Influence of UV-B radiation on Norway spruce seedlings (*Picea abies* (L.) Karst.)

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On the basis of the hypothesis that the ultraviolet radiation is one of the main causes for damage at higher altitudes, we have monitored the effect of UV radiation on Norway spruce for two and a half years. The influence of UV-B radiation on Picea abies (L.) Karst. seedlings cultured in pots in open greenhouses was examined by measuring photochemical efficiency of photosystem II, changes in chlorophyll a, b, and changes in anthocyanins. The seedlings were grown in a mixture of peat and vermiculite (4:1). We used Osram ultravitaluks bulbs as a source of UV-B radiation. In the experiment plants were treated with 21.24 ± 3,5 kJ/m^2 and 31.9 ± 2,5 kJ/m^2. The control plants were grown under ambient conditions in the greenhouse without artificial source of UV-B radiation. The mean yearly values were as high as 11,5 ± 5,2 kJ/m^2. The photochemical efficiency of photosystem II (PS II) in experimental plants did not vary during the experiment. It showed obvious decrease in the winter period, due to low temperatures and physical draught. The decrease in chlorophyll a and b, was already detected after one year of treatment with simultaneous changes in a/b ratio. An increase of anthocyanins amount was detected as well.

Key words: trees-radiation effects; ultraviolet rays; seeds growth and development; chlorophyll; anthocyanins

Introduction

UV-B radiation is a short wave length, non ionizing radiation (less than 400 nm) which represents about 7% of total solar radiation. It consists of UV-A (320-400 nm), UV-B (280-320 nm) and UV-C (below 280 nm). UV-C radiation is very harmful to organisms, but it is filtered in the ozonosphere.[1] UV-B radiation represents only 0.3% of the radiation reaching the earth surface, but it is still on the increase.[2] The effect is harmful, because UV-B radiation is absorbed by macromolecules, as are also proteins and nucleic acids.[3]

Numerous researches showed that the effect of UV-B radiation under experimental or natural conditions was species specific[1,3] and that it exerted different changes. In *Phaseolus vulgaris* the changes in chlorophyll were observed.[4] In one year seedlings of *Pinus taeda* L., the decrease of photosynthesis was established at the beginning, but later on it reached the normal level. Simultaneously, the formation of total UV-B absorbing sub-

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stances were detected.[5] The increase of UV-B radiation in *P. taeda* caused a significant decrease in photochemical efficiency of PS II.[6]

Pinus banksiana Lamb., *Picea mariana* Mill. B.S.P. and *Picea glauca* Moench showed higher production of total UV-B absorbing substances when treated with an increased level of UV-B radiation.[7] *Pinus pinea* L. and *Pinus halepensis* Mill. exhibited slight changes in variable and maximal fluorescence ratio (Fv/Fm) in one-year treatment under experimental conditions.[8]

The aim of our research was to estimate the effect of UV-B radiation on photochemical efficiency of PS II, chlorophyll *a* and *b* and anthocyanins contents in the seedlings of *Picea abies* (L.) Karst.

Materials and methods

Seeds of Norway spruce were sown in clay pots in a peat-vermiculite mixture (4:1). The greenhouses were opened at both sides to assure air circulation. The seedlings were irradiated for 8 hours a day with different quantities of UV-B radiation. The source of UV-B radiation was 300W Osram Ultra-Vitalux bulbs with a spectrum similar to sunlight in mountainous areas (Technical documentation, OSRAM). The level of radiation was measured by means of UV-B sensor (peak sensitivity 313 ± 2 nm, bandwidth - full width at half maximum 26 ± 1 nm, Delta T Devices). Over 8-hour period the measured values were as follows: 11,7 ± 5,2 kJm^{-2} (control), 21,24 ± kJm^{-2} (experimental level I) and 31,9 ± 2,5kJm^{-2} (experimental level II). The environmental conditions in the greenhouses were similar to those outside. We have monitored the effect of UV radiation on Norway Spruce for two and a half years.

In vivo chlorophyll fluorescence was measured with a PSM fluorometer (Plant Stress Meter, Biomonitor, Sweden). Before the measurements, plants were kept in the dark for 30 min. Measurements were performed once or twice a month from December 1992 to October 1994. Fast (5 seconds) kinetics was measured.

The photochemical efficiency of photosystem II can be estimated by the F_v/F_m ratio, where F_m stands for peak or maximum fluorescence and F_v is variable fluorescence (peak level minus initial level, $F_m - F_o$).[9] Excitation energy harvested by the photosystem II antennae is transferred and utilised by photosystem II reaction centres for photochemistry of photosystem II.

Every two months chlorophyll contents were determined in one-year old needles. The chlorophyll was extracted from fresh material with 100% acetone.[10] The chlorophyll content was expressed in mg per unit of dry weight of needles.

Relative contents of the anthocyanins were extracted in one-year old needles using 1% HCl in methanol.[11] The samples were being shaken for two days in the shaker at a temperature of 2-5 °C in the dark.

The significant differences between control and treated plants were tested with Student's t-test.

Results

Measurements of fluorescence kinetics showed no differences among the groups after two and a half years of treatment (Figure 1). The decrease of photochemical activity due to physical draught in the winter period which had already been observed in the first year of measurements was detected the following year as well.

Figures 2 and 3 present the results of chlorophyll content analyses in the monitored period. The differences among the three groups of plants indicated an obvious influence of UV-B radiation. In the first year the amount of chlorophyll *a+b* decreased in

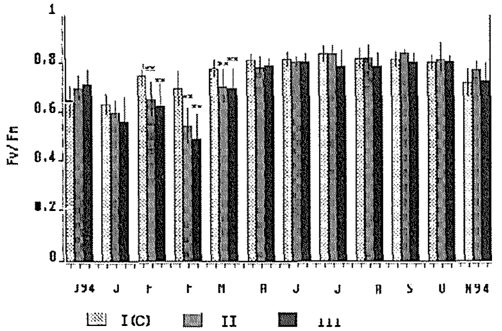

Figure 1. Variable versus maximal fluorescence (relative units) determined in spruce seedlings in the course of time. Values are the average of measurements on ten samples; vertical bars represent standard error; * P≤ 0.05, ** P≤ 0.01, without asterisk- not significant).

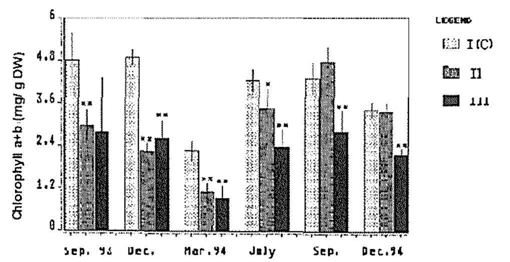

Figure 2. The amount of chlorophyll a+b (mg g^{-1}DW) in the needles of spruce seedlings. Values are the average of 5 samples (SE means standard error; * P≤ 0.05, ** P≤ 0.01, without asterisk - not significant).

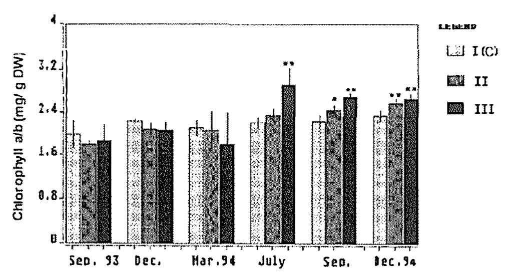

Figure 3. The chlorophyll a/b ratio in the needles of spruce seedlings. Values are the average of 5 samples (SE means standard error; * P≤ 0.05, ** P≤ 0.01, without asterisk - not significant).

treated plants in comparison to the control ones, but on the other hand we observed an increase in chlorophyll *a/b* ratio. In the second year the differences were even more evident and they showed similar trend during the whole period.

Relative anthocyanins contents in the needles (Figure 4) measured at the end of the experiment increased only in the plants treated with the highest UV-B radiation treatment. The other two groups showed negative values. This is not the indication that the anthocyanins are not present in the other two groups, but that the product of the chlorophyll decay prevailed in comparison with the anthocyanins.

Discussion

Our measurements of fluorescence kinetics are not in agreement with previous researches of Shawna[6] who observed a decrease of F_v/F_m ratio in *Pinus taeda*. In the same investigation no effect on photosynthesis were observed, what was also the conclusion of the investigations of Sullivan[5] and our previous researches.[12] In the case of additional stress exerted by drouhgt the significant changes of Fv/Fm in *Pinus pinea* and *Pinus halepensis* were detected.[8] The same pattern was observed during our winter measurements, when Fv/Fm showed the decrease in control and in treated plants as well. This decrease seems to be the consequence of the

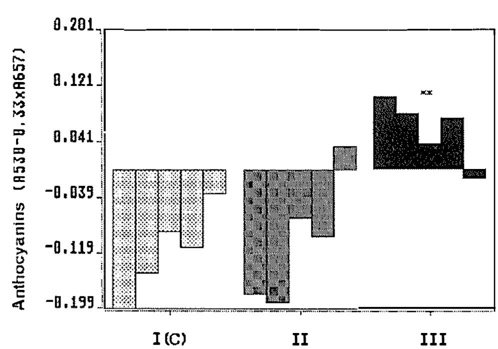

Figure 4. The variability of anthocyanins among five spruce seedlings for each treatment. (* P≤ 0.05, ** P≤ 0.01, without asterisk - not significant).

physical draught due to freezing of the substrate in the pots.

The increase of chlorophyll a/b ratio in the second year corresponds to the results of an earlier research[4] which investigated the influence of UV-B radiation on beans. In the same plant the decrease of total chlorophyll content was observed.[13] Significantly lower chlorophyll *a+b* were obtained in our experiment already in the first year[12] and, the following year, these differences were even more obvious. This is surprising since several authors who investigated other conifer species reported no influence on chlorophyll content.[5,8]

The reason for the lack of differences in photochemical efficiency of PS II between treated and control plants could be due to higher contents of anthocyanins, which offer the protection against enhanced UV-B radiation.[1,5] Different researchers measured higher amounts of UV-B absorbing substances in treated plants[14,5,7] what was also the case in our experiment. In *Pinus taeda* seedlings these substances were formed after six weeks of treatment, what could be the reason for an unchanged level of photosynthesis.[5] Higher level of UV-B absorbing substances were detected in *Abies lasiocarpa* and *Picea engelmanii* under UV-B treatment.[15] Low sensitivity to UV-B radiation is in close relation with the penetration of this part of light spectrum into the leaf. This is more expressed in conifers which are better protected with UV-B absorbing substances in comparison to herbal plants,[16,17] what could also be the reason for differences in photosynthesis. Only a small quantity of UV-B radiation was proved to penetrate in the mesophyll tissue of newly expanding leaves of two subalpine conifer species.[18] All the measurements on conifers including ours, showed great plasticity in response to UV-B radiation.[12,15,18]

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