# Physiological indication of stress reactions in trees

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The paper discusses changes in some physiological indications, biochemical reactions to vitality of the Norway spruce under different intensity of stress both in the process of spruce growth and development while crown closing in coenosis takes place but also while coniferous trees have been exposed to artificial loads of pollutants. It is found that the forming of the forest ecosystem from individual trees causes stress situations in trees. It is supposed that a certain typical deflection in cell metabolism under stress reveals itself through considerable changes in the composition of proteins and phytohormones, namely b-indolil acetic acid (IAA). Differences were determined both in proteins composition and their functional activity at various stages of spruce adaptation to the new environment. This has been accomplished by defining the composition of the quality and quantity of proteins, the permeability of cell membranes, the concentration of proline in needles of trees of various growth and development - classes A,B,C according to the tree classification (Kairiūkštis 1969) and by applying radioactive signs and phytohormones IAA (Skuodienė, Darginavičienė 1981).

In our experiments on tree at different stages of its growth and development as well as in coenosis formation and artificially pollution we raise the following tasks:

a) to trace the deviation from the standard quantity of simple and functionally significant proteins;

b) to restore the function of tree growth in different phases of the growth and development with the aid of radioactive substances of protein synthesis and applying artificial introduction of IAA;

c) to find out the acceptable physiological methods for early assessment of forest decline.

**Keywords:** Norway spruce, growth, cell metabolism, composition of proteins, radioactive substances.

## Introduction and methods

For investigation the buds and shoots of trees (*Picea abies (L.) Karst, Populus tremula L., Quercus robur L., Betula pendula L., Pinus sylvestris L.*) of various classes of development (Kairiūkštis 1969) have been used. Trees differed in length and intensity of the growth, they possessed different morphological and physiological indications, their ability to use solar energy in the process of plant growth was different, their biological production was also different. In some experimental plots the trees have been artificially exposed to different lighting conditions as well as affected by industrial emission. The distance of trees from the source of contamination (1-12 km) was taken into consideration. The source of contamination selected was a factory of nitric fertilizers, the emissions of which mainly consist of: sulfurous anhydride, nitric oxides, fluorine compounds, ammonium as well as formaldehyde, methanol, carbomide dust, nitrophosphate and amonium nitrate. The trees of the same species growing under normal conditions of background air pollution (control stands) were also considered.

The composition of proteins was determined after Safonov and Safonova (Safonov 1969), and the quantity of proteins after Lawry O.H. et. all. (Lawry 1951).

Radioactive adenin $8^{-14}C$ and glycine $8^{-14}C$ processing was carried through basal shoot ends using radioisotopic water solution, the concentration being 4 mg/ml. The processing lasted for 17 hours. Shoot processing by $IAA^{14}C$ was carried through donor blocks, the concentration of IAA $10^{-14}M$ was considered to be optimal for spruce. Changes in the composition of proteins were tested by a set of operations conducted according to the techniques mentioned above (Safonov 1969, Lawry 1951, Merkys 1973).

The early response of trees to the environmental changes was estimated according to $K^+$ions transport via cellular membranes by using our elaborated methods (Skuodienė 1988). The physiological function of $K^+$ions transporting via cell membranes was determined with the help of ionometer, applying highly sensitive electrodes. The quantity of $K^+$ was calculated according to the standard curves for KCl, the concentration of which from $10^{-1}M$ to $10^{-6}M$. The optimum magnitude, the volume of the ionize and duration of measurements were determined experimentally taking into account electrode sensitivity (from $10^{-1}$ M to $10^{-6}M$).

A method of L.S.Butes and all (Butes 1973) for proline isolation was tested. It was ascertained that proline better "summed" up the effect of anthropogenic factors on trees. Its quantity did not change rapidly in needles of trees aged two, three years. In our conditions early spring and late autumn was the best time for analysing needles. Samples have been taken from one third of the upper crown of trees aged 30-35 years.

## Studies on physiological characteristics of spruce during the process of ecosystems formation

It is common knowledge that the forming of forest biocoenosis is a complex process. It occurs due to the influence of external factors and the interaction between individuals of different geno and phenotypical peculiarities to one or another extent. Both groups of factors making common cause – result in stress responses of trees frequently before closing of crowns (Kairiūkštis 1975). The caused responses of stress and adaptation directly correlate with physiologo-biochemical changes and differ in character and extent of deviation from the standard. The process of adaptation to stressors depends upon their strenght, the age of tree and the level of generative readiness of individual tree to enter the coenotic interaction. The situation of stresses started before crown closing and the process of adaptation to them followed by crown closing determines the intensity of tree differentiation in the growth and development classes and even death of separate individuals in case absolute disorder of metabolic reactions occurs. The individuals already being at various stages of differentiation ranks in coenosis subsequently react to stresses differently.

In adaptation process of spruce in coenosis light is of extraordinary importance. As a result of artificial creation of different conditions of lighting (5%, 30%, 70% and full daylight) for spruce of 9 years of age we traced the differentiation of trees according to the growth in the period of several years. We succeeded in elucidating the correlation between the conditions of lighting and changes in one of the main phytohormone of growth - IAA in a free form and combined with proteins (Kairiūkštis, Skuodienė 1973).

According to their functional significance the proteins are the basis of the vital processes of a plant cell. There are different kinds of proteins. The most important structural elements of a cell are simple proteins. Complex structural albumins comprise a separate group. Structural proteins contain nuclein acids RNA and DNA which provide storage and transmission of the genetic information and immediately take part in the biosynthesis of proteins.

Currently, in studying the process of plant growth a special emphasis is laced on the correlation between the phytohormone IAA on the one hand and a synthesis of nuclein acids and proteins on the other (Merkys et. all, 1975; Udovenko, 1979). It is supposed that the effect of IAA on the growth is realized through nuclein metabolism. Besides, the inhibition of protein synthesis is a major indication of discoordination of metabolic reactions under the impact of stress which leads to the losses of tree increment (Ilkun 1978).

The results of the experiments on proteins in trees at different stages of adaptation to coenosis indicated that high concentration of structural proteins is typical of the period of intensive shoot growth. At that time on shoots of trees we found 1.5 times more structural proteins than during their forced rest in early spring and twice more as compared with their quantity at the beginning of a complete rest. The same tendency was determined for weakly developed and suppressed trees (Cl. – B and C). The obtained data revealed that during the process of spruce growth structural proteins play an important role.

A certain correlation was elucidated between the content and composition of proteins and between the state-of-art of trees in coenosis. Buds and shoots of the suppressed trees of class C, which were under the impact of stress for a long time, differed by the least quantity of simple and structural proteins per one gram of dry substance investigated. Approximately only 1.860 mg/g of simple proteins were found in buds and shoots of trees of class C or 2.2 and 1.5 times less than in these well (Cl. - A) and weakly developed trees (Cl. - B). Structural proteins also amounted to 13.379 mg/g or 1.2 and 1.4 times less than in shoot of trees of classes A and B. Analogical correlation between the simple and structural proteins was noted during the whole vegetation period of these trees. The mean annual height increment of trees of class C was 6.4 cm while that of classes B and A 14.2 cm and 50.4 cm respectively.

Certain peculiarities of protein metabolism have been observed in weakly developed trees. These trees according to

the quantity of simple proteins during the whole vegetation period as well as according to the height increment are in the intermediate state between well developed and suppressed trees. However, according to the quantity of structural proteins (18.923 mg/g) weakly developed trees exceed the well developed and suppressed ones 1.3 and 1.4 times, respectively. Bearing in mind the functional significance of structural proteins it is feasible to suppose that trees at the given stage of adaptation (Cl. B) have a great potential possibility of the growth if the stressor is eliminated. This was proved by subsequental measurement of increment.

A radioactive adenin – $8^{-14}C$ as a part of molecules RNA and DNA and glycine – $8^{-14}C$ as amino acid which is found in the molecules of protein have been used. They permitted us to determine the effect of IAA on the level and speed of protein metabolism and on the intensity of shoot growth.

By artificial introduction of IAA together with radioactive adenin and glycine we attempted to diminish the stress impact in the process of spruce adaptation and found out that IAA inclusion enhanced the joining of glycine in the total quantity of proteins. The very tendency is characteristic of all classes of trees (A, B and C). However, shoots of trees of class B possess the highest activity of glycine joining. The joining of glycine amino acid into proteins of shoots of trees of class B is fourfold in comparison with the data without IAA. The joining of glycin in shoots of trees of class A was only 1.8 times more. Injection of radioactive glycine into spruce shoots of class C under the impact of IAA was of minor significance.

Shoot processing by adenin gave analogical results. It was found that the activity of adenin introduced into nuclein acids RNA and DNA under the impact of phytohormone IAA (in shoots of class A) increased 2.3 times while in class B - 3.7 times. The greatest effect of the growth was observed namely in the given phase of adaptation due to the increase in protein metabolism and the activity of nuclein acids influenced by phytohormones IAA. The height increment of trees of class B under the influence of IAA augmented 4.3 times, as compared with the control (Skuodienė, Darginavičienė 1981).

The results of our experiments have indicated that the interaction between trees entering coenosis create a stress situation. It influences the reactions of protein metabolism including high-molecular proteins, as well as nuclein acids and IAA biosynthesis. In protein metabolism the deviation from the standard depends on the extent of tension and on the duration of the impact of a stressor. It reflects in the subsequent process of adaptation and differention of trees in classes of the growth and development. IAA in the given process is an agent which reduces the stress and enhances the growth of trees. The findings show that the light favours IAA biosynthesis in plants. The optimum thinning of stands may compensate for the lack of IAA in tissues of forest biocoenoses.

## Assessment of $K^+$ion transport via membranes as an initial signal response of trees to changes in a physical and chemical environment

The cell membranes perform their function by passive and active transport of substances. Active transport of substances is a more intricate process since it requires a biological energy source within the cells. The source of energy which catalyses the substance transport against the concentration gradient is a hydrolysis of the molecules of adenosinetriphosphate (ATP). However, for a continuous synthesis of ATP molecules, $K^+$ions are needed. According to the data gained in laboratory experience, the cells of plants lose potassium when toxic substances are added in the nutrition environment. This also holds true for the conditions of air pollution by chemical substances (Skuodienė 1984). With an increase in $K^+$ions transport through membranes of cells, the interrelationship between the biological processes requiring intensive metabolism is noted during the growth of pollen tubes in the early phases of seed germination. Owing to potassium losses the potential energy of substance transport decreases. Consequently, the organism is provided with less micro-and macro elements. Such a disorder in the energy balance of cells results in the delay of photosynthesis, breathing, protein metabolism and other functions of immense importance for the production process. Thus, the method based on the biophysical and physiological state-of-art of trees determined by measurement of the quantity of $K^+$ions transported via cell membranes was worked out and suggested for early assessment of tree reaction to environmental changes under the impact of stressors.

The initial-signal responses of trees to the changed situation in the environment are of importance in studying a tree as a system accepting the impact of ecological factors. M.I.Jaffre (Jaffre 1969) already revealed that the effect of indolyacetic acid (IAA) on the permeability of membranes occurs earlier than on the process of growth, i.e., changes in membrane permeability is the first unit of the initiating mechanism. A close correlation between the dynamics of root secretion depending on the permeability of cellular membranes and the dynamics of outbreaks in the sun was found by L.N.Dubrov (Dubrov 1973). Before that V.I.Zhalkovich and I.A.Shidlovskaja (Zhalkovich 1971) found that due to the impact of unfavourable environmental factors, the permeability of membranes of mitochondrium and chloroplasts are disturbed. This results in a variation of energy change in plants.

Our investigations of a tree as a system capable of indicating both: physical (while the trees are entering coenosis) and chemical (while trees are exposed to industrial pollutants) environmental changes have demonstrated that $K^+$ions transported via cellular membranes perfectly reflected these changes. Due to a stronger stressor the content of $K^+$ions penetrated via membranes ranged from 1.2 to 4.3 time as compared to the control.

It has been also ascertained that tree reaction to environmental changes (light, temperature, pollutants) depends on the initial state-of-art of the tree and on the rate of environmental changes themselves: the stronger the environmental changes and the weaker the initial state-of-art of the tree (according to development classes), the greater impact on the tree is observed. This situation perfectly indicated the function of $K^+$ions transport via cellular membranes. It is initial and the most sensitive reaction signals of the tree to environmental changes.

Insignificant fluctuations of permeability of cellular membranes to $K^+$ions is typical of trees growing under comparatively normal conditions. If trees are submitted to stresses as it occurs in the process of natural coenosis formation or in case of atmospheric pollution $K^+$ion transport immediately increases. The proximity of root systems, for example, and areal interaction between trees before crown closure already creates a stress situation for trees and increases $K^+$ions penetration by 2-4 times. The given situation appears by far prior to crown closure and remarkable decrease in lighting for individual trees.

Let us consider $K^+$ion penetration via cell membranes under the impact of chemical environmental changes, for example, environmental conditions. The well (Cl.A) and weakly (Cl.B) developed trees sensitively respond to stressors of physical and chemical nature. It confirms what Udovenko (Udovenko 1976) already pointed out: membrane permeability, bioelectric potentials and DNA structure are extremely sensitive reactions, rapidly responding to changes in factors of the environment.

The findings of the experiment and their theoretical basis as well as the data inferred from the literature allow one to conclude that the function $K^+$ion penetration via cellular membranes is a sensitive index of an environmental change. It can reflect the state-of-art of trees being stressed and their adaptation.

## Studies on proline in coniferous trees for early indication of forest decline

In search of the most stabile physiological markers of forest decline amino-acid proline in the needles of spruce and pine have been investigated. Artificial load of pollutants in stacionary and monitoring plots in Lithuania have been used.

It has been ascertained, that the activity of the synthesis of proline depends upon the concentration of chemical substances in the environment. In case the chemical load is the same level over a certain period (6 years have been artificial polluted trees) the synthesis of proline in the needles becomes more intensive already in the first year after the appearance of chemical substances in the environment. Maximally intensive synthesis is observed in the second year. At that time in the needles of conifers having the most significant chemical load the quantity of proline was 4 times larger (7.9 mg/100 gr f.m.) as compared to the control. Due to chemical effect the variation in the quantity of proline is typical of the stress adaptation reactions of biological systems. As it was proved above the same holds for $K^+$ion transport via cellural membranes under the influences of a physical stressor. Decrease in the intensity of proline synthesis later under a permanent chemical stressor may be associated with adaptation of an individual to new conditions and with the onset of its degradation.

Certain differences have been ascertained between the quantity of proline in needles and crown defoliation. In the needles of pine and spruces of moderate defoliation (25-60%) the quantity of proline comprised 30% more, as compared to that in the needles of conifers of slight defoliation (10-25%). The amount of proline in needles of the last and second years on average in monitoring plots has been determined for pine $2.68\pm0.15$; $3.21\pm0.17$ and for spruce $2.57\pm0.12$ and $2.70\pm0.12$ respectively.

The results of investigation enable us to state that the quantity of proline in needles of coniferous trees can characterized the state-of-art of a tree and most probably can be used for early indication of forest decline.

## Conclusions

1. The results of the longterm investigations on the trees entering coenosis as well as the trees artificially exposed to environment pollution enable us to recognize stress reactions at different levels of an organism over a long and intricate period of the growth and development of trees.

2. The situation of stress in trees started before crown closing and the process of adaptation to it followed after crown closing. A certain correlation was elucidated between the content and composition of proteins and between the state-of-art of trees being stressed: the deviation in protein metabolism from the standard depends on the extent of tention and on the duration of the impact of a stressor.

3. The function of $K^+$ions transport via cellular membranes in needles is a sensative index and it perfectly reflected environmental changes: due to a stronger stressor the content of $K^+$ions penetrated via membranes ranged from 1.2 to 4.3 time.

4. The synthesis of proline in needles depends upon the chemical load in the environment: in the needles affected considerable by chemical pollutants the quantity of proline was 4 times larger as compared to the control.

5. The investigations may be useful theoretically in supplementing the biological characteristics of trees in terms of stress whereas practically in observing forest decline in early stages, before remarkable defoliation occures.

At the same time the investigations clearly show how subtle the process of degradation of trees in process of forest decline is and how closely it is associated with the laws of forest coenosis forming and traits of anthropogenic-chemical factors. The presented findings do not concern wider dependences of the

process of degradation of an individual on the genetic peculiarities of an individual. However, as a rule, some genetic properties as well as diseases and insects accelerate degradation. The investigations have also demonstrated that only few physiological methods are applicable for indication of forest decline particulary in regional monitoring. For example, in case the stressor is weak or short lasting the physiological functions are reconstructed soon.

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# Физиологическая оценка стрессовой реакции у деревьев

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*Резюме*

В статье рассматриваются результаты многолетних исследований физиологических показателей у ели во время образования экосистемы (биоценоза), а также процесс деградации деревьев хвойных пород под воздействием атмосферного загрязнения и искуственного дождевания с определенными концентрациями загрязнителей. Установлено, что стрессовое состояние деревья испытывают до смыкания крон при образовании ценоза и их адаптации продолжается после смыкания крон. Функция выхода $K^+$ ионов через клеточные мембраны является чувствительным признаком стрессового состояния деревьев, тогда как более стабильным показателем является синтез и накопление пролина в хвое деревьев. По этим показателем оценено состояние деревьев в различных условиях загрязнения воздуха на площадях регионального мониторинга лесов.

**Ключевые слова:** ель обыкновенная, рост, метаболизм клеток, композиция белков, радиоактивные субстанции.