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Growth and physiological features of pea (*Pisum sativum* L.) of different morphotypes under ozone exposure

Rima Juozaitytė[1],

Asta Ramaškevičienė[1],

Algirdas Sliesaravičius[1],

Aušra Brazaitytė[2],

Pavelas Duchovskis[2],

Natalija Burbulis[1]

[1] *Lithuanian University of Agriculture, LT-53067 Akademija, Kaunas distr., Lithuania*
E-mail: Rima.Juozaityte@lzuu.lt

[2] *Lithuanian Institute of Horticulture, LT-54333 Babtai, Kaunas distr., Lithuania*

Two different morphotypes of peas (*Pisum sativum* L.), the leafy form 'Ilgiai' and the subleafy form 'Profi', were examined under ozone exposure. A day after exposure, adverse effects of ozone on 'Ilgiai' morphometric features were observed, while certain processes in 'Profi' were stimulated. After three days of exposure, the leaf area of leafy peas was reduced by 34%, while in subleafy peas it increased by 33% under the 160 μg m^{-3} ozone level. Chlorophyll and carotenoid content in leaves of leafy peas decreased with increasing exposition time and ozone concentration. However, a linear dependence between the content of photosynthetic pigments in leaves of subleafy peas and exposition time / ozone concentration was determined, and only the highest ozone level significantly decreased the content of those pigments.

The ratio of hexoses to sucrose increased in leafy peas after one day of ozone exposure, but it tendentiously decreased as the time of exposure increased. The same trends were observed also for subleafy peas, though the variation range was not so intense. However, pronounced changes in the ratio of soluble sugars were determined only after five days of exposure.

Key words: growth, ozone, peas, pigments, sugars

INTRODUCTION

Troposphere or ground-level ozone (O_3) is one of the main secondary air pollutants. The worldwide trend of increasing ozone concentration has been clearly defined [1, 3]. During the period 1981–1999, ozone level was increasing on average by 0.93 μg m^{-3} annually [2]. Although the critical ozone levels in Eastern Europe are not exceeded, transition of air masses from other countries, increased traffic and other primary pollution sources may rapidly raise the ground-level ozone concentration [4].

High levels of ozone are known to affect most of the plant's physiological and biochemical processes as well as growth and yield [5]. Ozone as a strong oxidizing agent reduces plant biomass, damages leaves, suppresses photosynthesis and stomata conductivity, and reduces productivity [6]. Plant reaction to ozone is defined by ozone soaking into plant leaves and further by the protective mechanisms of plant tissues, which are necessary for intoxication and the reproduction of damaged tissue [7]. Soluble sugars, including mono- and disaccharides, are important for cell structure and function [8]. Glucose and fructose are the main source of carbon and energy in eukaryotic cells. Sucrose is a product of photosynthesis in higher plants and is used in transportation, heterotrophy, and in complex with certain enzymes it takes part in development processes [9].

Plant resistance to ozone effects varies within and between the species due to different genetic and phenological features [1]. Sensitive plants such as legume crops are influenced even by a low levels of ozone, which inhibit the development and growth of these plants. Therefore, investigation of resistance and adaptation of two different morphotypes of peas (*Pisum sativum* L.) under various ozone concentrations was the primary objective of this study.

MATERIALS AND METHODS

Pea (*Pisum sativum* L.) was sown and grown in 5 l vegetative pots in neutral (pH 6.0–6.5) peat substrate, 25 plants per pot. Ozone concentrations of 30–40 (reference), 80 and 160 μg m^{-3} were maintained by the OSR-8 ozone generator (Ozone Solutions, Inc.) seven hours per day, five days per week. Ozone level was monitored with the portable OMC-1108 ozone sensor (Ozone Solutions, Inc.). All treatments were run in three replicates. Plants were sown and grown in greenhouse for one week after germination, then moved to phytotron chambers where a photoperiod of 14 h and a temperature of 21 °C-day / 14 °C-night were maintained. High-pressure SON-T Agro sodium lamps (Philips) were used for illumination. Shoot dry weight, leaf area, the content of photosynthetic pigments and sugars were being determined at 1, 3, 5 days during ozone exposure and at the end of experiment (9 days after the beginning of exposure). Photosynthetic pigments were analysed in 100% acetone extracts prepared according to the method of Wettstein [10]. Analyses of fructose, glucose and sucrose were performed employing the high-performance liquid chromatography (HPLC) system with a refractive index detector. Variations of sugar content were expressed as variations of the ratio of monosaccharides (glucose and fructose) to sucrose. Means and standard deviation were used as statistical measures in data presentation.

RESULTS

Biomass decrease of 20% was determined for leafy peas after one day of exposure. In contrast, a stimulating effect on subleafy peas was observed even after three days of exposure when biomass increase of 49% and 31% under 80 and 160 µg m^{-3} ozone levels, respectively, were determined versus the reference plants (Table 1). However, at the end of experiment (9 days after the beginning of exposure) the dry weight of subleafy peas was similar to that of reference plants at a 80 µg m^{-3} and by 13% lower at a 160 µg m^{-3} ozone level, while the dry weight of leafy peas was lower by 29% and 45%, respectively.

Leaf area in leafy peas decreased even after one day of exposure in comparison with reference plants, while a 35% increase in leaf area was determined for subleafy peas even after three days of ozone exposure (Table 2). However, leaf area in subleafy peas was reduced after a prolonged exposure, especially under the 160 µg m^{-3} ozone level.

The total chlorophylls (a + b) increased by 55% after one day of exposure to the 160 µg m^{-3} ozone level versus the reference plants. However, chlorophyll synthesis was suppressed under a prolonged exposure, and at the end of treatment the content of chlorophylls decreased by 23% and 53% under 80 and 160 µg m^{-3} ozone levels, respectively (Fig. 1). In general, the synthesis of chlorophylls (a + b) in subleafy peas was stimulated as the amount of these pigments increased by 22% and 29% under 80 and 160 µg m^{-3} ozone levels, respectively, in comparison with reference plants at day 5 of exposure. The adverse effect of ozone on pigment content in subleafy peas was observed only at day 9 after the beginning of exposure (Fig. 1).

The content of carotenoids mimicked the variation of chlorophylls, i. e. increased in leafy peas 'Ilgiai' only after one day of

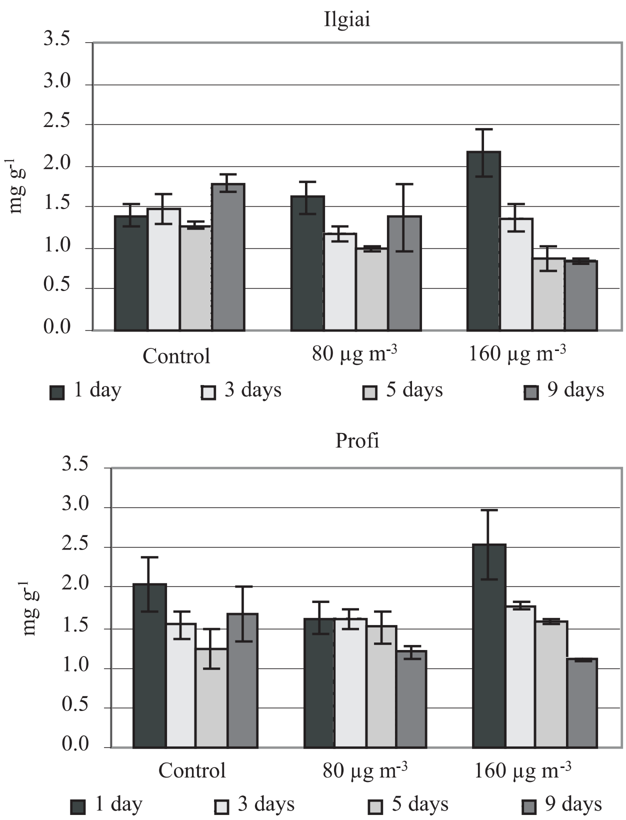

Fig. 1. Synthesis of chlorophylls (a + b) in different pea morphotypes under ozone exposure

Table 1. **Dry weight of different pea morphotypes under ozone exposure**

Days of exposure	Dry weight, g					
	O_3 concentration (µg m^{-3})					
	0	80	160	0	80	160
	'Ilgiai'			'Profi'		
1	2.49 ± 0.15	1.95 ± 0.05	1.97 ± 0.05	2.49 ± 0.11	2.81 ± 0.13	2.67 ± 0.07
3	2.15 ± 0.06	1.98 ± 0.03	1.69 ± 0.11	2.01 ± 0.03	2.99 ± 0.12	2.64 ± 0.08
5	5.18 ± 0.09	4.26 ± 0.06	3.49 ± 0.11	4.74 ± 0.11	4.65 ± 0.04	3.36 ± 0.11
9	8.40 ± 0.49	5.94 ± 0.55	4.65 ± 0.50	9.78 ± 0.31	9.09 ± 0.49	8.54 ± 0.47

Table 2. **Leaf area of different pea morphotypes under ozone exposure**

Days of exposure	Leaf area, cm²					
	O_3 concentration (µg m^{-3})					
	30–40	80	160	30–40	80	160
	'Ilgiai'			'Profi'		
1	84.01 ± 15.8	85.02 ± 7.84	62.74 ± 15.3	92.54 ± 9.22	91.66 ± 28.0	78.72 ± 15.2
3	78.22 ± 28.9	72.91 ± 25.8	51.85 ± 8.61	66.21 ± 19.7	89.80 ± 21.2	87.88 ± 14.0
5	157.7 ± 6.88	104.6 ± 25.0	87.77 ± 18.1	130.2 ± 29.7	103.4 ± 29.4	59.29 ± 7.81
9	163.7 ± 4.66	93.89 ± 8.53	88.48 ± 16.9	158.5 ± 8.63	124.5 ± 16.8	69.95 ± 9.05

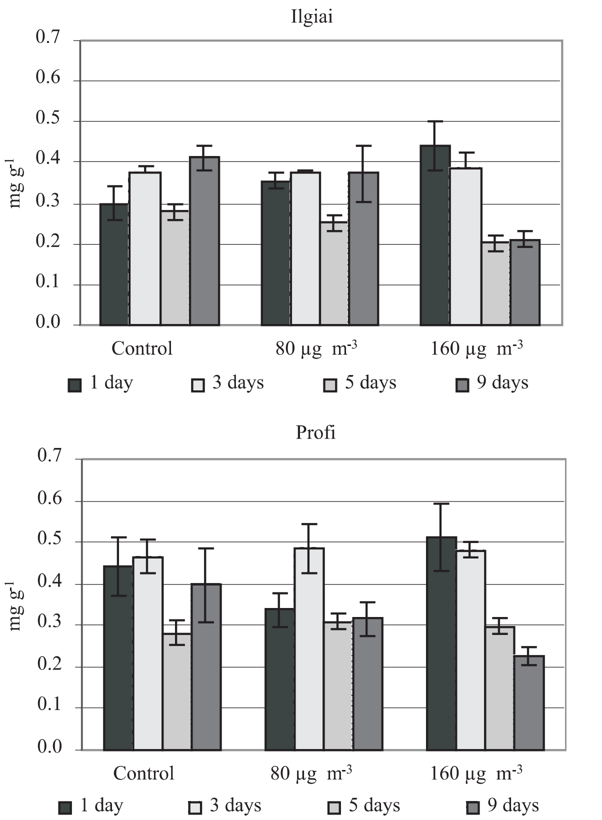

Fig. 2. Synthesis of carotenoids in different pea morphotypes under ozone exposure

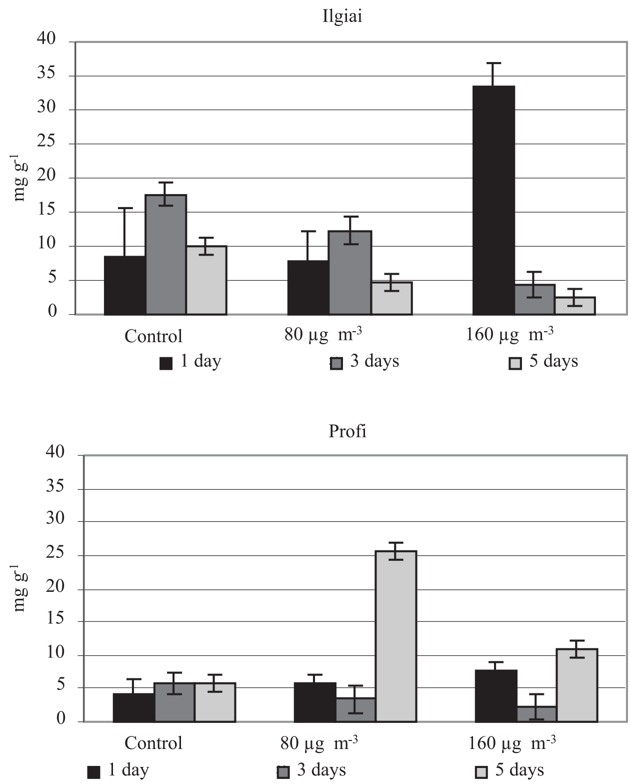

Fig. 3. Monosaccharide / sucrose ratio in different pea morphotypes under ozone exposure

exposure and gradually decreased afterwards. The strongest adverse effect of ozone on carotenoid content was determined in leafy peas 5 and 9 days after the beginning of exposure to the 160 µg m^{-3} ozone level. The level of carotenoids remained stable in subleafy peas during all 5 days of exposure, and only at day 9 of experiments a decrease by 20% and 43% under 80 and 160 µg m^{-3}, respectively, was observed (Fig. 2).

Hexoses-to-sucrose ratio in the leafy peas 'Ilgiai' increased by a factor of 4 after one day of exposure under 160 µg m^{-3}, though a gradual decrease of sugars' ratio was observed afterwards. At day 3 of exposure, the ratio decreased by a factor of 4 under 160 µg m^{-3} in comparison with reference plants. The ratio of sugars increased by 55% in the subleafy peas 'Profi' after one day of exposure to 160 µg m^{-3} of ozone and decreased by 40 % and 60% after three days of exposure to 80 and 160 µg m^{-3} of ozone, respectively. However, monosaccharide-to-sucrose ratio in leaves of the subleafy peas 'Profi' increased by a factor of 4.5 and 1.9 after 5 days of exposure to 80 and 160 µg m^{-3} ozone levels, respectively (Fig. 3). No sugars were observed at the end of experiment as the plants were severely damaged.

DISCUSSION

Plant biomass reduction under ozone exposure occurs due to stomata closure, what prevents further damage but suppresses photosynthesis productivity [11]. Consequently, the content of chloroplasts decreases, and chlorosis and necrosis of leaves is initiated even after a few days of exposure [12]. The state and function of the photosynthesis apparatus are strongly related to the total content of chlorophylls and carotenoids which are responsible for protection from antioxidative stress [13]. Our study revealed that the content of chlorophylls gradually decreased in leafy peas in accordance with the length and intensity of ozone exposure. However, the synthesis of chlorophylls and carotenoids was stimulated in leaves of subleafy peas, and only the highest level of ozone significantly reduced the content of these pigments at day 9 after the beginning of exposure. As expected, a linear dependence was determined between the content of chlorophylls and carotenoids. A number of authors [14–16] suggest, that carotenoids are vital for chlorophyll protection against photooxidative destruction as they stabilize the membranes, bind the free radicals formed by lipid peroxidation, and transfer excess energy from photo-excited chlorophyll. Moreover, Pfunde and Birgel [17] have stated that biosynthesis of carotenoids strongly correlates with the production of ascorbic acid. Such findings were proved in our study as well, as the greater amounts of carotenes were found in subleafy peas throughout the experiment, thus they remained protected from the adverse effect of ozone much longer than the leafy form of peas.

As reported in [18], signal transfer in plants is actualized via the content of sugars which are likely responsible for the process of leaf senescence due to reduced chlorophyll content and photosynthetic activity. In general, a low amount of sugars negatively affect photosynthetic activity, the accumulation and transportation of photosynthesis products, while higher contents of sugars stimulate plant growth and the accumulation of carbohydrates [19]. Sucrose is known to regulate cell division and the accumulation of energy resources, while hexoses are responsible for plant growth and metabolism [20]. Changes in glucose content markedly affect the process of plant leaf senescence [21].

In our study, the monosaccharide-to-sucrose ratio increased even after one day of ozone exposure, indicating an immediate plant response. However, such ratio of sugars and, as a consequence, plant resistance to ozone gradually decreased afterwards. Therefore, early reduction of leaf area, decrease of dry weight, leaf senescence and desiccation occurred in leafy pea 'Ilgiai' plants. The ratio of sugars changed in subleafy peas after one day of exposure as well, though the variation range was not so intense and, hence, dry weight and leaf area remained greater in comparison with leafy peas. This means that analysis of soluble sugars may be used for detecting the initiation of the processes of enhanced plant resistance to a certain stressor in advance. In general, homeostasis in subleafy pea plants appeared to be more inert in comparison with the leafy pea form because significant changes in sugars' ratio and a decrease in morphometric features were observed only at day 5 or even 9 of ozone exposure. The obtained data confirm the findings that the outcome of anthropogenic stress is very species-specific and, as proposed by Alexieva [16], depends on the duration and intensity of exposure.

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Rima Juozaitytė, Asta Ramaškevičienė, Algirdas Sliesaravičius, Aušra Brazaitytė, Pavelas Duchovskis, Natalija Burbulis

OZONO POVEIKIS SKIRTINGŲ MORFOLOGINIŲ TIPŲ ŽIRNIŲ AUGIMUI IR FIZIOLOGINIAMS RODIKLIAMS

Santrauka

Buvo ištirtas įvairių ozono koncentracijų poveikis dviejų skirtingų morfologinių tipų žirniams (lapuotiems 'Ilgiams' ir pusiau belapiams 'Profi'). Po vienos dienos poveikio lapuotų žirnių 'Ilgiai' morfometriniams parametrams nustatytas toksiškas ozono poveikis, o pusiau belapiams 'Profi' – stimuliacinis poveikis. Veikiant ozonu (160 μg m^{-3}) po trijų dienų lapuotų žirnių lapų plotas sumažėjo 34%, o pusiau belapių, palyginus su kontrole, 33% padidėjo. Ištyrus ozono poveikį chlorofilo a + b bei b karotino sintezei nustatyta, kad fotosintetinių pigmentų kiekis lapuotuose žirniuose mažėjo priklausomai nuo ekspozicijos laiko ir ozono koncentracijos, o pusiau belapių žirnių lapuose šių pigmentų kiekis buvo stimuliuojamas, tačiau stipriausia tirta ozono koncentracija ir ilgiausias ekspozicijos laikas patikimai sumažino fotosintetinių pigmentų kiekį. Lapuotų žirnių heksozių ir sacharozės santykis labai padidėjo po vienos dienos poveikio, o ilgėjant ekspozicijos laikui šis santykis tendencingai mažėjo. Pusiau belapių žirnių lapuose heksozių ir sacharozės santykis taip pat keitėsi jau po vienos dienos poveikio, tik pokyčio amplitudė buvo kur kas mažesnė. Labai ryškus tirpiojo cukraus santykio pasikeitimas nustatytas tik po penkių dienų.