A-5.1 Effects of enhanced UV-B on Forest Ecosystem

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Abstract

UV-B irradiation to young seedlings of Spruce and Beech or other tree spieses caused inhibition of height growth but volume of dry matter. Intensive UV-B irradiation to young seedlings of Spruce and Beech or others caused inhibition of height growth and volume growth of dry matter. Also the growth inhibition was caused in the condition without any UV-B irradiation. Moderate UV-B irradiation caused good results. No information has so far been available of the effect of UVB supplement on tree DNA. This is the first report to describe UVB-induced DNA damage in growing tree leaves under artificial lighting.

Intensities of two peaks in HPLC spectrum were clearly increased in extract of UV-treated Spruce, and their isolations and identifications are under progress now to determine their correlation with a sensitivity to the UV-B. The accumulated secondary metabolites were isolated and identified as kaenpferol-3-glucoside.

Total UV-B irradiance reached to ground was fairly larger at high altitude alpine zone absolutely and relatively. The difference of total UV-B irradiance depended on the direct component.

Key Words : UV-B, Forest Ecosystem, UV-B Stress

1. Introduction.

The depletion of the stratospheric ozone layer is actualized. Especially influences on living organisms are forecast that the influence is exerted on production, growth or the proliferation of the plant by the ozone layer destruction and a large increase of the amount of the ultraviolet B rays,UV-B.

There is an anxiety to the decline of the forest where buffer action to environmental changes is high and the research concerning the forest where many of biomasses are occupied is attached to importance in the influence evaluation to global ecosystems. Moreover, there are possibilities to receive the influence of ultraviolet rays increase more sensitively in the forest ecosystem postponing distributing even to Alpine area that the altitude is high.

It is thought that the examination of the adaptation mechanism etc. it to be necessary to contribute to the evaluation and measures of the tree influence by ultraviolet rays is pressing needs.

In this research, attention was paid to following points. To evaluate the influences on the forest plant, the irradiation experiment was executed to seedlings of some tree species.

2. Evaluation of influence on forest vegetation by enhanced UV-B

The UV-B irradiation experiment was executed to the plant influence investigation of ultraviolet rays. It is thought that the examination of the adaptation mechanism etc. it to be necessary to contribute to the evaluation and measures of the tree influence by ultraviolet rays is pressing needs.

[Examination method]

The UV-B irradiation examination to seedling was done with a ultraviolet rays irradiation device set up in artificial weather room and outdoor.

The influence evaluation by which the reaction of seedlings immediately after germination was done by the result of the irradiation examination for a short term done in artificial weather room.

The long period irradiation examination for seedling was provided and was tried was with a ultraviolet rays irradiation device in outdoor.

Mainly, species of Glehn's spruce Picea glehnii Mast.,White fir Abies mayriana,M.et K. and Seibold's beech Fagus crenata Bl. were treated in these experiments. Artificial UV-B sources were supplied by ultraviolet lamps(F40UVB). Irradiation strength was set from 0.15Wm-2 between 1.21Wm-2 of the strongest processing level in indoor.

The UV-B irradiation examination to seedlings was done with a ultraviolet rays irradiation device set up in outdoor. The processing level where the amount of about 35,000J/m2 expectation and the irradiation of about 2.5-3 times was given was set as the strongest processing with the multiplication natural light UV-B value observed at the summer fine weather of the experimental field.

Result

Influence appearance of seedlings by UV-B irradiation

The remarkable, visible influence appearance on seedlings was admitted in growth and the form. Especially, the form change in the seedling according to UV-B irradiation strength took place soon in spruce after germinating.

The leaves hanged downwards according to the increase of irradiation strength of UV-B while the developing leaves turned to the upper side in the control where ultraviolet rays was not irradiated.

Abnormality of the development leaf was confirmed to the strong irradiation level.

Remarkable visible influence appearance was also admitted in growing and the form of beech seedlings. The influence on leaf area per single leaf because of the form change of the expansion growth and the leaf of the leaf was shown as an example of the influence of the ultraviolet rays irradiation to growth of beech seedlings.

Influences of long-term UV-B irradiation on young trees were shown as differences in relative growth. The relative height growth was suppressed (The contcol level received damage by the pest in summer in beech). UV-B has accelerated diameter growth except White Fir.

3.Effects of UVB supplement on DNA damage in leaves of young trees growing under artificial lighting.

No information has so far been available of the effect of UVB supplement on tree DNA. This is the first report to describe UVB-induced DNA damage in growing tree leaves under artificial lighting.

[Plant materials]

Extraction of DNA and determination of damage

Extraction of DNA was performed in principle according to Doyle and Doyle [1] with slight modification. The damage of DNA was determined by ELISA using the monoclonal antibodies TDM-2 and 6-4M-2 [2], respectively, for cyclobutane pyrimidine dimers (CPD) and pyrimidine (6-4) pyrimidinone photoproduct (6-4PP). ELISA was always run at 6 different dosages of test DNAs and of UVB-irradiated salmon DNA provided from E. Wellmann, Freiburg University (FrDNA) on the same ELISA plate. To applied test DNAs, correspondingly different amounts of non-irradiated salmon DNA were suppl;emented to make the total amounts of DNA in a well to 50 ng and 500 ng for detecting CPD and 6-4PP, respectively. From the absorbances thus-obtained at the different dosages absorbances for 20 ng/well and 200 ng/well for CPD and 6-4PP, respectively, were calculated and expressed in FrDNA unit to give damage indexes. A FrDNA unit represents the amounts of the respective photoproducts contained in one ng FrDNA.

Results and discussion

ELISA which was repeated several times with test DNA extracted from one sample of leaves from each kind of trees. In all species tested except 6-4PP in Kuromatsu some definite amounts of CPD and 6-4PP were detected, and and increased with supplement of UVB (UVB+), while CPD in Touhi did not. In Kuromatsu CPD increased with further increase in intensity of UVB (UVB++). In Buna and Akaezomatsu under intense UVB (UVB++), however, detectable amounts of CPD and 6-4PP decreased sharply below those of the non-UVB-supplemented control. 6-4PP of Kuromatsu also showed a similar, but less marked, decline. In Touhi 6-4PP decreased even under weak UVB (UVB+). The cause of apparent decrease of damage at very intense UVB is not clear.

Kuromatsu was found to have characteristically lower levels of CPD and 6-4PP than the other species tested.

In all species tested the ratios of 6-4PP to CPD were lower under all UVB conditions than those in FrDNA. This may be due to possible conversion of 6-4PP to the Dewar type.

These results may suggest that (1) DNA damage is caused by very weak UVB contained in the visible light from non-UV fluorescent lamps, or is elicited by other cause; (2) supplement of UVB increases both types of DNA damage; (3) further increase of UVB supplement decreases detectable amounts of damage. In UVB-tolerant species like Kuromatsu seems to need more intense UVB for decline of detectiable DNA damage.

4.Identification of Flavonol glycoside induced by UV-B irradiation in woody plants as UV-B stress maraker.

In reacent years, there has been a steady decrease in the levels of stratospheric ozone as a result of industrial activities. Practically significant ozone depletion has been observed over Antarctica and the Southern Ocean as well as Australia. Due to depletion of ozone in the stratosphere, the influx of ultraviolet-B radiation (320-280nm) to the surface of the earth is likely to rise in the future. UV-B radiation cause a multitude of physiological and biochemical changes in organisms.

In plants UV-B exposure has pronounced effects on phtosynthetic; on growth; on crop yields. However, To minimize the damaging effects of UV-B on cellular components such as DNA, RNA or proteins, plants possess developed a variety of UV-B protective mechanisms, including increases in UV-B absorbing pigments, leaf thickening, or increase in photoactivated repair of DNA damage.

Flavonoids have been suggested to play an important biological role in the protecting of plants from harmful effects of UV-B, because flavonoids storongly absorb UV light in the critical range of 280-320nm. As with many species, UV-B radiation enhances concentrations of flavonids in the vacuoles of epidermal cells. On the enzyme level, PAL and CHS are key enzyme and play major roles in light regulation of the phenylpropanoid pathway. It has been reported that in young leaves of several plant species both enzymes are inducible by different light qualities including UV radiation at the mRNA and protein level, respectively.Recent work with mutants defective in phenylpropanoid metabolism provided the importance of these secondary metabolism in prevention of UV-B damage in plants.

Most experiments investigating flavonoids induced by UV-B irradiation have been carried out on cultivated plants, while a few have been performed on trees. This paper reports (a) an identification of UV-B absorbing flavonol glucoside on woody plants in response to UV-B exposure; (b) a strong correlation between the flavonol glucoside content and its degree by UV-B irradiation on forest trees; (c) a suggestion that flavonol glucoside is usefull of a maker as UV-B stress.

Results

Structure of flavonol gycoside as UV-B stress compound.

After two months of UV-B irradiation the aerial parts of the plant were extaracted with 70%MeOH as described in Methods and Materials. Fig. 1 and Fig. 2 shows the absorbance at 320nm of 70%MeOH extracts of control and treated plants on Picea glehnii and Fagus crenata, respectively. A clear increase in the absorbance of irradiated plants is observed at A, B and C. We have isolated A and identified kaempferol-3-O-β–D-glucoside (1). Its strucuture were assigned by comparison with literature.

The UV spectrum of 1 in methanol showed two maxima at 265nm and 350nm. The 1H NMR spectrum indicated a presence of a four aromatic proton signals at δ 6.20 (1H, d, H-6), 6.40 (1H, d, H-8), 6.87 (2H, d, H-3' and H-5'), and 8.04 (2H, d, H-2' and H-6'). The 1H NMR spectrum also supported the presence of a β-glucopyranoside (H-1'' at δ 5.25, d, J=7.5Hz). The 1H-1H COSY experiment enabled the total assignment of the sugar protons. In the HMBC spectrum, a cross peak between H-1'' and C-3 of the agricon . and then 1 was identified according to the standard of kaempferol-3-O-β–D-glucoside.

Induction of kaempfrol-3-O-glucoside in UV-B treated plants.

The amount of kaempfrol-3-O-glucoside in woody plants treated with UV-B are summarized in Fig. 1. This result leads to the conclusion that the production of kaempfrol-3-O-glucoside is dependent on dose of UV-B irradiation.

Physiological effects

The heighest decrease is observed in Abies veitchii (39%) in UV-B++ compared with controls. There is also a significant decrease in Picea jezoensis (28%) and in Pinus thunbergii (19%). However, The height of Pinus densiflora and Picea jezoensis have been not changed values in treated plants compared with controls. Especially The height of Larix leptolepis has increased values in UV-B+ compared with control. As to effect of shoot fresh weight, The heighest decrease is observed in Abies veitchii (70%) in UV-B++ compared with controls. There is also a significant decrease in Picea jezoensis (42%), in Picea jezoensis

Carr.var.hondoensis Rehd (36%), in Pinus thunbergii (31%), in Pinus densiflora (31%). However, The shoot fresh weight of Larix leptolepis has increased values in UV-B+ compared with control (32% increase). As to effect of root fresh weight, There is a significiant decrease in woody plants (including Larix leptolepis).

These results lead to the conclusion that UV-B effects different parts on different trees.

5.Studies on the distribution of UV-B effect for forest ecology

The goal of this study is to make a hazard map of UV-B effect for forest ecology.To reach the goal it is needed to conduct a lot of observations of direct and diffuse UV-B radiations at various places,that is,at different altitude and at different latitude.Then it is needed to make a model to calculate UV-B radiation based on the data.At the last stage,the UV-B mesh data is overlay on the map of forest and a hazard map is completed after making clear the effect of UV-B for a forest.

In the first year (1996) of this research project,observation of UV-B by radiometer(MS-210D and MS-330B) was started at the top of Mt.Daikoku (2772m) in Mt.Norikura area and data for 11 days were obtained.Data of diffuse UV-B were also obtained by shading the radiometer.In 1997 and 1998,UV-B radiometer(MS-210D) was set at the Norikura Corona Observatory(2876m) and UV-B was continuously observed from August to October. Also continuous observation of UV-B by MS-210D was conducted at Forestry and Forest Products Research Institute(FFPRI) from 1996.Direct and diffuse UV-B observation by a simple radiometer(MS-330I) was started at Matsudo for fine weather from autumn in 1997. UV-B observations were also conducted at other places(Mt.Akagi,Mt.Asama,Shimoda in Izu,so on) under different conditions.Observation of solar radiation was also conducted in all the place.A lot of data are summarized in some report.

Direct UV-B radiation can be obtained by Lambert-Bear's law.We considered Rayleigh scattering,absorption by Ozone and attenuation by aerosol.Diffuse radiation in our model was calculated by a formula obtained by experiences.The coefficients for the formula were obtained by observations.Based on the model,we obtained gloval,direct and diffuse UV-B 500m mesh data of all over Japan for fine weather.But it is needed to exclude the assumptions and approximations in the model by a lot of observations in various conditions.Especially,it needs to make clear the aerosol effect in atmospheric boundary layer and the difference between inland and the seaside.And it needs to make a UV-B model for cloudy and climatological condition.

6.Reference

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