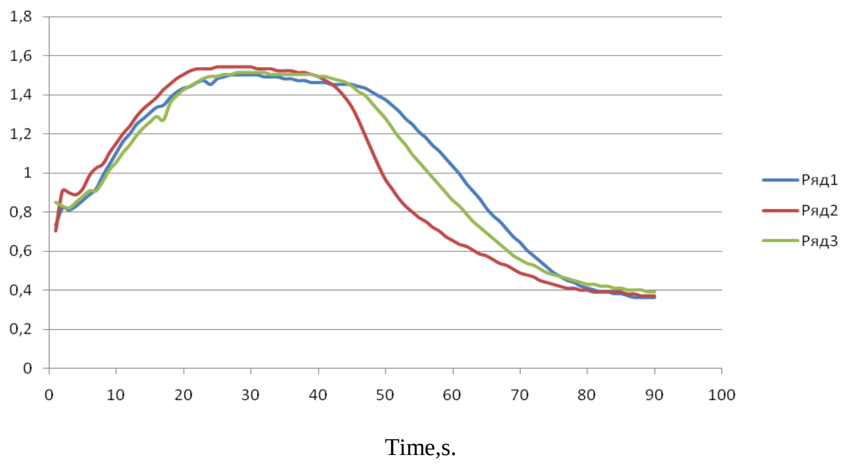


Fig. 1. Typical curves fotoinduktsiyi flyuorystsentsiyi Horse Chestnut leaf chlorophyll red moth resistant minuyuchoyi where number 1 - control number 2 - Parklands, number 3 - street stands near highways.

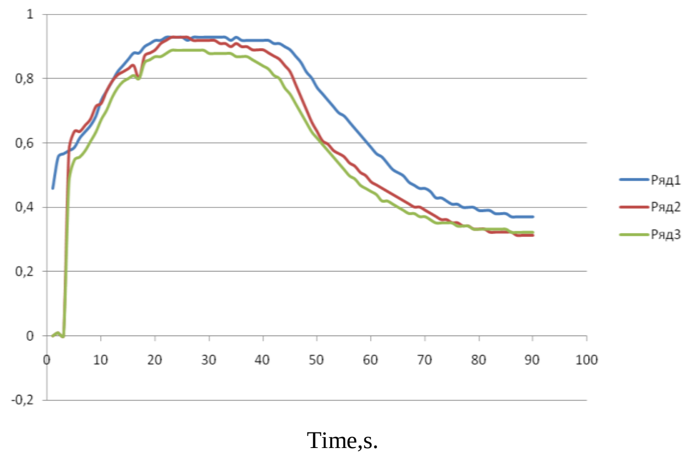


Fig. 2. Standard curves fotoinduktions flyuorystsentsions chlorophyll leaves Aesculus hippocastanum, where number 1 - control number 2 - Parklands, number 3 - street stands near highways.

The dependence of the functioning of the photosynthetic apparatus Aesculus hippocastanum leaves and red depending on locations

| characteristic phenotype | Fv | Fv/Fo | (Fpl-Fo)/Fv | Fmax/Fst | Ki | Fv/Fmax | (Fpl-Fo)/Fmax | (Fmax-Fpl)/Fmax |
|---|---|---|---|---|---|---|---|---|
| Aesculus hippocastanum | 0,517 ± 0,006 | 0,868 ± 0,022 | 0,340± 0,009 | 3,677 ± 0,014 | 0,728 ± 0,010 | 0,465 ± 0,017 | 0,158 ± 0,011 | 0,307± 0,007 |
| Street planting near | 0,625 ± 0,013 | 1,163 ± 0,024 | 0,375± 0,014 | 3,500 ± 0,008 | 0,714 ± 0,012 | 0,538 ± 0,008 | 0,202 ± 0,004 | 0,336± 0,018 |
| highways | 0,664 ± | 0,782 ± | 0,388± 0,006 | 3,876 ± | 0,742 ± | 0,439 ± | 0,039 ± | 0,400± 0,013 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 0,014 | 0,016 | | 0,024 | 0,020 | 0,017 | 0,002 | |
| Aesculus hippocastanum | 0,772 ± 0,009 | 1,054 ± 0,026 | 0,114± 0,013 | 4,163 ± 0,032 | 0,760 ± 0,017 | 0,513 ± 0,008 | 0,058 ± 0,004 | 0,455± 0,013 |
| control | 0,840 ± 0,012 | 1,195 ± 0,034 | 0,244± 0,006 | 4,158 ± 0,036 | 0,759 ± 0,027 | 0,544 ± 0,014 | 0,076 ± 0,003 | 0,468± 0,014 |
| Aesculus hippocastanum | 0,684 ± 0,021 | 2,121 ± 0,028 | 0,328± 0,017 | 3,552 ± 0,042 | 0,718 ± 0,013 | 0,680 ± 0,011 | 0,291 ± 0,004 | 0,388± 0,012 |

The most significant changes occur with the intensity of dark photosynthetic processes that reflect the slow changes in chlorophyll fluorescence induction. Against the background of general decline in chlorophyll fluorescence emission in resistant plants observed steady growth of Fst 52.2%. Under these conditions, the fluorescence induction coefficient Ki is reduced compared to susceptible plants by 73%. This indicates blocking the flow of photosynthetic processes in chloroplasts of leaves Aesculus hippocastanum.

Among the parameters of chlorophyll fluorescence induction factor Plateau Kpl = (Fpl-Fo) :( Fmax-Fo) = dFpl / Fv (where: dFpl = Fpl-Fo - amplitude plateau fluorescence; Fv = Fmax-Fo - variable fluorescence) explicitly characterize resistance Aesculus hippocastanum to biotic factors such as their pathological effects on functional status of plants. Established a significant increase in Kpl Aesculus hippocastanum of street trees near the highway compared with the same version of Red Horse Chestnut (0.340 and 0.114 respectively). This value dFpl / Fv (Kpl), which characterizes the relative amount of inactive reaction centers with respect to total reaction centers, defined as a test indicator for high adaptability of plants Horse Chestnut red.

The value of the parameter dFpl / Fv, which corresponds to the relative amount of non-renewable Qb-PS2 complexes that do not participate in the linear electron transport increases (respectively versions experiment with Aesculus hippocastanum 2.9 and 2.7 times). Note that chlorophyll fluorescence emission increases and slow phase induction curve at maximum Fmax 15.5 - 19.4%, and its stationary level Fst - more than 2.2 times. Accordingly, the ratio of fluorescence induction is reduced by 57-

58%, indicating a significant inhibition of both photophysical and photochemical processes of photosynthesis in susceptible plants. The lowest rates were reduced in these plants of both species sampled in the park areas, indicating a much lower human impact on them than those who were on the roads. And, then, the plants and the park area was under the best overall physiological condition.

Thus, the plant species studied in three ecological zones differ widely in terms of induction of chlorophyll fluorescence changes that reflect the energy conversion process in the early stages of photosynthesis. Thus, the parameter Fo in Aesculus hippocastanum susceptible to pests increases, which characterizes increase in the number of molecules of chlorophyll, which do not transfer energy to the reaction centers of PS2. These rates were highest in plants that were selected among street trees near highways city.

The increasing degree of reduction of PS2 reaction centers and chloroplasts suggests increasing the maximum level of chlorophyll fluorescence Fmax.

**Conclusions.**

Thus, there is every reason to believe that the induction of chlorophyll fluorescence is promising for practical use at an early rapid diagnosis of plant state. But the relationship between changes in fluorescence parameters and photosynthetic apparatus of plants as a whole is not fully installed. The primary stage of photosynthesis of plants under the influence of environmental factors are not static, but actively regulated cells according to their physiological state, which leads to complex changes in fluorescence parameters.

As a test indicator for early selection of resistant forms Horse Chestnut to the environmental impact of several factors recommended ratio Plateau Kpl = (Fpl-Fo) :( Fmax-Fo). Kpl ≥0,4-0,5 value indicates susceptibility genotypes to Horse Chestnut of negative environmental factors, including a breakdown chestnut moth. Establishing relationships between the state of the photosynthetic apparatus and mechanisms of plant resistance to biotic factors would contribute to the understanding of photosynthesis of plants in the wild, and allow plenty of informative studies to monitor environmental conditions and plant status using fluorescence methods.

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