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Investigation of the Photosynthetic Activity of Bark Phelloderm of Arboreous Plants Using the Fluorescent Method

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Abstract—Seasonal changes in the characteristics of chlorophyll fluorescence were studied in the bark of several species of trees originating in various climatic zones: Siberian cedar (*Pinus sibirica*), larch (*Larix sibirica*), eastern arborvitae (*Thuja occidentalis*), pendent white birch (*Betula pendula*), wild black cherry (*Padus virginiana*), horse chestnut (*Aesculus hippocastanum*), red oak (*Quercus rubra*), Manchurian catalpa (*Catalpa bungei*), linden (*Tilia cordata*), goat willow (*Salix caprea*), Amur cherry (*Padus maakii*), and apple Korichnaya (*Malus domestrica* B.). Tree bark has a sufficient amount of chlorophyll for measuring the parameters of chlorophyll fluorescence throughout the year. The relative yield of the variable fluorescence of chlorophyll (F_v/F_m) can be used to assess seasonal changes in the physiological state of various trees.

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One of the adaptations of arboreous plants for survival of unfavorable winter conditions is their ability to pass over to a dormant state. An important role in the formation of the tolerance of a plant to frost is played by the dynamics of the dormant period. In some studies, it was shown that, when arboreous plants pass over from the active state to the state of winter dormancy, the rate of photosynthetic transport of electrons decreases (Janssen and Hasselt, 1990) as a result of blockage of electron transport between photosystem 2 (PS2) and photosystem 1 at the level of plastoquinon (Oquist et al., 1980). This may depend either on the destruction of plastoquinon or modification of its surroundings. Inhibition of photosynthesis in needles increasing at decreasing temperatures was observed (Hawkins and Lister, 1985).

Photosynthesis of bark becomes especially important under conditions of loss of leaves by trees in the cold or dry season. The layer of phelloderm in the bark of arboreous plants contains much chlorophyll and has photosynthetic activity. The content of chlorophyll in the bark per unit of area may be as high as in leaves (Pearson and Lawrence, 1958; Solhaug et al., 1995). Due to the presence of the photosynthetic apparatus of phelloderm of arboreous plants and shrubs all year round, they are convenient for investigation of the physiological state of these plants (Nielsen, 1995; Kharouk and Middleton, 1995).

Earlier, we demonstrated that the parameters of phelloderm are convenient for observation of the state of plants preparing for dormancy (Ortoidze et al., 1987). In the period of transition of a grapevine to dormancy, the photosynthetic activity decreases in correlation with the degree of dormancy, the value of the inductive maximum of the retarded fluorescence of the chlorophyll (RF) of the phelloderm decreases (Ortoidze et al., 1989), and the maximum dependence of the RF on the temperature shifts from 50 to 35°. This indicates a decrease of the thermostability of membranes under such conditions (Ortoidze et al., 1987).

In bark cells, a low ratio of Chl *a*/*b* takes place, which reflects the adaptation of photosynthesis in the bark to shading by leaves and by the layer of phelleme (Larcher et al., 1988; Muthuchelian and Haugen, 1998). In the absence of leaves in the cold season, the intensive illumination may, under a low temperature, cause photoinhibition (Huner and Oquist, 1993) leading to decrease of the photochemical activity of PS2, of the relative output of the alternating fluorescence of chlorophyll F_v/F_m, and to development of processes of peroxide oxidation of lipids (Ortoidze ey al., 1988; Havaux, 1992; Solhaug and Haugen, 1998).

At present, highly sensitive fluorescent methods are used for measurement of photosynthetic processes in leaves of plants and in algae (Matorin and Venediktov, 1990; Kazakov and Matorin, 1998; Venediktov et al., 2000; Havaux, 1992). The physiological state of plants is estimated by the ratio F_v/F_m characterizing the efficiency of utilization of excitation energy in the reaction center of PC2. $F_v = F_m - F_0$ is called alternating fluorescence. Permanent fluorescence F_0 corresponds to the oxidized state of the primary acceptor of electrons of PS2 and the maximum level of F_m—to its reduced state. Some studies on plants indicate a proportional relationship between F_v/F_m and the values of the photosynthesis determined by the rate of evolution of oxygen or by fixation of carbon dioxide (Renger and Schreiber, 1986).

The present study is aimed at a comparison using the parameters of the chlorophyll fluorescence to indi-

Values of the relative output of alternating fluorescence of chlorophyll (F_v/F_m) in bark and buds of arboreous plants in the winter and summer

Species	F_v/F_m			
	Bark		Buds	t_{act} (month)
	summer	winter		
Aesculus hippocastanum	0.70	0.33	0.50	7.5
Pinus sibirica	0.72	0.46	–	7.8
Malus domestrica	0.79	0.39	0.30	5.3
Thuja occidentalis	0.73	0.33	–	6.4
Padus virginiana	0.75	0.39	0.61	6.6
Padus maakii	0.81	0.08	0.1	4.2
Tilia cordata	0.76	0.07	0.06	6.6
Larix sibirica	0.73	0.23	–	7.5
Quercus rubra	0.81	0.33	0.22	6.0
Betula pendula	0.78	0.17	0.07	8.0

cate the seasonal changes in the activity of the photosynthetic apparatus in the bark of various species of trees.

METHODS

The objects of the investigation were parts of the bark and buds of sprouts of arboreous plants of 12 species growing under natural conditions in the vicinity of the Department of Biology of MSU: Siberian cedar (*Pinus sibirica*), larch (*Larix sibirica*), eastern arborvitae (*Thuja occidentalis*), pendent white birch (*Betula pendula*), wild black cherry (*Padus virginiana*), horse chestnut (*Aesculus hippocastanum*), red oak (*Quercus rubra*), Manchurian catalpa (*Catalpa bungei*), linden (*Tilia cordata*), goat willow (*Salix caprea*), Amur cherry (*Padus maakii*), and apple korichnaya (*Malus domestrica* B.). Samples of the tissues of the bark of the trees were taken once a month all year round, and those of the buds were taken only in the winter. Sprouts of trees of the same year and buds were taken from the southern side of the trees at a height of 1.5–2 m from the ground surface. Immediately prior to the analysis, a part of the bark (0.5–1 cm^2) was separated from the sprouts from the same place along the length.

The activity of the photosynthetic apparatus was recorded by the fluorescence parameters using a one-ray pulse fluorimeter (Lyadskii et al., 1987). The fluorescence (F_0) was excited by pulses of weak light (50 μs, 0.5 Hz) from a flash lamp through SS-4 and SZS-22 light filters. The intensity of the maximum fluorescence (F_m) at the shut reaction centers PC2 was measured in the same way but in the presence of 10^{-5} M diuron and an additional three-minute permanent background illumination of 7 W/m^2. The relative output of the alternating fluorescence was described by the ratio F_v/F_m, where $F_v = F_m - F_0$.

The content of chlorophyll (Chl) "*a*" and "*b*" in the tissues of the bark was determined spectrophotometrically after the extraction of the chlorophylls with 80% acetone. The optical density was measures using a SPECOL-211 spectrophotometer (Germany). The concentration was calculated using the equations C_a (mg/l) = $11.63 \cdot D_{665} - 2.39 \cdot D_{649}$, C_b (mg/l) = $20.11 \cdot D_{649} - 2.39 \cdot D_{665}$, where C_a and C_b are the concentration of Chl "*a*" and Chl "*b*" respectively, in g of chlorophyll per liter of extract. D_{665} and D_{649} are the values of the optical density of the extract at 665 and 649 nm, respectively.

The meteorological and phenological data were supplied by the meteorological station of MSU and are taken from the bulletin of the Botanical Garden of MSU.

RESULTS AND DISCUSSION

The values of the relative output of alternating fluorescence F_v/F_m characterizing the photosynthetic activity were maximum in the summer in the phelloderm of all the species and were from 0.7 in *Aesculus hippocastanum* to 0.81 in *Padus maakii* (Table). Such values are typical of photosynthetically active tissues. In winter, the alternating fluorescence was decreased in the bark of all the species indicating the inactivation of PS2 in this season. In the winter, the maximum values of the ratio F_v/F_m were from 0.07 in *Tilia cordata* to 0.46 in *Pinus sibirica.*

Figure 1a demonstrates the typical dynamics of the changes of the parameters of the fluorescence (of the intensity of the permanent (F_0) maximum fluorescence and alternating fluorescence (F_v/F_m) in the bark of birch during the year). Figure 1 shows that, from April till July, the intensity of the permanent and maximum fluorescence increased and, from July till November, their intensity decreases. F_0 changed by two–three

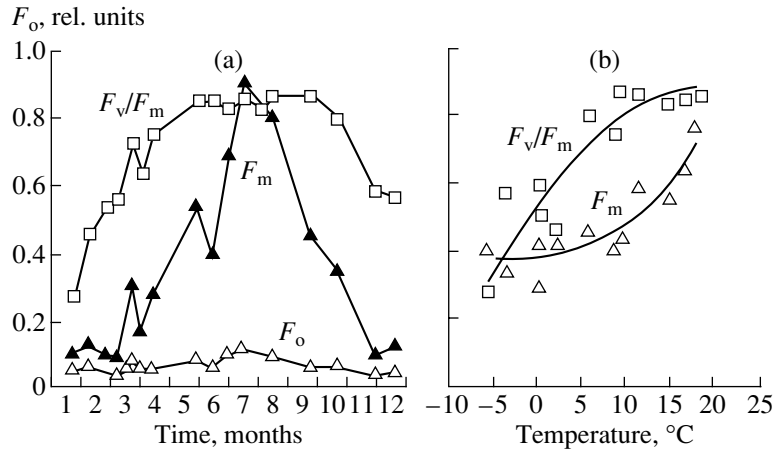

Fig. 1. Seasonal dynamics of the parameters of the chlorophyll fluorescence (F_0, F_m, F_v/F_m) in the bark of birch (A) and their correlation with the average monthly temperature (B) (1990).

times and F_m by over eight times. As a consequence, the relative output of the alternating fluorescence increased from 0.4 in the winter to 0.8 in the summer.

The content of chlorophyll in the phelloderm of the birch bark (from 0.18 in May to 0.44 mg/g in July) changed approximately at the same level as the F_0. This means that the changes of the intensity of F_0 seem to depend mainly on the increase of the content of chlorophyll in the bark until the middle of the summer and its subsequent decrease in the second half of the summer. The correlation of the intensity of the fluorescence F_0 with the content of pigments of the photosynthetic apparatus performing light collection of light energy was previously described for algae (Matorin et al., 2004).

The differences in the seasonal dynamics of the permanent F_0 and alternating fluorescence F_v/F_m draw attention. The intensity of F_0 changed continuously in the summer, attaining the maximum value in July. On the contrary, during the summer, the relative output of alternating fluorescence retained its permanent high value. The decrease in the intensity of the alternating fluorescence took place later (from October till January) than the decrease of F_0, and the increase took place earlier (in March–April). Figure 1a shows that the inhibition of the alternating fluorescence in the winter is related principally to the decrease of the output of the maximum fluorescence F_m, i.e., with extinction of the fluorescence in the shut reaction centers of FS2.

The difference in the seasonal dynamics of various parameters of the chlorophyll fluorescence clearly manifests itself in the plot of their correlation with the average temperature of the month (Fig. 1b). F_v/F_m linearly correlated with the temperature at its decrease during the autumn–winter below +10° and the corresponding increase in the spring. However, at changes of the average monthly temperature within the range from +10 to +18°, i.e., its increase from May to July and its subsequent decrease from July till September, the relative output of alternating fluorescence did not change. In contrast to the alternating fluorescence, the constant fluorescence F_0 did not change at temperature fluctuations below +10° but was an almost linear function of the temperature at its changes within the range above +10°.

The different species of trees significantly differed by the intensity and duration of the inhibition of the photosynthetic activity (F_v/F_m) in the bark in the winter (table and Fig 2). In most of the investigated species, the output F_v/F_m in the period from September till January decreased gradually attaining a value of 0.3–0.4. The inhibition of the alternating fluorescence was the most rapid in *Padus maackii, Salix caprea*, and *Tilia cordata*: the decrease in the ratio of F_v/F_m started already in August and finished within one–two months with F_v/F_m attaining 0.1–0.15. Later than other species (from November) and to a lesser extent (up to 0.45), the alternating fluorescence was inhibited in the bark of the pine. The seasonal dynamics of the alternating fluorescence in the bark of the other species was intermediate between these extreme types.

In the spring, the alternating fluorescence rapidly restored during March–April. The maximum value of F_v/F_m was attained by most species in May. In the bark of the larch, the reactivation of alternating fluorescence started in February (earlier than in other species) and principally already finished in April, while, in *Thuja occidentalis* and *Catalpa bungei*, the reactivation started in April and finished only in early June.

A certain correlation ($r = 0.67$) should also be noted between the value of the F_v/F_m ratio in the summer and the duration of the winter inhibition of the alternating fluorescence (Fig. 3). The duration of this period was determined as the distance between the descending and ascending parts in the seasonal dynamics of the F_v/F_m ratio at the level equal to half of the difference between the minimum winter value and the maximum summer value (Fig. 2). The highest value of F_v/F_m in the sum-

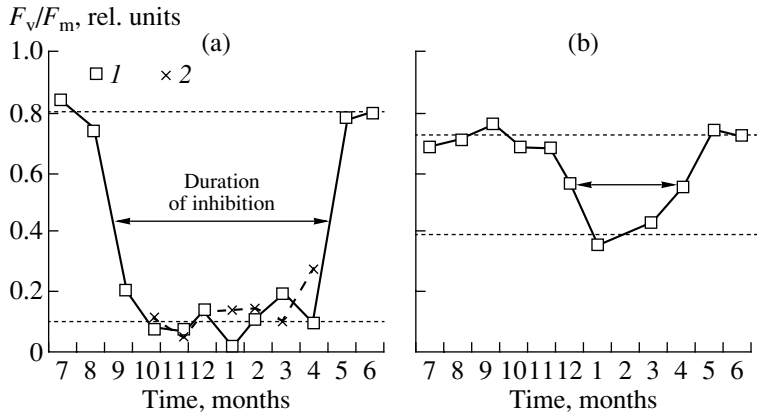

Fig. 2. Seasonal dynamics of the alternating fluorescence of chlorophyll (F_v/F_m) in the bark (1) and buds (2) of *Padus maackii* (A) and in the bark of *Pinus sibirica* (B). Examples of the determination of the duration of the winter inhibition are shown (1990).

mer and the most prolonged inhibition of the activity in the winter was in *Padus maackii* differing in the earliest leaf fall and the lowest values of F_v/F_m. A prolonged active period was noted in *Aesculus hippocastanum*, which retained foliage until late autumn, and in the conifers, pine, and larch.

The photosynthetic activity according to F_v/F_m in the buds is also inhibited in the winter. The level of this inhibition in different species is close to that in the bark (Table). However, in *Aesculus hippocastanum* and *Padus*, the alternating fluorescence in the buds was, in the winter, significantly higher than in the bark; in the willow and birch, it was lower. Among the investigated species, two groups may be discerned: in one group (*Aesculus hippocastanum, Malus domestrica, Padus*, and oak) the relative output of the alternating fluorescence in the bark is identical (0.33–0.39) and, in the buds, it is from 0.25 to 0.6 depending on the species. In the other group (*Padus maackii, Tilia cordata*, willow, and birch), the output of the alternating fluorescence was strongly inhibited in the bark and buds. It was somewhat higher in the bark than in the buds.

The plots of the correlation of the F_v/F_m ratio with the average monthly temperature (Fig. 4) demonstrate that there is a characteristic range of temperatures within which the alternating fluorescence of the chlorophyll in the bark of various species is inhibited. Thus, in *Padus maackii*, the alternating fluorescence in the bark is inhibited beginning from +15° and attains the maximum value (about 0.1) at +5°. Similar characteristics are recorded in *Tilia cordata* and *Salix caprea.* In pine, the inhibition begins at a temperature of about 0° and, even at –17°, the F_v/F_m ratio stays at the 0.4 level. The correlation of the alternating fluorescence with the average monthly temperature is close to that in pine and in larch. In other species, the winter inhibition of the alternating fluorescence is intermediate between the described extreme variants.

The dates and level of the winter inhibition of the activity and the dates of the spring restoration of the photosynthesis vary depending on the year. Figure 5 shows the data for birch, for which observations were made for two–four years. The winter of 1986–1987 was cold, the average temperature in January was –17.2°, and the transition above 0° of the air temperature took place only on April 26. This retarded the development of the arboreous plants by two–three weeks in relation to the average long-term dates. The F_v/F_m ratio in the birch bark decreased in February–March to 0.1–0.15 and started increasing only in April.

On the contrary, the winter of 1989–1990 was exceptionally warm. The average temperature in January was –5 to 6°. In February, 70% of the days had a positive temperature, and, in late February, the temperature definitely crossed +5° resulting in circulation of sap in the birch. In this winter, the F_v/F_m ratio decreased up to January to 0.3, which is similar to the value of 1987, but, already in February, it was increasing and attained in March a value of 0.7 (Fig. 5).

Irrespective of the time from which the F_v/F_m ratio was increasing, its maximum value was attained in the birch by the beginning of May, which corresponds to

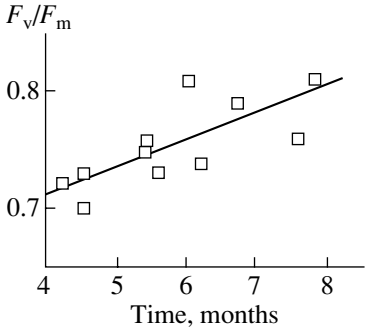

Fig. 3. Correlation between the value of the alternating fluorescence F_v/F_m in the summer and the duration of the winter inhibition of photosynthetic activity according to F_v/F_m in the bark of different species.

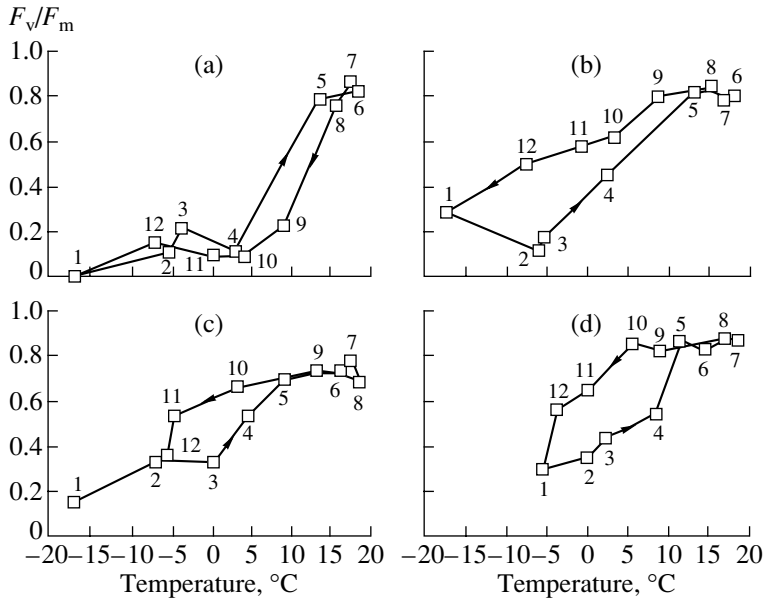

Fig. 4. Correlation of the alternating fluorescence of chlorophyll (F_v/F_m) in the bark of different species and the average monthly temperature of the air. (A) *Padus maackii*; (B) *Betula pendula*; (C) *Larix sibirica*; (D) *Catalpa bungei.* The numbers near the dots designate the months.

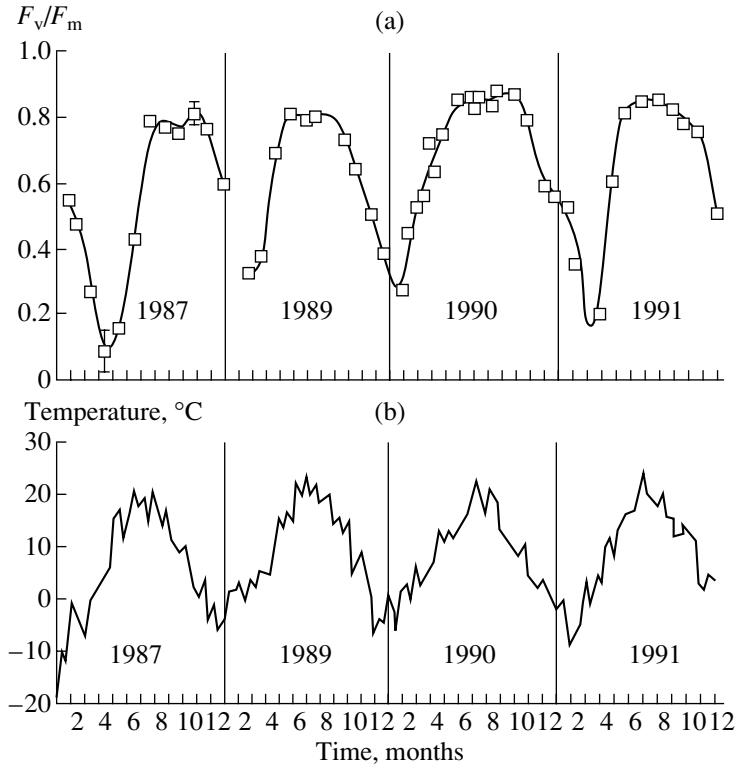

Fig. 5. Seasonal dynamics of the relative output of the alternating fluorescence of chlorophyll (F_v/F_m) in the bark of birch sprouts (A) and changes of the average air temperature of ten-day periods (B) in the vicinity of MSU.

the average date of the bud swelling and bud bursting (May 1 and 7, respectively), and the decrease of the ratio started in late September immediately after the leaf falling (the average date of the mass leaf falling for the birch is September 28). In *Catalpa bunge*, the leaves appeared, according to our observations, two–three weeks later than in the birch, and the leaf falling continued until the frosts. Accordingly, the maximum

value of the F_v/F_m ratio was attained later (up to the middle or end of May) and the decrease of this ratio started only in early November.

After our experiments, the seasonal changes of the photochemical activity of photosystem 2 determined by the value of the F_v/F_m ratio were investigated in the bark of *Populus tremula* L. (Solhaug and Haugen, 1998). These authors also demonstrated that the highest decrease occurs after cold periods. The photochemical activity restores in late April, approximately a month before the appearance of leaves.

CONCLUSIONS

Thus, the investigations of the seasonal changes in the various parameters of the chlorophyll fluorescence in the bark and buds of 12 species of trees growing near the Department of Biology of MSU demonstrated that all the investigated objects manifest a complex of characteristic changes of the rapid fluorescence depending on the modification of the state of the photosynthetic apparatus during the year and reflecting the inactivation of the reaction centers of PS2 in the cold period. Different species of trees in the winter significantly differ in the intensity and duration of the inhibition of photosynthesis as estimated by the parameter of alternating fluorescence F_v/F_m. In most of the investigated species, the relative output of the alternating fluorescence decreases gradually in the period from September till January. In the spring, the alternating fluorescence rapidly restores during March–April. The maximum value of F_v/F_m in most of the species is attained in the middle of May. For all the investigated species, the duration and level of the winter inhibition of activity and the seasonal dynamics of the F_v/F_m ratio are determined. Meanwhile, it is found that the dates and level of the winter inhibition of activity and the dates of the spring restoration of photosynthesis changed depending on the climatic conditions of a particular year, as was well seen by the F_v/F_m ratio. This demonstrates that, by means of measurements of the parameters of the chlorophyll fluorescence in the bark of trees, the dynamics of the transition of plants into the state of winter dormancy, the level of damager of plants by the cold in winter, and their transition from the state of dormancy in the spring may be easily observed. This is highly promising for express testing of changes in the physiological state of arboreous stands under the action of natural and anthropogenous factors. Incorporation of this method in monitoring systems opens possibilities for prognostication of both the state of each tree and of urban stands generally, the more so as this method permits determinations during a period significantly exceeding the vegetation season. Application of biophysical methods in ecological investigations is promising, as they provide the possibility to work on intact plants and to obtain unbiased information within a short time.

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REFERENCES

1. M. Hawaux, Stress Tolerance of Photosystem II *in vivo*. Antagonistic Effects of Water, Heat and Photoinhibition Stress. Physiol. Plant. **58**, 424–432 (1992).
2. C.D.B. Hawkins and G.R. Lister, In vivo Chlorophyll Fluorescence as a Possible Indicator of the Dormancy Stage in Douglas-Fir Seedlings, Can. J. Forest Research **15** (4), 607–612 (1985).
3. N.P.A. Huner and G. Oquist, Photosynthesis, Photoihnhibition and Low Temperature Acclimation in Cold Tolerant Plants, Photosynth. Res. **37**, 19–39 (1993).
4. L.H.J. Janssen and P.R. Hasselt, Influence of Growth Conditions of Chlorophyll a Fluorescence in Cold Tolerant Plants, Physiol. Plant. **79** (2), 107 (1990).
5. L.K. Kazakov and D.N. Matorin, Indication and Estimation of Ecological Situations in Industrial Regions, Ekologiya i promyshelnnost Rossii, No. 5, 32–36 (1998).
6. V.I. Kharouk and E.M. Middleton, Aspen bark Photosynthesis and Its Significance to Remote Sensing and carbon Budget Estimates in the Boreal Ecosystem. Water Air Soil Pollution **82**, 483–497 (1995).
7. W. Larcher, C. Lutz., M. Nagele, and M. Bodner, Photosynthetic Functioning and Ultrastructure of Chloroplasts in Stems Tissues of *Fagus sylvatica*, Physiol. Plant. **132**, 1731–737 (1988).
8. V.V. Lyadskii, M.A. Gorbunov, and P.S. Venediktov, Pulse Fuorometer for Investigation of Primary Photophysical Processes in Green Plants, *Biol. Nauki,* No. 11, 31 (1987).
9. D.N. Matorin, T.K. Antal, M. Ostrowska, A.B. Rubin, D. Ficek, and R. Majchhrowski, Chlorophyll Fluorometry as a Method for Studying Light Absorption by Photosynthetic Pigments in Marine Algae, Oceanologia, no. 4, 519–531 (2004).
10. D.N. Matorin and P.S. Venediktov, Luminiscence of Chlorophyll in Cultures of Microalgae in Natural Populations of Phytoplankton, Itogi nauki tekhn., Ser. Biophysics, **40**, 49–100.
11. K. Muthuchelian, Photosynthetic Characteristics of Bark Tissues of the Tropical Tree of *Bombax ceiba* L., Photosynthetica **26**, 633–636 (1992).
12. E.T. Nielsen, Stem Photosynthesis: Extent, Patterns, and Role in Plant Carbon Economy, Ed. B.L. Gartner. *Plant Systems: Physiology and Functional Morphology* (San Diego, 1995), pp. 223–240.
13. T.V. Ortoidze, D.N. Matorin, and P.S. Venediktov, Investigation of Functioning of Photosynthetic Apparatus of Bark Phelloderm of One-Year Old Shoot of Grape in the Period of Deep Dormancy by Method of Retarded Fluorescence, Fiziol. Biokhim. kulturnykh rastenii **19** (2), 165–169 (1987)
14. T.V. Ortoidze, D.N. Matorin, and P.S. Venediktov, Effects of Deep Dormancy on the Primary Processes of Photosynthesis in Vine (*Vitis vinifera*) shoots, Biochem. Physiol. Pflanzen **183** (4), 301–305 (1988).

15. T.V. Ortoidze, D.N. Matorin, and P.S. Venediktov, Modification of Fluorescence of Chlorophyll of Phelloderm in the period of Dormancy in Connection with Tolerance to Frost in Grape Plants, Phyziol. rastenii **36** (4), 802–806 (1989).
16. G. Oquist, L. Brunes, and J.-E. Hallenger, Effects of Artificial Frosty Hardening and Winter Stress on Net Photosynthesis, Photosynthetic Electron Transport and RUBR-Carboxylase Activity in Seedlings of *Pinus silvestris*, Physiol. Plantarum **48** (4), 526–637.
17. I.C. Pearson and D.B. Lawrence, Photosynthesis in Aspen bark, Amer J. Bot. **45**, 383–387 (1958).
18. G. Renger and U. Schreiber, *Practical Application of Fluometric Methods to Algae and Higher Plant Research* (Acad. Press, 1986), **4**, pp. 578–617.
19. K.A. Solhaug, Y. Gausla, and J. Haugen, Adverse Effects of Epiphytic Crustose Lichens upon Stem Photosynthesis and Chlorophyll of *Populus tremula* L., Bot Acta **108**, 233–239.
20. K.A. Solhaug and J. Haugen, Seasonal variation of Photoinhibition of Photosynthesis in bark from *Populus tremula* L., Photosynthestica **35** (3), 411–417 (1998).
21. P.S. Venediktov, Yu.V. Kazimirko, T.E. Krendeleva, G.P. Kukarskikh, V.V. Makarova, S.I. Pogisyan, O.V. Yakovleva, and A.B. Rubin, Investigation of Physiological Conditions of Arboreous Plants by Characteristics of Fluorescence in Bark of One-Year Old Shoots of Trees, Ekologiya, No. 5, 338–342 (2000).

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