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The competition between winter rape (C_3) and maize (C_4) plants in response to elevated carbon dioxide and temperature, and drought stress

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Abstract

The aim of this study was to estimate the effects of drought stress under elevated CO_2 and temperature on the competition between winter rape and maize plants. The experiment was conducted in a controlled-environment growth chamber at the Institute of Horticulture, Lithuanian Research Centre for Agriculture and Forestry. Plants grown in monoculture (100:0) or in mixture (winter rape:maize, 50:50) irrigated and non-irrigated (drought stress), were exposed to ambient environment conditions – day/night temperature 21/14°C, CO_2 – 350 ppm (CO_2 T), and enhanced environment conditions – day/night temperature 30/23°C, CO_2 – 700 ppm (+CO_2 T). Maize (C_4) grown in mixture with winter rape (C_3), had significantly smaller assimilating leaf area and produced less fresh mass at CO_2 T and +CO_2 T, as compared to monoculture, and water deficit strengthened the negative effect. Comparison of the data obtained for monoculture revealed that elevated CO_2 and temperature in the conditions of sufficient moisture supply had significantly positive effect on maize assimilating leaf area (increased ~21%) and fresh mass accumulation (increased ~22%). Assimilating leaf area and fresh mass of winter rape and maize monocultures significantly decreased in response to water deficit both at CO_2 T and +CO_2 T. Water shortage had a stimulating effect on chlorophylls in leaves of winter rape in monocultures and mixtures. At +CO_2 T and sufficient water supply, maize in monoculture and mixture with rape significantly reduced the content of chlorophyll *a*. Carotenoid content significantly decreased in maize leaves (monoculture and mixture) under +CO_2 T in both irrigated or non-irigated substrates. Winter rape, in monoculture and mixture, accumulated significantly higher content of carotenoids under water deficit and ambient CO_2 T. However, a significant decrease in carotenoids was determined in rape leaves under +CO_2 T. The elevated contents of ascorbic acid, total phenolics and antioxidant potential indicate high capacity of winter rape to counter oxidative stress.

Key words: antioxidants, *Brassica napus*, growth indices, photosynthetic pigments, *Zea mays*.

Introduction

The intensive use of fossil fuel and deforestation have driven global atmospheric CO_2 concentration from a preindustrial concentration of 280 µmol mol^{-1} to a current concentration of about 380 µmol mol^{-1}, with a projected increase to 550 µmol mol^{-1} by 2050 and top 700 µmol mol^{-1} by the year 2100 (IPCC, 2014). As a consequence, the surface temperature is projected to rise over the 21st century and it is very likely that heat waves will occur more often and last longer which in turn will affect the growth, development and yield of agricultural crops (IPCC, 2014). For terrestrial plants and ecosystems, increases in the atmospheric CO_2 concentration and air temperature as well as changes in precipitation patterns are expected to have strong impacts on the carbon balance. Globally, water availability is a key factor limiting plant productivity and crop yield (Mu et al., 2007). Regional climate models predict enhanced temperature and a change in the pattern of precipitation, resulting in longer summer drought periods and the occasional incidences of high amounts of precipitation (Meehl et al., 2007). Plant growth is affected by environmental changes (Juknys et al., 2011) and a common plant response to a variety of environmental stressors is the accumulation of antioxidants and secondary metabolites. Several environmental factors are associated with metabolism and thereby alter the composition and concentration of plant chemical compounds (Manochai et al., 2010; Sakalauskaitė et al., 2012).

Under stressful environmnetal conditions the activity of antioxidant enzymes and the amount of antioxidants are elevated in plants and are species-specific (Nayyar, Gupta, 2006) or even cultivar-specific (Pérez-López et al., 2009; Uzilday et al., 2011).

Plant species have varying ability to deal with oxidative stress that govern their differential sensitivity to various environmental stressors (Nayyar, Gupta, 2006).

Examination of reaction of plants with different photosynthetic pathways to global change has great economic importance because significant alteration is expected in the balance between plants with different photosynthetic pathways (Bernacki, 2012). It is possible that a complex of interrelated factors (increasing CO_2 level, temperature, water and nitrogen availability) lead to predominance of both C_4 and C_3 plants, increase of CO_2 level will prefer C_3 photosynthesis, but shift of temperature will stimulate C_4 photosynthesis (Ward et al., 2009). Understanding the different responses of C_3 and C_4 plants to increasing atmospheric CO_2 and temperature, more frequent drought periods might be especially important for crop competition within agroecosystems. The lack of a consistent, direct enhancement of photosynthesis and yield in species across a broad range of growing conditions diminishes the extent that elevated CO_2 will offset global yield loss resulting from other aspects of environmental change, even if elevated CO_2 acts locally to ameliorate stress associated with greater drought and temperature (Leakey et al., 2009).

Winter rape and maize were chosen for the present study as representatives of C_3 and C_4 plants which differ functionally in their carbon fixation. Winter rape (*Brassica napus* L.), a C_3 plant, is one of the most important crops because of its wide use in oil, animal fodder, and biodiesel production. In some cases, winter rape can act like a weed and pose a serious threat for other crops. Rape seeds can persist in the soil for many years and thus volunteers can emerge several years after the last rape crop has been grown. Maize (*Zea mays* L.) is the most globally important C_4 grain crop, which is grown in over 160 countries (http://faostat.fao.org/). It is an important crop in sustaining human life in terms of its role as a major grain and feed commodity, as well as significant bioethanol energy resource. Maize is a dominating crop for biogas production and is considered to have one of the highest yield potential among field crops (Amon et al., 2007). As forecasted for Lithuania, warmer and longer growing season provides benefits for C_4 crops such as maize. Maize has been introduced in Lithuania as a silage crop; however, nowadays a number of short-season varieties suitable for grain production under Lithuanian climate conditions are available on the market. The increases in ambient temperature accelerate plant development, allowing completion of phenological stages in a shorter time (Povilaitis et al., 2013).

Effects of environmental changes such as elevated CO_2, temperature, and precipitation on plants and ecosystems have primarily been investigated as effects of a single factor or two factors in combination (Hyvönen, 2011; Miri et al., 2012). Since all environmental changes occur concurrently, in order to investigate potential interactions among factors, it is important to study the effects of the individual factors as well as their combinations. The aim of this study was to determine the interactive effect of elevated CO_2 and temperature, and water supply on winter rape (C_3) and maize (C_4) plants in monocultures and mixtures and to test which of these crops can benefit from changing environment in terms of their competitive ability. Maize is very sensitive to weed competition especially in the early growth stage (Olorunmaiye, Olorunmaiye, 2009) and due to the weed competition maize green material yield decreases by 2–10 times (Auškalnienė, 2006).

Materials and methods

The research was carried out in controlled-environment growth chambers at the Institute of Horticulture, Lithuanian Research Centre for Agriculture and Forestry in 2011. Two separate chambers (each 24 m^2) were used for the study. Winter rape (*Brassica napus* L., cv. 'Valesca') and maize (*Zea mays* L., cv. 'Auxxel') were sown and grown in a neutral peat substrate in 5 L pots (21 cm in diameter). Ten days after germination, seedlings were thinned to 8 plants per pot and adjusted to the target ratios (winter rape:maize) of 100:0, 50:50 and 0:100. For each treatment, there were 10 pots of replication. Prior to germination and treatment, the plants were grown in a greenhouse at an average temperature of 16–20ºC under natural solar radiation. Plants at the stage of 3–4 fully expanded leaves were transferred to growth chambers with 16 h photoperiod and 21/14ºC day/night temperature, and left for 2 days to get accustomed to the new environment. High-pressure sodium lamps SON-T Agro (Philips) at a PPFD (photosynthetic photon flux density) of ~300 μmol m^{-2} s^{-1} were used for illumination. The two different water stress treatment were: 1) control, where pots were watered regularly to maintain 75–80% of the soil moisture capacity, and 2) drought, where irrigation was stopped for 10 days. The soil moisture content was maintained by using a soil moisture sensor HH2 (Delta-T Devices, UK). Irrigated (control) and non-irrigated (drought) plants were exposed to ambient environment conditions – day/night temperature 21/14°C, CO_2 – 350 ppm (CO_2 T); and enhanced environment conditions – day/night temperature 30/23°C, CO_2 – 700 ppm (+CO_2 T). Plants were grown under such conditions for 10 days.

Assimilating leaf area, fresh and dry mass was determined for winter rape and maize plants upon treatment completion. Ten randomly selected plants of winter rape and maize were taken from each experimental treatment. Assimilating leaf area was measured using a leaf area meter WinDIAS (Delta-T Devices, UK). Dry mass content was determined following forced air convenion drying at 105ºC to a constant weight (air oven method).

Plants were randomly sampled from pots of each treatment for biochemical analysis. Photosynthetic pigment content, ascorbic acid, total phenolic compounds, DPPH* radical scavenging activity were analysed on fresh samples of the fully unfolded mature leaves of randomly-selected plants. The amount of chlorophylls and carotenoids in green leaves was determined by spectrophotometry in a 100% acetone extract (Gavrilenko, Zigalova, 2003), using a spectrophotometer Genesys 6 (ThermoSpectronic, USA). Ascorbic acid (vitamin C) content was also evaluated using spectrophotometry (Janghel et al., 2007) again with a spectrophotometer Genesys 6. One gram of plant leaves was homogenised in 10 ml 5% oxalic acid, in order to prevent the loss of ascorbic acid, and then centrifuged (5 min at 1691 × g). A 1 ml aliquot of the extract was mixed with 2 ml 0.1% methyl viologen and 2 ml 2 M sodium hydroxide. The solution was shaken gently and allowed to stand for 2 min. The coloured radical ion was measured at 600 nm against the radical blank.

The total content of phenolic compounds was determined in plant leaf methanol extracts (1 g of fresh leaf sample was ground frozen in liquid nitrogen using mortar and pestle and diluted with 10 ml 80% methanol) using calorimetric method (Ragaee et al., 2006). The extract was shaken for 30 min, then centrifuged at 2012 × g for 20 min. A 1 ml aliquot of the sample extract was diluted with 1 ml 10% (w/v) Folin-Ciocalteu reagent (diluted with bi-distilled water) and 2 ml 1 M Na_2CO_3 solution. The absorbance was measured after 20 min at 765 nm with spectrophotometer Genesys 6 against a water blank. Gallic acid was used as a standard and the total phenolics were expressed through the calibration curve.

The antioxidant (DPPH radical-scavenging) activity of the methanol extracts (1 g of fresh leaf sample was ground frozen in liquid nitrogen using mortar and pestle and diluted with 10 ml 80% methanol) was evaluated spectrophotometrically by their 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging capacity (Ragaee et al., 2006). A spectrophotometer Genesys 6 was used again for this analysis. The extract was shaken for 30 min, after which it was centrifugated at 2012 × g for 20 min. The absorbance, scanned at 16 minute at 515 nm, was used to calculate the ability of the plant material to scavenge DPPH free radicals (μmol g^{-1}).

All data were analysed using *ANOVA* for *MS Excel*, version 3.43, and Fisher's LSD test ($p \leq 0.05$).

Results

In this experiment, drought stress, at ambient or elevated CO_2 and temperature resulted in a significant decrease (68–81%) in the fresh mass of winter rape grown in monoculture (Table).

Table. Assimilating leaf area, fresh and dry mass of winter rape and maize plants in monocultures (100:0, 0:100) and mixtures (50:50) exposed to ambient (CO_2 T – CO_2 350 ppm, T 21/14°C) and enhanced (+CO_2 T – CO_2 700 ppm, T 30/23°C) environment conditions and different substrate moisture

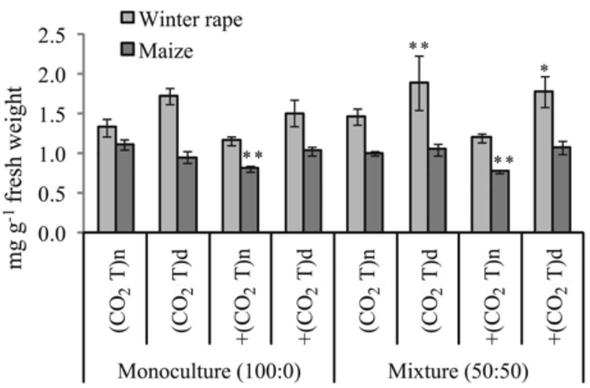
Rape:maize	Substrate	Carbon dioxide and temperature	Assimilating leaf area cm²		Fresh mass g		Dry mass g	
			rape	maize	rape	maize	rape	maize
100:0	n_r	CO_2 T	**194.6**	–	**12.9**	–	**1.5**	–
	d		113.3*	–	4.2*	–	0.9	–
	n	+CO_2 T	165.8	–	12.5	–	1.7	–
	d		42.9*	–	2.5*	–	0.8	–
0:100	n_m	CO_2 T	–	**487.4**	–	**25.1**	–	**1.7**
	d		–	295.0*	–	12.8*	–	1.1
	n	+CO_2 T	–	593.3*	–	30.7*	–	2.4
	d		–	136.3*	–	11.2*	–	1.7
50:50	n	CO_2 T	330.4*	196.3*	18.1*	10.9*	1.5	0.8
	d		170.9	50.9*	6.6*	4.4*	1.0	0.7
	n	+CO_2 T	545.7*	219.3*	28.1*	14.8*	3.8*	2.6
	d		157.8	56.6*	5.6*	7.9*	1.3	2.7
LSD_{05}			68.8	78.1	2.7	3.9	0.9	1.2

Notes. Bold data in line means reference plants: n_r – winter rape, n_m – maize; n – normal (irrigated), d – drought (non-irrigated). Significant differences from reference plants is denoted by an asterisk * – $p \leq 0.05$.

A lack of substrate moisture significantly reduced the accumulation of fresh mass in maize monoculture independent of CO_2 concentration and temperature. The +CO_2 T conditions under sufficient water supply significantly increased total fresh mass of maize monoculture in comparison to control conditions. The investigated plants grown in mixture (winter rape:maize, 50:50) responded differently to applied environment conditions. A significantly greater amount of fresh mass was observed for winter rape under ambient or elevated CO_2 and temperature and sufficient substrate moisture content as compared to reference winter rape. Moisture deficit had negative effect on winter rape fresh mass, regardless of CO_2 concentration and temperature. Winter rape caused strong competition to maize, irrigated plants or plants experienced the water shortage under CO_2 T or +CO_2 T environment conditions accumulated less mass compared to reference maize plants. Experimental factors did not exert any significant effect on plant dry mass, except for winter rape which grew in a mixture with maize plants, when substrate water was sufficient under +CO_2 T conditions (Table).

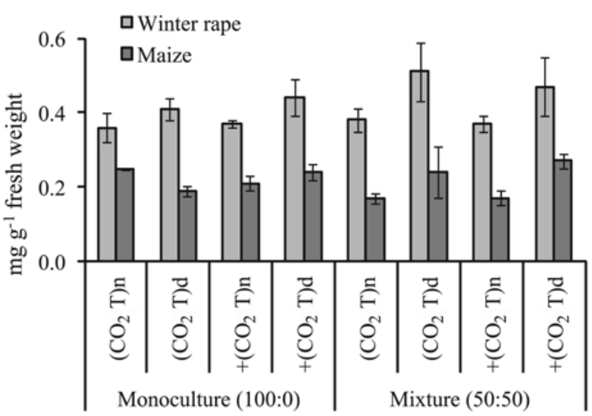
Moisture deficit had negative effect on assimilating leaf area of winter rape monoculture, it decreased 78% at +CO_2 T and 42% at CO_2 T (Table). Winter rape in mixture produced significantly greater leaf area than monoculture plants, where substrate moisture was sufficient, and elevated CO_2 and temperature had a greater positive effect. Elevated conditions (+CO_2 T) had a significant positive effect on assimilating leaf area of maize monoculture, when substrate water was sufficient. However, substrate drought had a significant negative impact on the assimilating leaf area of maize monoculture. Maize plants, grown in a mixture with winter rape, had a significantly reduced assimilating leaf area both in CO_2 T and +CO_2 T conditions as compared to maize monoculture, also insufficient water content had a greater negative effect.

In the leaves of winter rape plants grown in monoculture, insignificant increase in chlorophyll *a* content was observed for plants exposed to water deficit under CO_2 T and +CO_2 T conditions (Fig. 1). Whereas, winter rape in mixture accumulated a significantly greater amount of chlorophyll *a* than reference plants, when there was insuficient water content in substrate at both CO_2 T and +CO_2 T conditions. Interestingly, maize plants accumulated significantly lower content of chlorophyll *a* both in monoculture and mixture under elevated (+CO_2 T) conditions, when water supply was sufficient. No significant impact on chlorophyll *a* was determined for other treatments.



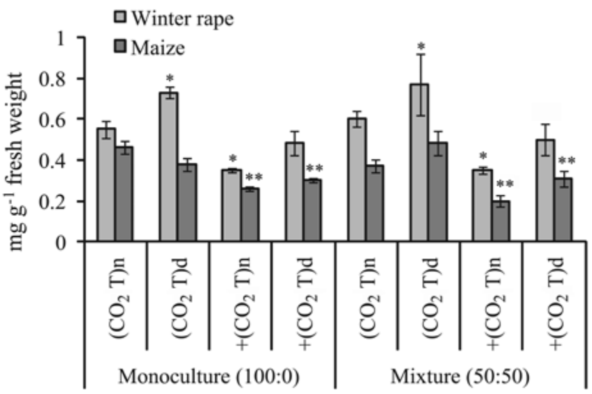
Note. Significant differences from reference plants (CO_2 T)n monoculture are denoted by asterisks * – $p \leq 0.05$ and ** – $p \leq 0.01$; n – normal (irrigated), d – drought (non-irrigated).

Figure 1. Chlorophyll a content of winter rape and maize plants in monocultures (100:0, 0:100) and mixtures (50:50) exposed to ambient (CO_2 T – CO_2 350 ppm, T 21/14°C) and enhanced (+CO_2 T – CO_2 700 ppm, T 30/23°C) environment conditions and different substrate moisture



n – normal (irrigated), d – drought (non-irrigated)

Figure 2. Chlorophyll *b* content of winter rape and maize plants in monocultures (100:0, 0:100) and mixtures (50:50) exposed to ambient (CO_2 T – CO_2 350 ppm, T 21/14°C) and enhanced (+CO_2 T – CO_2 700 ppm, T 30/23°C) environment conditions and different substrate moisture



Note. Significant differences from reference plants (CO_2 T)n monoculture are denoted by asterisks * – $p \leq 0.05$ and ** – $p \leq 0.01$; n – normal (irrigated), d – drought (non-irrigated).

Figure 3. Carotenoid content of winter rape and maize plants in monocultures (100:0, 0:100) and mixtures (50:50) exposed to ambient (CO_2 T – CO_2 350 ppm, T 21/14°C) and enhanced (+CO_2 T – CO_2 700 ppm, T 30/23°C) environment conditions and different substrate moisture

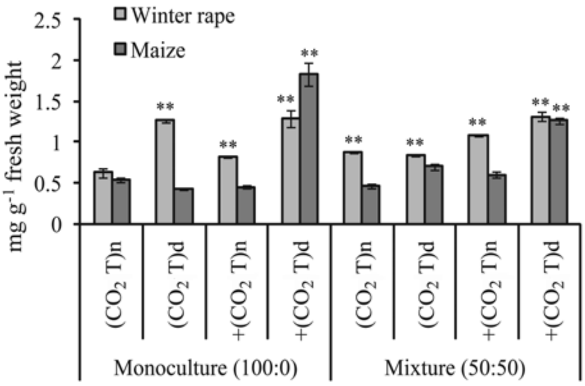
No significant differences in chlorophyll *b* content were determined either in winter rape or maize plants in monoculture and mixture among treatments (Fig. 2).

Drought conditions in the substrate significantly increased the carotenoid content in winter rape leaves grown in monoculture and mixture under CO_2 T conditions (Fig. 3). However, in winter rape monoculture or mixture a significantly lower content of carotenoids was observed when plants were grown in irrigated substrate and +CO_2 T conditions. Maize plants, in monoculture or mixture, grown under +CO_2 T conditions had a significantly lower carotenoid content both in irrigated and non-irigated substrate as compared to reference maize plants.

In winter rape leaves, the ascorbic acid content was determined significantly greater in monoculture and mixture plants grown under CO_2 T or +CO_2 T environment conditions independet of soil water treatment (Fig. 4). In maize monoculture or mixture, a significantly greater amount of ascorbic acid, as compared to the reference maize plants, was determined only in plants grown under +CO_2 T and exposed to water stress conditions.

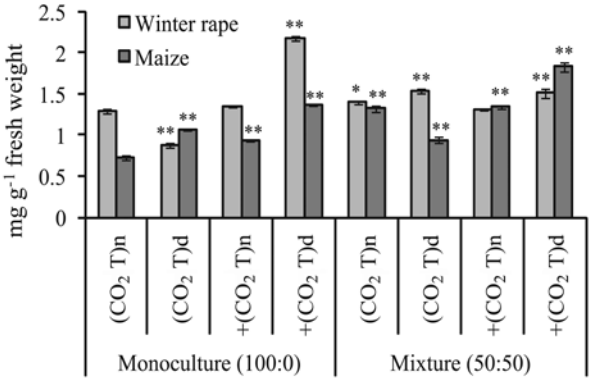
In the leaves of winter rape plants grown in monoculture, significant decrease in total phenolic compounds was observed for plants exposed to water deficit under CO_2 T conditions. On the contrary, significant increase in total phenolic compounds was observed in winter rape monoculture leaves, under water deficit and +CO_2 T conditions (Fig. 5).

Compared to the reference plants (monoculture; CO_2 T), winter rape grown in mixture with maize, had a



Note. Significant differences from reference plants (CO_2 T) n monoculture are denoted by an asterisk ** – $p \leq 0.01$; n – normal (irrigated), d – drought (non-irigated).

Figure 4. Ascorbic acid content of winter rape and maize plants in monocultures (100:0, 0:100) and mixtures (50:50) exposed to ambient (CO_2 T – CO_2 350 ppm, T 21/14°C) and enhanced (+CO_2 T – CO_2 700 ppm, T 30/23°C) environment conditions and different substrate moisture

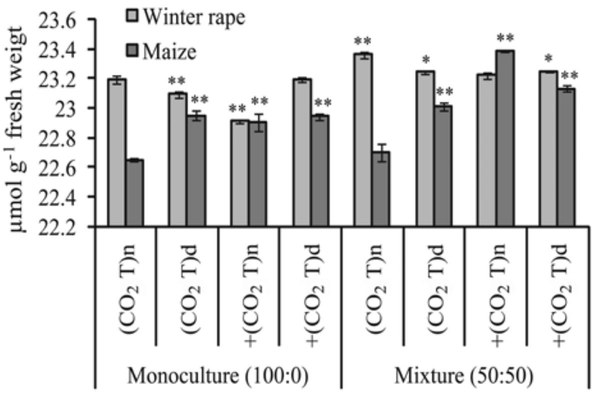


Note. Significant differences from reference plants (CO_2 T)n monoculture are denoted by asterisks * – $p \leq 0.05$ and ** – $p \leq 0.01$; n – normal (irrigated), d – drought (non-irigated).

Figure 5. Total phenolic compound content of winter rape and maize plants in monocultures (100:0, 0:100) and mixtures (50:50) exposed to ambient (CO_2 T – CO_2 350 ppm, T 21/14°C) and enhanced (+CO_2 T – CO_2 700 ppm, T 30/23°C) environment conditions and different substrate moisture

significantly greater content of total phenolic compounds in their leaves. In maize monoculture and mixture, significantly greater content of total phenolic compounds as compared to reference plants (monoculture, CO_2 T) was determined in plant leaves independet of CO_2 T and water treatment

The antioxidant activity in plants was measured by free radical scavenging – DPPH method. The results showed that the winter rape plants differed in their capacity to quench or inhibit free radical DPPH under different environment conditions (Fig. 6). Maize plants grown in monoculture or mixture had a greater capacity to quench DPPH radicals as compared to the reference maize plants.



Note. Significant differences from reference plants (CO_2 T)n monoculture are denoted by asterisks * – $p \leq 0.05$ and ** – $p \leq 0.01$; n – normal (irrigated), d – drought (non-irigated).

Figure 6. DPPH-free radical scavenging capacity of winter rape and maize plants in monocultures (100:0, 0:100) and mixtures (50:50) exposed to ambient (CO_2 T – CO_2 350 ppm, T 21/14°C) and enhanced (+CO_2 T – CO_2 700 ppm, T 30/23°C) environment conditions and different substrate moisture

Disccusion

Elevated CO_2 and temperature, and water availability in the substrate influenced the growth of winter rape (C_3) and maize (C_4) plants in monocultures and mixtures. According to the obtained results, elevated CO_2 and temperature increased the fresh mass of maize grown in monoculture but not of winter rape, under sufficient water supply (Table). It can be argued that enhanced CO_2 and temperature, and soil water availability influenced winter rape mass production similarly in monocultures and mixtures, conversely, growth response of the maize plants to CO_2 and temperature enrichment and water availability was reduced in mixtures compared to monocultures. Winter rape was a strong competitor to maize when grown in mixture. Assimilating leaf area is important in capturing light and is a key factor in competitive outcomes between plants. In winter rape and maize mixture (50:50), elevated CO_2 and temperature significantly increased the assimilating leaf area of winter rape under sufficient moisture content in substrate (Table). The increment of winter rape leaf area significantly reduced the competitive ability of maize plants. As a consequence, the potential loss in maize assimilating leaf area resulting from competition with winter rape actually increased under elevated CO_2 and temperature, moreover, the lack of soil moisture enhanced this effect. When comparing the data obtained for winter rape and maize monoculture, sufficient water supply, elevated CO_2 and temperature had greater effect on maize assimilating leaf area as compared to winter rape. It is generally assumed that growth of C_4 plants will not respond to elevated atmospheric CO_2 concentrations. However, a number of studies have shown enhanced photosynthesis and/or growth of C_4 plants under elevated atmospheric CO_2 (Cousins et al., 2001; Rogers et al., 2008; Souza et al., 2008). Typically, the increased growth response to elevated atmospheric CO_2 of C_4 plants is attributed to the indirect CO_2 effect of stomatal closure and subsequent increased water use efficiency and water potential (Ghannoum, 2009). Also it is possible that enhanced productivity of C_4 crops is in part due to the fact that younger leaves are more C_3-like and are thus more sensitive to elevated CO_2. Indeed, it has previously been observed that C_4 photosynthesis in young fully expanded *Sorghum bicolor* leaves was more responsive than older leaves to growth under elevated atmospheric CO_2 in a free-air CO_2 enrichment (FACE) experiment (Cousins et al., 2001).

Aboveground performance of individual C_4 (maize) but not C_3 (winter rape) plants was reduced in mixtures compared to monocultures, implying that winter rape was the superior competitor in mixtures (Table). Contrary to our results, Derner and co-authors (2003) predict that in monoculture, CO_2 enrichment increased height, leaf area, above-ground mass and reproductive output of cotton (C_3), but not in sorghum (C_4), and was independent of soil water treatment. In mixtures of cotton and sorghum, above-ground mass and height of cotton were generally reduced compared to monocultures, across both CO_2 and soil water treatment. Other authors also predict that environmental stresses (e.g., soil water, nutrient availability) generally reduce the response of C_3, but not C_4, plants to CO_2 (Oliver et al., 2009; Bernacki, 2012), suggesting that C_4 plants will maintain their competitive advantage over C_3 plants in CO_2-enriched environments. Thus interspecific competition may moderate the growth response of plants to CO_2 and temperature enrichment, but the magnitude of the effects is likely influenced by soil water conditions.

Photosynthesis is one of the main physiological processes that determine plant productivity. An efficient operation of the photosynthetic apparatus ensures optimal photosynthetic pigment content and ratio. Photosynthetic pigments (chlorophyll *a*, *b* and carotenoids) are indicators for the mass and the capacity of photosynthesis, their concentrations also reflect the physiological status of plants. In our experiments, the substrate drought factor had a stimulating effect on chlorophylls in leaves of winter rape in monocultures and mixtures (Figs. 1–2). This indicates that plants experience stress and to survive in these conditions, accumulate higher levels of photosynthetic pigments. For maize plants in monoculture, lower content of photosynthetic pigments was determined in the leaves under elevated CO_2 and temperature; however, efficient photosynhetic apparatus capacity was ensured (Figs. 1–3).Winter rape was a serious competitor for maize plants in a mixture, the same, or in some cases lower photosynthetic pigment content in maize leaves in comparison with reference maize plants was unable to warrant an effective activity of photosynthesis. There are a number of reasons why maize plants might not have the same ability to acclimate to elevated CO_2, temperature and drought stress and to compete with winter rape when grown in mixture. C_4 plants have substantial acclimation potential, but in most cases lag behind the acclimation responses in C_3 plants. Certain features unique to C_4 photosynthesis may reduce the potential for phenotypic plasticity and photosynthetic acclimation to environmental change relative to what is possible with C_3 photosynthesis. During acclimation, the structural and physiological integrity of the mesophyll-bundle sheath (M-BS) complex has to be maintained if C_4 photosynthesis is to function efficiently in the new environment. Disruption of the M-BS structure could interfere with metabolic co-ordination between the C_3 and C_4 cycles, decrease metabolite flow rate between the tissues, increase CO_2 leakage from the bundle sheath, and slow enzyme activity. For example, some C_4 species are unable to maintain high quantum yields when grown in low-light conditions. Others fail to reduce carboxylase content in shade, leaving substantial over-capacity of Rubisco and phosphoenolpyruvate (PEP) carboxylase in place (Sage, McKown, 2006). Maize plants are sun-adapted; therefore when grown in mixture they may not acclimate to lower light because of winter rape shading and may not be able to complete their life cycle in the shade of abundant winter rape canopy. However, the metabolic and structural requirements of the C_4 pathway may have decreased the ability of maize to overcome winter rape plants and to modify the photosynthetic apparatus in order to acclimate and improve performance in changed environment conditions.

Differential sensitivity of winter rape and maize plants to changed environmental factors might be associated with their variable capacity to deal with oxidative stress. Changes in environmental factors can easily disturb the steady-state redox balance in plants by causing a rapid increase in reactive oxygen species generation. Mechanisms of active oxygen species detoxification exist in all the plants and include activation of enzymatic (superoxide dismuatase, catalase, ascorbate peroxidase, peroxidase and glutathione reductase) as well as non-enzymatic (ascorbic acid, carotenoids, glutathione and tocopherols) antioxidants (Ashraf, 2009). The antioxidant properties revealed that the both plant species had varying ability to deal with oxidative stress that might govern their differential response to elevated CO_2, temperature and substrate drought conditions. The increase of ascorbic acid was noticeable both in winter rape monoculture and in mixture under elevated CO_2 and temperature in all water regimes (Fig. 4). Ascorbic acid is one of the most abundant antioxidants in plant tissue and high levels of ascorbic acid are essential to oxidative stress tolerance (Chen, Gallie, 2005). Maize plants, either in monoculture or mixture, accumulated greater amount of ascorbic acid at elevated CO_2 and temperature when water content in substrate was insuficient. In maize plants phenolic compound accumulation and DPPH-free radical scavenging capacity were more expressed than in winter rape plants (Figs. 5–6). The degree to which the activities of antioxidants enzymes and the amount of antioxidants are elevated under stressful conditions is extremely variable among several plant species (Nayyar, Gupta, 2006) and even between cultivars of the same species (Pérez-López et al., 2009; Uzilday et al., 2011). An increase in phenolic compounds and antioxidant activity was detected in winter rape when grown in mixture. The higher levels of ascorbic acid, total phenolic compounds and changes in total antioxidant capacity in winter rape reflected their greater involvement in winter rape plants to counter oxidative stress as compared to maize plants when plants were grown in mixture.

Conclusions

1. Under sufficient water supply, elevated CO_2 and temperature significantly increased the assimilating leaf area (21%) and total fresh mass (22%) of maize monoculture but the effect on dry mass was insignificant. The water deficit, irrespective of CO_2 and temperature level, resulted in a significant reduction of assimilating leaf area and fresh mass of maize and winter rape monoculture and had insignificant effect on their dry mass.

2. In mixture (winter rape:maize, 50:50), winter rape (C_3 plant) posed a strong competition to maize (C_4) plants in early growth stages. Assimilating leaf area and fresh mass per plant of maize were reduced in mixtures compared to monocultures, independent of water availability in substrate and environment conditions (CO_2 350 ppm, T 21/14°C – CO_2 T or CO_2 700 ppm, T 30/23°C – +CO_2 T). A significantly greater assimilating leaf area and amount of fresh mass were observed for C_3 (winter rape) under ambient or elevated CO_2 and temperature and sufficient substrate moisture content. The total fresh mass of winter rape was significantly reduced when water content was insuficient in substrate regardless of CO_2 concentration and temperature.

3. The different ability of winter rape and maize plants to modify the photosynthetic apparatus in order to acclimate and improve performance in changed environment conditions, may influence the competition. In mixture, winter rape plants accumulated greater amount of chlorophyll *a* and carotenoids when substrate water content was deficient under CO_2 T or +CO_2 T conditions. Maize plants accumulated signifficantly less chlorophyll *a* and carotedoinds under +CO_2 T and sufficient water supply and significantly less carotenoids under +CO_2 T and water deficit.

4. The different total antioxidant properties were responsible for the varying ability of winter rape and maize grown in mixture to deal with oxidative stress. The higher levels of ascorbic acid and total phenolic compounds in winter rape (C_3) reflect their greater involvement to counter oxidative stress. In maize (C_4) plants, phenolic compound accumulation was more expressed than in winter rape plants.

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Žieminių rapsų (C_3) ir kukurūzų (C_4) konkurencija esant didesnei anglies dioksido koncentracijai ir temperatūrai bei vandens trūkumui

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Santrauka

Tyrimo tikslas – įvertinti sausros streso įtaką žieminių rapsų ir kukurūzų konkurencijai, kai aplinkoje buvo didesnė CO_2 koncentracija ir temperatūra. Tyrimai atlikti Lietuvos agrarinių ir miškų mokslų centro Sodininkystės ir daržininkystės instituto reguliuojamo klimato kamerose. Eksperimento metu monokultūriniai augalai (100:0) arba žieminių rapsų ir kukurūzų mišiniai (50:50) augo skirtingose aplinkose: 1) esamo klimato sąlygomis, kai temperatūra buvo 21/14 °C dieną/naktį, CO_2 – 350 ppm (CO_2 T) ir 2) kintančio klimato sąlygomis, kai temperatūra buvo 30/23 °C dieną/naktį, CO_2 – 700 ppm (+CO_2 T). Abiejose kamerose auginamiems augalams buvo palaikomi du skirtingi substrato drėgnio lygiai.
Kukurūzai (C_4) konkurencijoje su žieminiais rapsais (C_3) išaugino žymiai mažesnį asimiliacinį lapų plotą ir sukaupė šviežios masės kiekį, palyginus su monokultūriniais augalais abiejose aplinkose (CO_2 T ir +CO_2 T), o neigiamą įtaką dar sustiprino nepakankamas kiekis vandens. Lyginant žieminių rapsų ir kukurūzų monokultūrinius augalus, +CO_2 T veiksniai, kai substrate buvo pakankamas kiekis drėgmės, turėjo žymią teigiamą įtaką kukurūzų asimiliacinio ploto augimui (padidėjo ~21%) ir masės kaupimuisi (padidėjo ~22%). Kai substrate buvo nepakankamas kiekis vandens, žieminių rapsų ir kukurūzų monokultūriniai augalai, nepriklausomai nuo aplinkos, išaugino žymiai mažesnį asimiliacinį lapų plotą ir sukaupė žalios masės. Vandens trūkumas substrate skatino kauptis chlorofilus lapuose žieminių rapsų, kurie augo kaip monokultūra arba konkuruodami su kukurūzais. Nustatytas reikšmingai sumažėjęs chlorofilo *a* kiekis kukurūzų monokultūrinių augalų lapuose +CO_2 T aplinkoje, kai vandens kiekis substrate buvo pakankamas. Esant +CO_2 T aplinkai kukurūzai (monokultūros ir mišinio) lapuose kaupė mažesnį kiekį karotenoidų ir trūkstant vandens, ir substrate esant pakankamam jo kiekiui. Žieminiai rapsai kaip monokultūra arba konkuruodami su kukurūzais kaupė reikšmingai didesnį kiekį karotenoidų, kai substrate trūko vandens, o CO_2 ir temperatūra buvo nepakitę. Esant +CO_2 T aplinkai, kai substrate buvo pakankamas kiekis vandens, rapsų augalai (monokultūros ir mišinio) sukaupė žymiai mažesnį kiekį karotenoidų.
Nustatyti didesni kiekiai askorbo rūgšties bei fenolinių junginių ir antioksidacinis potencialas rodo didesnę žieminių rapsų gebą kovoti su oksidaciniu stresu.

Reikšminiai žodžiai: antioksidantai, augimo rodikliai, *Brassica napus*, fotosintezės pigmentai, *Zea mays*.

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