SCIENTIFIC WORKS OF THE LITHUANIAN INSTITUTE OF HORTICULTURE AND LITHUANIAN UNIVERSITY OF AGRICULTURE. SODININKYSTĖ IR DARŽININKYSTĖ. 2008. 27(2).

Effects of UVB radiation on photosynthesis pigment system and growth of pea (*Pisum sativum* L.)

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The object of the work was to determine the effects of UV-B radiation on growth of peas (*Pisum sativum* L.) and photosynthesis pigment system. Research was carried out in phytotron complex at the Lithuanian Institute of Horticulture and Laboratory of Agro Biotechnology at Lithuanian University of Agriculture. Plants were grown in 5 L vegetative pots in neutral peat substrate (pH 6.0–6.5), 25 plants per pot. A photoperiod of 16 h and temperature of 21 °C/17 °C (day/night) was maintained throughout the experiment. High-pressure sodium lamps 'Son-T Agro' (Philips) were used for illumination. UV-B radiation was generated using UV-B lamps (TL 40W/12 RS UV-B Medical, Philips). Plants were treated with UV-B radiation for 5 days. There were investigated UV-B radiation doses of 0 (control), 1, 3, 5, 7 and 9 kJ m^{-2} each day. Shoot height, dry weight, stomata density and leaf area were measured at 1, 3, 5 day of experiment immediately. Content of photosynthesis pigments were determined spectrophotometrically in 100 % acetone extracts. After the first day of UV-B radiation there was established exposition toxic UV-B effect on above-ground length, dry biomass and number of leaves. It was established that increasing UV-B radiation doses and expositions, there was tendency of pigment content decrease.

Key words: growth, peas, photosynthetic pigments, UV-B radiation.

Introduction. Stratospheric ozone layer absorbs the most of space UV radiation (100 to 400 nm) including solar and other sources, thus protecting plants and other living organisms from harmful UV radiation effect. The thickness of ozone layer is rapidly decreasing and intensity of UV radiation is increasing since seventies of the last century (Krizek, 1998). Shortest UV waves are most detrimental to the living organisms. Usually, UV radiation is divided to UV-A (315–400 nm), UV-B (280–315 nm) and UV-C (100–280 nm) spectral ranges. UV-C radiation is completely absorbed by ozone layer while UV-A radiation is unaffected, though, such radiation does no harm to plants. However, the UV-B radiation intensity is mostly affected by the thickness of stratospheric ozone layer and exactly this type of radiation is most harmful to plants. Recently, research on UV-A radiation also showed some adverse effect on plants (Helsper et al., 2003; Krizek, 2004).

Many authors have determined the wide range of UV-B radiation effects on plants. Higher than normal levels of UV-B causes various damages to plant such as DNA and membrane injuries, photosynthetic or hormone systems' disorders (Rozema et al., 1997; Jansen et al., 1998; Hollosy, 2002). Molecular-level changes affect other processes including gene activity, metabolism, photosynthesis intensity and, consequently, the growth of the whole plant. Some genes are identified as "UV-B dependent genes" and are responsible for synthesis of UV screening compounds, DNA repairs and activation of ant oxidative enzymes (Brosche, Strid, 2003).

Continuous measurements of UV radiation level were initiated in Lithuania only since 2000. Lithuanian Hydrometeorology Service does such measurements at Kaunas meteorology and Palanga aeronautical meteorology stations. Obtained data shows that UV-B radiation intensity depends on season, daily time and meteorology. Average daily dose during fine weather reaches 2.5 kJ m^{-2} (Jonavičienė, 2005).

Objectives to determine UV-B radiation effect on pea (*Pisum saivum* L.) growth and photosynthetic pigment system were raised.

Object, methods and conditions. Research was carried out at Lithuanian University of Agriculture, Laboratory of Agro biotechnology while vegetative research took place in phytotron complex at Lithuanian Institute of Horticulture. Research object – pea (*Pisum sativum* L.), species 'Ilgiai' (leafy).

Pea was grown in 5 L vegetative pots in neutral peat substrate (pH 6.0–6.5). Research was done in three replications, each of 25 plants. Pea was germinated and grown for one week in a greenhouse and then carried to growth chambers were photoperiod of 16 hours and temperatures at 21/17 °C (day/night) were maintained.

UV-B daily doses of 0 (reference), 1, 3, 5, 7 and 9 kJ m^{-2} were investigated. UV-B radiation was generated by TL 40 W/12 RS UV-B Medical lamps (Philips, Austria). Irradiation experiment lasted 5 days.

Shoot height, dry mass, number of stoma and leaf area (Win Dias meter) were measured after 1, 3 and 5 days of exposure. Photosynthetic pigments were analyzed by spectrophotometer in 100 % acetone extract according to Vetstein method (Wettstein, 1957). Average values and SDs were calculated by MS Excel.

Results. Shoot height has decreased even after the first day of exposure to UV-B (Fig. 1 A). Lowermost shoots were observed after the fifth day of exposure to 7 and 9 kJ m^{-2} daily doses, which reached only 58 and 59 % of reference height, respectively.

Shoot dry mass followed the pattern of shoot height and decreased even after the first day of exposure (Fig. 1 B). It reached only 55 % of reference value after the fifth day of exposure under 9 kJm^{-2} of UV-B.

Adverse effect of UV-B radiation on leaf area was also determined (Fig. 2). Leaf area decreased even after the first day of exposure. Most reduced leaf area values were observed after the fifth day of exposure to 7 and 9 kJ m^{-2} and reached only 52 and 63 % of reference values, respectively.

Number of stoma has increased up to 23 % after the first day of exposure to 1 kJ m^{-2} (Fig. 3). Other dosage resulted in zero effect. However, as the exposure time increased, the significant decrease of stoma counts was observed.

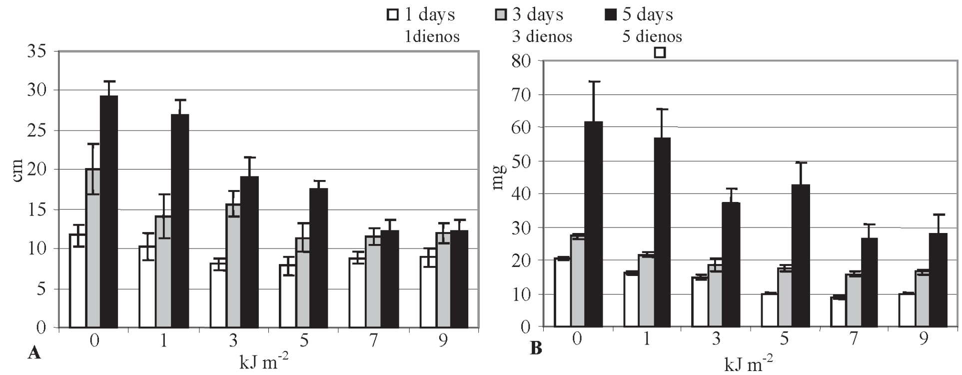

Fig. 1. Effect of UV-B radiation on pea (*Pisum sativum* L.) growth parameters: A – height growth, B – above-ground dry biomass

1 pav. UV-B spinduliuotės poveikis sėjamojo žirnio (*Pisum sativum* L.) augimo parametrams: A – aukščio didėjimui, B – antžeminės dalies sausai biomasei

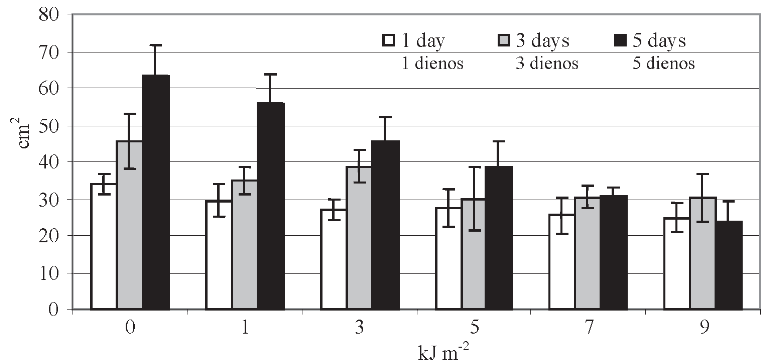

Fig. 2. Effect of UV-B radiation on pea (*Pisum sativum* L.) leaf area

2 pav. UV-B spinduliuoutės poveikis sėjamojo žirnio (*Pisum sativum* L.) lapų plotui

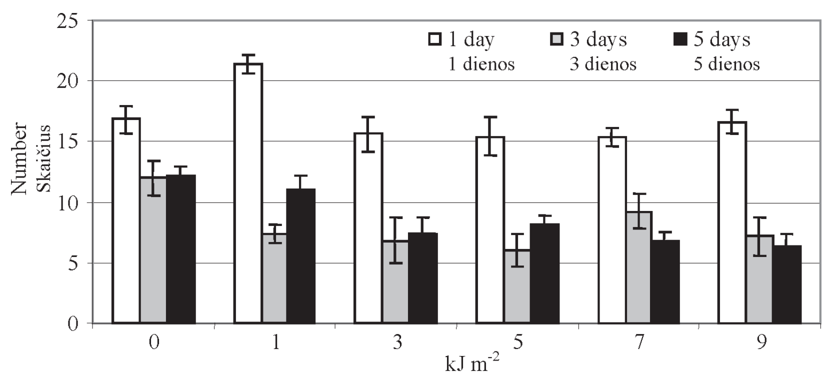

Fig. 3. Effect of UV-B radiation on stomata content in pea (*Pisum sativum* L.) leaf area

3 pav. UV-B spinduliuotės poveikis sėjamojo žirnio (*Pisum sativum* L.) žiotelių skaičiui lape

First day of exposure to UV-B radiation had no effect on chlorophyll *a* amounts in pea leaves (Table 1). However, a decrease in chlorophyll *a* was observed after the third and fifth day of exposure.

1 Table. Effect of UV-B radiation on chlorophyll *a* content
1 lentelė. UV-B spinduliuotės poveikis chlorofilo *a* kiekiui

Exposure Poveikio trukmė	Chlorophyll *a* Chlorofilas *a*					
	UV-B (kJ m^{-2})					
	0	1	3	5	7	9
			'Ilgiai'			
1 day 1 diena	1.25 ± 0.04	1.26 ± 0.03	1.26 ± 0.02	1.29 ± 0.07	1.21 ± 0.03	1.18 ± 0.04
3 days 3 dienos	1.37 ± 0.06	1.16 ± 0.01	1.11 ± 0.09	1.00 ± 0.03	0.92 ± 0.05	1.03 ± 0.08
5 days 5 dienos	1.52 ± 0.04	1.15 ± 0.05	1.19 ± 0.06	0.95 ± 0.03	0.97 ± 0.01	0.95 ± 0.06

Amount of chlorophyll *b* remained steady even after the third day of exposure (Table 2). The most significant decrease in chlorophyll *b* amount was observed after the fifth day of exposure to 5 kJ m^{-2} daily doses.

2 Table. Effect of UV-B radiation on chlorophyll *b* content
2 lentelė. UV-B spinduliuotės poveikis chlorofilo *b* kiekiui

Exposure Poveikio trukmė	Chlorophyll *b* Chlorofilas *b*					
	UV-B (kJ m^{-2})					
	0	1	3	5	7	9
			'Ilgiai'			
1 day 1 diena	0.41 ± 0.01	0.39 ± 0.01	0.39 ± 0.02	0.42 ± 0.03	0.40 ± 0.02	0.40 ± 0.02
3 days 3 dienos	0.44 ± 0.02	0.40 ± 0.01	0.38 ± 0.04	0.40 ± 0.01	0.39 ± 0.03	0.46 ± 0.03
5 days 5 dienos	0.54 ± 0.02	0.44 ± 0.02	0.51 ± 0.02	0.42 ± 0.02	0.50 ± 0.03	0.48 ± 0.04

The first day of exposure had no effect on chlorophyll *a* and *b* ratio (Table 3). However, *a* and *b* ratio have decreased as the time of exposure have increased.

Table 3. Effect of UV-B radiation on chlorophyll *a/b* ratio
3 lentelė. UV-B spinduliuotės poveikis chlorofilo *a/b* santykui

Exposure Poveikio trukmė	Chlorophyll *a/b* Chlorofilas *a/b*					
	UV-B (kJ m^{-2})					
	0	1	3	5	7	9
			'Ilgiai'			
1 day 1 diena	3.05 ± 0.09	3.26 ± 0.10	3.21 ± 0.11	3.05 ± 0.09	3.05 ± 0.14	2.98 ± 0.05
3 days 3 dienos	3.10 ± 0.13	2.91 ± 0.04	2.93 ± 0.05	2.52 ± 0.09	2.41 ± 0.09	2.26 ± 0.01
5 days 5 dienos	2.80 ± 0.06	2.58 ± 0.09	2.35 ± 0.07	2.25 ± 0.09	1.95 ± 0.10	1.99 ± 0.05

4 Table. Effect of UV-B radiation on carotenoid content
4 lentelė. UV-B spinduliuotės poveikis karotinoidų kiekiui

Exposure Poveikio trukmė	Carotenoids Karotinoidai					
	UV-B (kJ m^{-2})					
	0	1	3	5	7	9
			'Ilgiai'			
1 day 1 diena	0.38 ± 0.01	0.40 ± 0.01	0.41 ± 0.01	0.41 ± 0.01	0.39 ± 0.01	0.38 ± 0.01
3 days 3 dienos	0.44 ± 0.02	0.38 ± 0.01	0.40 ± 0.03	0.42 ± 0.02	0.40 ± 0.02	0.44 ± 0.03
5 days 5 dienos	0.46 ± 0.01	0.39 ± 0.02	0.40 ± 0.02	0.37 ± 0.02	0.42 ± 0.01	0.43 ± 0.04

Carotenoid content remained steady or even decreased even after the first day of exposure (Table 4). However, carotenoid content have decreased after the fifth day of exposure to UV-B radiation.

Discussion. This research revealed that pea growth pattern is very sensitive to UV-B radiation. The adverse effect of UV-B radiation was observed after the first day of exposure. Other scientist also shows significant UV-B radiation effect on barley growth parameters including stem height, sprout count, leaf area and biomass (Correia et al., 1999, Nasser, 2001). Plant sensitivity to UV exposure might be determined by direct damage to cell structural and functional elements or by ineffective acclimatization process (Smith et al., 2000).

As aforementioned, shoot height, dry biomass and leaf area decreased even after the first day of exposure. UV-B radiation may induce leaf differentiation and senescence processes via modification of leaf structure (Kakani et al., 2003, 2004). Plants are capable to accommodate to certain UV-B radiation as well as to light intensity, though tolerance range are determined by plant species, age, duration of exposure and other factors. If the UV-B dosage exceeds the limits of tolerance, plant leaf anatomy is changed and biomass is decreased (Coleman, Day, 2004, Kakani et al., 2003, Zhao et al., 2003). Nevertheless, other authors (Liu et al., 1995; Stephen et al., 1999; Schmitz-Hoerner and Weissenbock, 2003; Valkama et al., 2003) show that biomass or photosynthetic pigment content does not change under the exposure to UV-B radiation or such variation is insignificant. Hakala et al. (2002) determined sensitivity of various agricultural plant species including barley, wheat, oat, clover, timothy, fescue and potato to exposure to UV-B radiation (as if ozone layer would be decreased by 30 %) and found no significant variation of biomass accumulation or yield. Thus, it was shown that C_4 type of plants are resistant to UV-B radiation.

Stomata formation and permeability is determined by leaf age and other factors including UV-B radiation intensity (Day, Neale, 2002). In our study, stomata counts on pea (*Pisum sativum* L.) leaves remained unchanged only after the first day of exposure to UV-B radiation. Similar results are presented by Day and Neale (2002) where stomata counts and permeability were changing if exposure period and intensity is increasing.

Photosynthesis is very important process in plants as it determines biomass increase, thus, is a subject for UV-B exposure research. Photosynthesis process is based on chlorophylls' system and, if such system is altered by UV-B radiation, biomass increase is hindered; hence, such feature might be used to determine UV-B sensitive plants. Accordingly, plants, which are able to keep chlorophylls' system unchanged, are far more resistant to UV-B radiation (Smith et al., 2000). During our study, amount of chlorophyll *a* remained unchanged after the first day of exposure, but significantly decreased if the exposure period and intensity increased. Amount of chlorophyll *b* was more stable, but decreased at the end of experiment. Some authors have stated, that content of chlorophyll *a* and carotenoids remains unchanged under the exposure to UV-B, while amount chlorophyll *b* decreases (Barsig and Malz, 2000). However, our study revealed that chlorophyll *a* was more sensitive to UV-B radiation than chlorophyll *b*. Such variation could be based on the injury of thylakoid lumen, where the center of light harvesting system – chlorophyll *a* – is being damaged and disintegrates (Rengel et al., 1989). Decrease of chlorophylls' *a* to *b* ratio under exposure to UV-B radiation was also shown by other authors (Smith et al. 2000).

Carotenoids called xanthophylls are the main protective agents dissipating excess energy and protecting photoreaction center from auto-oxidation (Yamamoto, Bassi, 1996). Our study revealed that content of carotenoids remained unchanged during first three days of exposure, but varied at the fifth day of exposure accordingly to radiation intensity.

In general, pea growth and biomass accumulation was hindered even after the first day of exposure and the plant response intensity was adequate to the duration and intensity of exposure to UV-B radiation. Thus, it could be concluded that pea plants are very sensitive and hardly adapt to the increased UV-B radiation.

Conclusions. 1. An adverse UV-B effect on pea (*Pisum sativum* L.) growth was observed even after the first day of exposure.

2. Decrease of amounts of photosynthetic pigments in pea (*Pisum sativum* L.) leaves indicates plant sensitivity to UV-B radiation.

Acknowledgement. The research was funded by Lithuanian State and Studies Foundation under the priority research project "Complex effect of anthropogenic climate and environmental changes on vegetation of forests and agro ecosystems".

Gauta 2008 04 23
Parengta spausdinti 2008 04 29

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SODININKYSTĖ IR DARŽININKYSTĖ. MOKSLO DARBAI. 2008. 27(2).

UV-B spinduliuotės poveikis sėjamojo žirnio (*Pisum sativum* L.) augimui ir fotosintezės pigmentų sistemai

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Santrauka

Darbo tikslas – nustatyti UV-B spinduliuotės poveikį sėjamojo žirnio (*Pisum sativum* L.) augimui ir fotosintezės pigmentų sistemai.

Bandymas vykdytas kontroliuojamose sąlygose Lietuvos sodininkystės ir daržininkystės instituto fitotrone bei Lietuvos žemės ūkio universiteto Agrobiotechnologijos laboratorijoje. Augalai auginti neutralaus rūgštumo substrate (Ph 6–6,5), 5 L talpos vegetaciniuose induose, po 25 augalus. Fotoperiodas – 16 val., temperatūra – 21 °C/17 °C (dieną/naktį), šviesos šaltinis – „SON-T Agro“ (PHILIPS) lempos). UV-B spinduliuotę skleidė UV-B lempos (TL 40W/12 RS UV-B Medical, Philips). Augalai UV-B spinduliuote švitinti 5 dienas. Tirtos tokios UV-B spinduliuotės dozės: 0 (kontrolė), 1, 3, 5, 7 ir 9 kJ m^{-2} per dieną.

Po 1, 3, 5 dienų UV-B spinduliuotės poveikio nustatytas antžeminės dalies aukštis, sausoji biomasė, žiotelių skaičius, lapų plotas (WinDias matuoklis). Fotosintetiniai pigmentai nustatyti spektrofotometriniu būdu 100 % acetono ištraukoje pagal Vetšteiną.

Atlikus tyrimą jau po 1 dienos poveikio nustatytas toksiškas UV-B poveikis antžeminės dalies ilgio, sausos biomasės ir lapų ploto augimui. Didėjant UV-B spinduliuotės dozėms ir poveikio laikui, nustatytas tendencingas fotosintetinių pigmentų kiekio mažėjimas.

Reikšminiai žodžiai: augimas, fotosintetiniai pigmentai, UV-B spinduliuotė, žirniai.