Original Research

Response of Three Conifer Species to Enhanced UV-B Radiation; Consequences for Photosynthesis

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Abstract

Investigations were carried out to find whether enhanced ultraviolet radiation influences the *Taxus, Thuja* and *Juniperus* genera used in garden architecture. Seedlings a few years old were subjected to UV-B at a 16 kJ m^{-2} day^{-1} dose for several weeks. Carbohydrate contents (monosaccharides and sucrose), levels of chlorophylls and chlorophyll *a* fluorescence were analyzed, and the variable/maximal fluorescence (F_v/F_m) and the Rf_{d} vitality index were calculated. In *Taxus* and *Juniperus* no negative effects were found in carbohydrate accumulation and even increased chlorophyll *a* and *b* levels were noted. After 6-9 weeks of irradiation the amount of these pigments in *Juniperus* needles was as many as twofold higher. Under the influence of UV-B a 50% reduction of monosaccharide accumulation was found only in *Thuja,* after 3 weeks irradiation, and an approx. 30% reduction in chlorophylls after 6-9 weeks of irradiation. No changes in the F_v/F_m parameter were found in either species, but the vitality index (Rf_d) under the first weeks of radiation stress showed even an increase in *Thuja* leaves. These results indicate that shoot blight of conifers is not caused by enhanced UV-B radiation.

Keywords: UV-B radiation, carbohydrates, chlorophyll, American arborvitae, common yew, juniper

Abbreviations: rubisco, ribulose bisphosphate carboxylase-oxygenase; PhAR, photosynthetically active radiation; DMSO, dimethyl sulfoxide

Introduction

Due to damage of the stratospheric ozone layer the level of ultraviolet radiation reaching the biosphere, especially in the range of UV-B (280-320 nm), is increasing. Strong absorption of UV-B photons by biologically important macromolecules, *i.e.* proteins and nucleic acids, has a large effect on plant and animal metabolisms [10].

The effects of UV-B on plants include inhibited growth, morphological changes and an increase in the level of phenolic pigments [12, 19, 27]. Inhibition of photosynthesis belongs to the key factors responsible for physiological disorders and a decrease in the biomass of crop plants. The deleterious effect of UV-B on the efficiency of this process can be attributed to specific reductions in expression of important photosynthetic genes [18], a reduction in Rubisco activity [29], changes in ion-permeability of thylakoid membranes [7.12] and in the level of chlorophyll and carotenoids [8, 27]. Simultaneously, many anatomical and morphological changes were observed, such as the reduction of plant height and leaf length/area [6, 23], leaf bronze, glazing, chlorosis or necrotic spots [14].

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There have been well-documented effects of UV-B radiation on crop plants: barley [26], wheat [1, 32, 33] oats (Yuan *et al.* 1999), maize [3, 25], soybean [30] and cotton [9] *e.t.c*. These studies were conducted under field conditions, in greenhouses and closed chambers, with varying sources of light radiation.

Coniferous plants, because of their wide geographic and ecological distribution, have developed special adaptations to environmental conditions [16]. Above all, highly adapted species are important for forestry, including the genera of *Pinus*, *Picea*, *Abies*, and *Pseudotsuga*. Moreover, many conifers with large cultivars and ornamental forms are widely used in park and garden plantings at different biotop conditions and changeable solar radiation. Fully grown needles with glaucous waxy surfaces and thick epidermal cells can be well protected against UV-B exposure. However, it is not known whether young emerging needles during the early spring period of enhanced UV-B radiation are sufficiently tolerant. Moreover, the sensitivity to UV-B of photosynthetically active tissues depends on synergism with other environmental factors, such as PhAR density, temperature and nutrition [28].

This study describes the effect of elevated UV-B radiation on conifers used in garden architecture, *i.e. Taxus*, *Thuja* and *Juniperus* genotypes, which differ in leaf morphology, phytochemical properties and in the adaptation to insolation. The effects of UV-B under the experimental conditions on the levels of carbohydrates and chloroplast pigments and the photochemical quenching coefficient, *i.e.* vitality index (Rf_d), have been studied. The Rf_d index related to chlorophyll fluorescence parameters was important owing to its correlation with potential photosynthetic net CO_2 assimilation [2, 23]. It was hypothesized that the enhanced UV-B radiation under field conditions can affect the shoot blight of conifers.

Material and Methods

Plant Material, Growth Conditions and Experimental Treatments

Experiments were conducted using 3 or 4-year-old seedlings of *Taxus x media 'Hicksii'* – common yew, *Thuja occidentalis* – American arborvitae and *Juniperus communis* – juniper. Plants were grown in 12 or 16-cm pots containing high peat and pine bark ground in a 1:1 (v:v) mixture at pH 4.5-5.5. Experiments were conducted in a growth chamber at day/night 22/18^{0}C temperature and 14/10 h photoperiod at a photosynthetic photon flux density (PPFD) of 120 μmol m^{-2} s^{-1} and irradiation of 10.4 kJ m^{-2} d^{-1} using fluorescence Philips 58 W/84 sun lamps. UV-B was supplied by TL 20W/01 RS Philips lamps at 185 μmol m^{-2} s^{-1} and 16 kJ m^{-2} d^{-1} irradiation at the canopy level, with max. transmission of 315 nm. The PhAR radiation was measured by FF-01 Sonopan (Poland) quantum sensor and UV-B with a VLX 3W radiometer.

Shoot sections were taken 1, 2, 3, 6 and 9 weeks after UV-B irradiation. Samples of 100 or 200 mg were frozen in liquid nitrogen and stored until use.

Carbohydrate Analysis

Frozen samples were ground in 80% ethanol and extracted in closed teflon tubes at 80°C for 1 h. After cooling the extracts were centrifuged (10 min 12,000 *g*) and evaporated in a speed vacuum concentrator (Heto Lab Equipment A/S, Denmark). The residues were resuspended in 0.1 mM CaEDTA. The HPLC analysis was performed by Waters Alliance with a Sugar Pack I column [22]. Parameters concerning temperature and the mobile phase flow were used according to Waters' protocol, whereas quantitative analysis was based on standard curves.

Chlorophyll Determination

Pigments were extracted according to Hiscox and Israelstam [11]. One hundred milligrams of leaf tissues were placed in vials containing 5 ml DMSO (Sigma Chemicals Ltd., Germany) and left for about 24 hours. The liquids were used for OD reading at 645 and 663 nm with a spectrophotometer. Chlorophyll contents were calculated following the equation used by Arnon's method and expressed in mg g^{-1} fresh leaf weight.

Fluorescence Measurements

Intact leaf fluorescence was measured using a Plant Efficiency Analyser (PEA), Hansatech Ltd., King's Lynn, Norfolk, UK. Measurements were carried out in the morning, at constant temperature of 22°C ± 1°C after 30 minute dark adaptation, at exposure to a 1340 μmol m^{-2} s^{-1} flash of actinic red light at λ_{max}=650 nm. The following parameters were determined: F_o (initial fluorescence; all reaction centers of PS II open), F_m (maximum fluorescence; all reaction centers of PS II closed), F_v (variable fluorescence), F_s (steady state chlorophyll fluorescence). The $(F_m – F_o)/F_m$ ratio, also termed variable/maximal fluorescence (F_v/F_m) and Rf_d: $(F_m–F_s)/F_s$ as the vitality index or rate of fluorescence decrease were calculated [17, 23].

Statistical Analysis

Experimental data were subjected to a one-way analysis of variance (ANOVA) and significant differences between means were determined through the use of Tukey multiple range test.

Results

Carbohydrate Contents

Taxus x media showed the highest constitutive levels of accumulated carbohydrates among the investigated species (Fig. 1). Fructose contents were 17-18 mg g^{-1} fw and exceeded those of glucose (10-12 mg g^{-1} fw) and sucrose (12-13 mg g^{-1} fw). In the *Th. occidentalis* leaves glucose content fell within the range of 3-5 mg g^{-1} fw and was much lower than those of fructose (8-12 mg) and sucrose (9-10 mg). In *J. communis* the level of all investigated carbohydrates were similar, *i.e.* 5-10 mg g^{-1}fw.

Under the influence of UV-B radiation a 50% reduction in monosaccharide accumulation was found only in *Th. occidentalis,* after 3 weeks irradiation. In *Taxus* needles after 9 weeks of irradiation even some increase in fructose and glucose was noted.

Chlorophyll Changes

Similarly to carbohydrates, *Taxus x media* showed the highest constitutive levels of the chloroplast pigments (Fig. 2) and the UV-B radiation caused an increase of chlorophyll *a* and *b* over the first two weeks of irradiation. On the contrary, in *Th. occidentalis* at first weeks of irradiation the level of pigments were stable, while after 6-9 weeks an approx. 30% reduction was observed. Elevated UV-B level in *J. communis* stimulated chlorophyll synthesis, to a limited extent in the first weeks of irradiation and after 9 weeks the level of pigments was 2-fold higher than in the control plants.

Chlorophyll Fluorescence

This parameter was measured for *Taxus x media* and *Th. occidentalis.* In both species no changes in the F_v/F_m

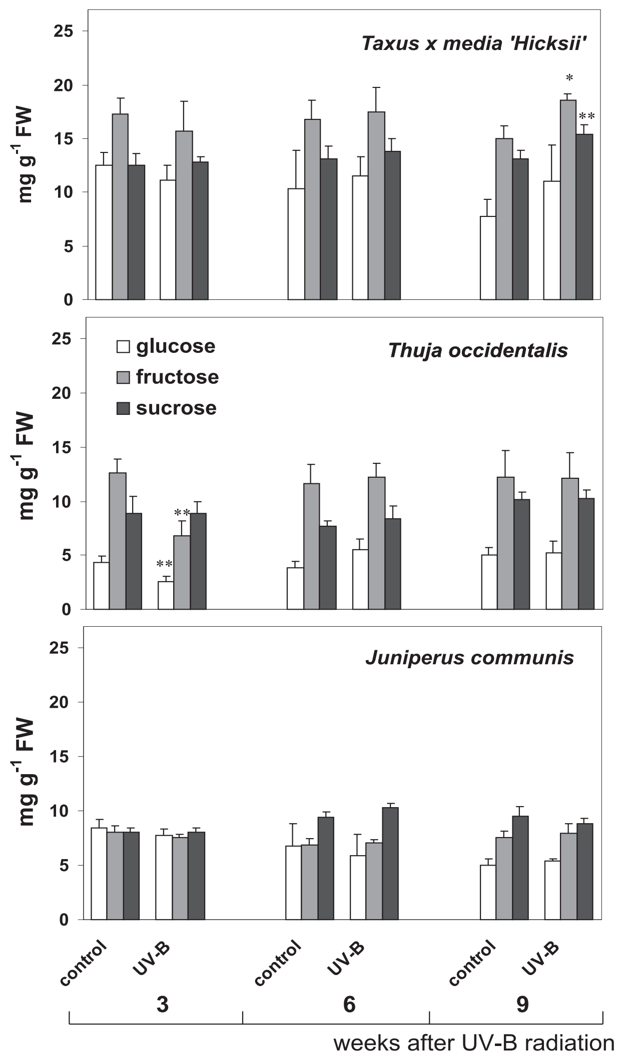

Fig. 1. Effects of UV-B radiation on carbohydrate accumulation in *Taxus x media 'Hicksii'*, *T. occidentalis*, and *J. communis* needles. Mean ± SD. Data significantly different from control: *P< 0.05, ** P< 0.01

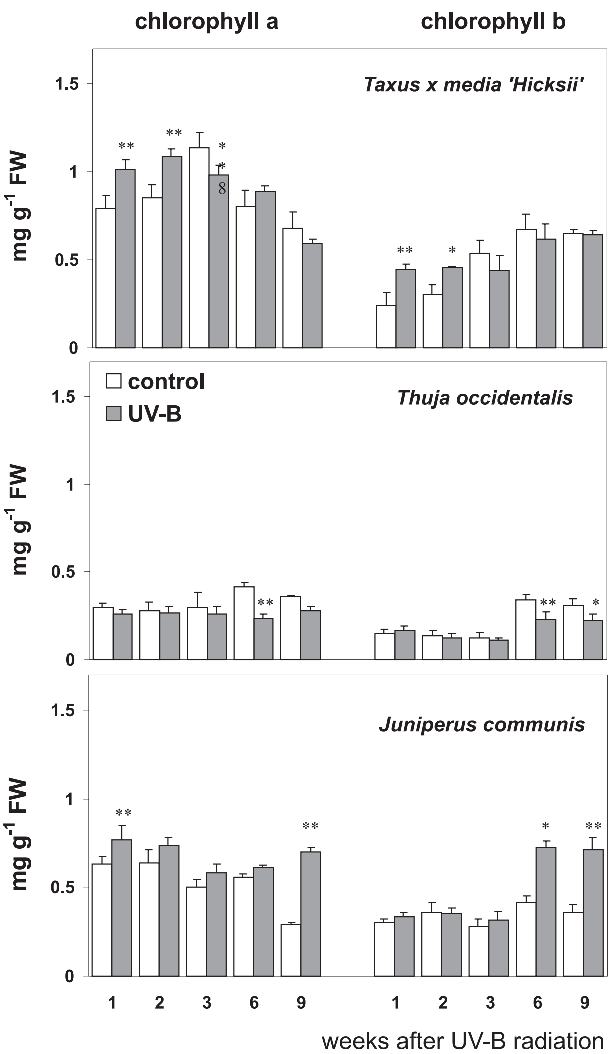

Fig. 2. Effects of UV-B radiation on chlorophyll accumulation in *Taxus x media 'Hicksii'*, *T. occidentalis* and *J. communis* needles. Mean ± SD. Data significantly different from control: *P< 0.05, ** P< 0.01

parameter were found under the influence of elevated UV-B radiation (Fig.3). An increase in the vitality index (Rf_d) was observed in the *Th. occidentalis* leaves under the first weeks of radiation stress.

Discussion

Over the last decades numerous studies have been published on the effect of elevated UV-B radiation on terrestial plants. In a vast majority these studies concern cultivated plants [14, 32, 33], while only a few experiments have involved trees and several conifer species [4, 16, 24, 28].

Adverse environmental factors, including UV-B radiation, may affect metabolic and pathological changes in coniferous plants used in park and garden plantings. Necrobiosis of several-year old ornamental species of *Thuja*, *Taxus* and *Juniperus* is quite frequently observed in Poland. Although the phenomenon might be caused by many factors, the response of these plants to elevated ultraviolet-B radiation has not been investigated.

Based on carbohydrate accumulation essential for plant metabolism, *i.e.* monosaccharides and sucrose, and the estimation of contents and activity of photosynthetic pigments a relatively small effect on the plants was found of radiation at 315 nm. If increased UV-B radiation could have a negative effect on photosynthesis, a decrease in carbohydrate contents was expected. *Th. occidentalis* turned out to be a species in which elevated UV-B lowered in fact the level of reducing sugars, but only during a short period of enhanced irradiation. No changes in sucrose level were observed. However, in *Taxus* x *media* needles not only no disturbances in carbohydrate levels were observed, but even after the 9-week irradiation some increase in fructose and sucrose was noted. In irradiated maize leaves, Barsig and Malz [3] also did not find significant effect of UV-B on sucrose content. Instead they reported a decrease in glucose level. Similar results were obtained by Yue *et al.* [31], who observed the reduction of soluble sugars in *Triticum aestivum* under UV-B influence. In experiments of Newsham *et al.* [21] an increase was found in the content of soluble carbohydrates, *i.e.* arabinose and glucose in *Quercus robur*. Enhanced UV-B radiation did not change the level of soluble sugars in *Juniperus* seedlings, most likely rich in natural screening mechanisms. Thus, similarly to the *Taxus* species, also this coniferous tree exhibits a very high tolerance to ultraviolet radiation.

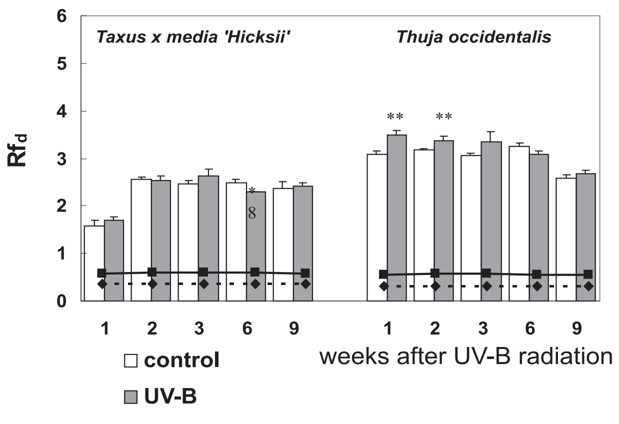

Fig. 3. Effects of UV-B radiation on vitality index (Rf_d) and photochemical efficiency of PS II (F_v/F_m) [◆–◆ control, ■–■ UV-B] of *Taxus x media 'Hicksii'* and *T. occidentalis* needles. Mean ± SD. Data significantly different from control: *P< 0.05, ** P< 0.01

Changes in contents of chloroplast pigments are further evidence of high UV-B tolerance of the analyzed plants. Two species reacted with increased contents of both chlorophyll *a* and *b*. In *Juniperus* needles after 9 weeks of irradiation even two-fold increase was found in the level of chlorophylls. A similar response, but after a short irradiation time, was noted in *Taxus* needles. Such an effect has been previously reported in literature sources, since an increase in chlorophyll following enhanced UV-B was observed in *Bromus catharticus* leaves [6] and in wheat [5]. Chlorophyll synthesis, although dependent on light absorbed by the phytochrome, may probably be a result of the co-action between UV-B and phytochrome photosensors [20].

Th. occidentalis was the only species with a lowering of chlorophyll content; however, it occurred after a long UV-B radiation, *i.e.* 6–9 weeks. Such changes were generally observed in UV-B sensitive plants. On the other hand, the chlorophyll fluorescence ratio Rf_d, a parameter directly correlated to net photosynthetic CO_2 assimilation, which may limit starch accumulation, was slightly enhanced. No changes were found in the F_v/F_m parameter. These observations indicate high PS II tolerance, including the capacity to restore damage of photochemical apparatus, e.g. in common spruce – a species resistant to radiation stress, a lowering in the vitality index was found [4, 24].

Summing up, the increased UV-B radiation, considerably exceeding the intensity under natural conditions, had inconsiderable effect on the photosynthetic activity of investigated coniferous species. In *Taxus x media* and *J. communis* even a stimulation of chloroplast pigments synthesis was noted. Thus, we confirmed that the conifer needles can be recognized as having a very effective UV-screening potential [13]. Negative effects on *Th. occidentalis* probably were suppressed by increased accumulation of UV-absorbing compounds [15]. So, we can conclude that the enhanced radiation does not contribute to coniferous shoot blight.

Acknowledgements

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