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The Influence of UV-B Irradiation on the Mitotic Activity in *Picea abies* (L.) Karst.

By

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Summary

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The influence of an artificial source of UV-B irradiation of 21.2 ± 3.4 kJ/m^2 and 32 ± 2.5 kJ/m^2 on Norway spruce (*Picea abies* (L.) Karst.) seedlings has been under observation for two and a half years. The controls were irradiated with normal 11.7 ± 5.2 kJ/m^2. The results show that 3 year old seedlings are sensitive to irradiation, as can be first seen from their decreased mitotic activity and lesser vitality.

Introduction

The stratosphere is particularly important with regard to the amount of UV irradiation reaching the Earth, or more particularly its lower lying ozonosphere, with highest ozone concentrations.

Due to damaging human activities, i.e. production of substances causing destruction of the O_3 molecules, the ozone layer has been thinning (BLUMTHALER & AMBACH 1990). UV irradiation represents 7% of solar radiation (CALDWELL 1981), is of short wavelength, nonionizing, it can be divided into 3 groups, UV-C (less than 280 nm wavelength), UV-B (280-320 nm) and UV-A (320-390 nm). While extremely harmful to organisms, UV-C does not reach the Earth's surface. UV-B induces specific though not necessarily noxious damage to organisms, and UV-A, which is the least harmful of the three (WELLMAN 1983).

UV-B irradiation is increasing due to thinning of the ozone layer (BLUMTHALER & AMBACH 1990). As it is absorbed by some biologically important molecules, such as proteins and nucleic acid, its effect is all the more important (CALDWELL 1981).

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To mention only conifers, tests on different species show different sensitivities to UV-B irradiation. Some noted visual changes such as yellowing and bronzing of the needles, decreased and slower growth of the seedlings and their roots, changes in dry weights with a reduction of the biomass of individual species, while others showed no changes at all. (SULLIVAN & TERAMURA 1988, 1994).

In our test, Norway spruce (*Picea abies* (L.) Karst.) seedlings were exposed for two and half years with artificial source of UV light to determine whether the spruce was sensitive to UV irradiation or not.

Material and Methods

The object of our experiments were 3 year old Norway spruce (*Picea abies* (L.) Karst.) seedlings, all from the same locality near greater Mozirje, at an altitude of 1250-1300 m. The seeds were sown in clay pots with a diameter of 30 cm into a mixture of peat and vermiculite (4:1). They were placed into open green houses to eliminate rain pollutants. 100 ml of Knopp's solution was added once a week to each pot, otherwise they were watered as needed with tap water. The first greenhouse with no artificial source of irradiation represented the control; the second contained 2 Osram ultravitalux 300 W elongated light bulbs; in the third were 4 light bulbs of the same strength. The bulbs gave a light similar to that found at higher altitudes. They were turned on every day from 9 AM to 5 PM regardlees of the sun. Four months after planting, irradiation of the plants was started in August, 1992. The plants were placed 0.5 m from the light source. Measurements with UV-B sensor (Delta T devices) showed that in 8 hours the controls received 11.7 ± 5.2 kJ/m^2 (treatment I). The greenhouse with 2 light bulbs recived 21.2 ± 3.4 kJ/m^2 (treatment II), and that with 4 bulbs 32 ± 2.5 kJ/m^2 (treatment III). Such ups and downs are due to varying weather conditions (sunny, cloudy) and seasons of the year. The temperature was similar to that outside as the greenhouses were kept open on 2 sides to permit circulation of air. Single visual changes were recorded regularly.

The root apices were first removed for analysis in August, 1993. They were fixed in glacial acetic acid and ethanol in a ratio of 1:3.Until used all materials were kept in a freezer. The roots were prepared for analysis by being hydrolized for 3 minutes at 60°C in 3N HCl, and then stained according to Feulgen. To determine the mitotic activity, they were macerated in acetocarmine, and studied under a Zeis NU2 light microscope. The mitotic index was determined on 1000 cells per each seedling. Five to ten plants were used per treatment. All data obtained were tested with the Student's T test with 8 -- 18 degrees of freedom.

Results

Our results show that in its early growth phase the Norway spruce is sensitive to UV irradiation. After a year of exposing them to UV-B irradiation, i.e. in August 1993, the samples compared to the controls, seemed to show no differences in the mitotic activity. A mild trend was indicated only at excesive treatment, but the difference was not statistically perceivable. In October of the same year, howewer a significant difference in comparison to the control, was recorded at the highest intensity of irradiation (treatment III). Concerning the weaker intensity, the number of samples to be analysed was too small, so no data are stated. Throughout the following year, a characteristically diminished mitotic activity was maintained in comparison to the controls. The sole exception was the first removal in June where at treatment II the difference was not statistically confirmed, though the trends towards decrease was already perfectly clear (Fig. 1).

Beside reduced mitotic activity, the seedlings began showing some other visible changes a brief description of which is given herafter. During the first

winter, in 1992/ 93, the 1 year old seedlings of the 2 treated groups appeared better than the controls. The difference lay particularly in the intensity of their green color;the controls showed paler yellow-green nuances. This was reversed in the spring of 1993 when the controls continually improving, showed greater vitality than the treated plants. In the winter of 1993/94 noticeable chloroses appeared, which then led to necroses in the 2 years old seedlings. The winter of 1994/95 was quite similar to that of 1993/94, only the differences between the controls and the treated plants became even more marked. The controls were dark green, and were of extreme vitality, while the treated plants were yellowish-green, also showing chloroses and necroses of the youngest needles.

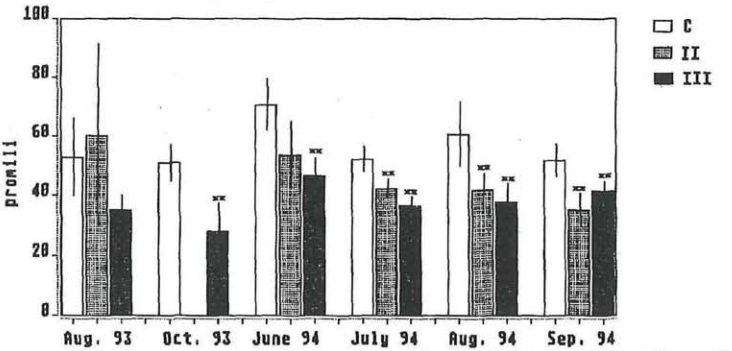

Fig. 1. Mitotic index in different phases of growth after UV-B irradiation. The vertical bar represent ± standard deviation. *.* - indicate significant differences from the controls ($p<0.01$). C - control, II - treatment II, III - treatement III.

Discussion

Our results are similar to those of some other authors dealing with different plants. DICKSON & CALDWELL 1978 state that in *Rumex patientia* UV-B radiation retard cells division. Cell division of cotyledons of cucumber seedlings (*Cucumis sativus*) decreased in relation to UV-B intensity in three weeks. In protoplasts of *Petunia hybrida* a decrease in mitotic activity was noticed after 48 hours (STAXEN & al. 1993). WELLMAN 1983 is of the opinion that slowing of mitoses is a protective plant reaction, as DNA is most sensitive to UV-B during replication. Our results likewise point to a decrease in mitotic activity. The only difference lies in that in our experiment the decrease in cell divisions becomes obvious only after more than a year. This could be put down to the fact that the roots grow in substrate and are not exposed to direct irradiation. A similar decrease in mitotic activity was observed in shoots growing under germicidal lamps but here it appeared very early, before 21 days (BAVCON & al. 1993) This could have been the consecqunce of direct exposure to irradiation, as the shoots were grown on filter paper and thus their roots were exposed.

Our visual observation are similar to those some other authors saw on different evergreens. Seedlings of 10 species, *Picea engelmanni, Pinus contorta, Abies fraseri, Picea glauca, Pinus strobus, Pinus resinosa, Pinus edulis, Pinus taeda, Pinus nigra, Pinus sylvestris* all exposed to UV-B irradiation of 12.4 and 19.1 kJ/m^2 after 22 weeks showed individual changes. Visual changes such as yellowing and bronzing of the

needles were noted in three: *Pinus contorta, Pinus resinosa* and *Pinus taeda* ; others showed no changes (SULLIVAN & TERAMURA 1988, 1994). Similar findings were also reported by FERNBACH & MOHR 1992 in *Pinus sylvestris*. In our experiments, we first noted visual signs, particularly changes in color, during the first winter, but the controls were a paler green-yellow. This could be explained as due to a period of cold winter temperatures when the irradiated plants had a higher day temperature of approximately 1 to 2 degrees.

A similar process of needle yellowing, browning, and then decay was seen in the 2 following winters.These changes are more marked in the winter; during the growth season they are reflected by a lesser number of needles on older shoots and a paler color of needles when compared to the controls. Several leaf abnormalities, such as poor growth and yellow discolorations, have been found in other plants, not only conifers (WELLMAN 1983). SISSON & CALDWELL 1976 state that as the effects of UV-B irradiation are cumulative, plants with long lived needles - such as the evergreens - are particularly at risk.

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