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Interactive effects of elevated CO₂, O₃, and soil water deficit on spring wheat (*Triticum aestivum* L. cv. Nandu)

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Abstract – This study investigated the interactive effects of elevated carbon dioxide (CO₂), ozone (O₃), and soil water deficit on spring wheat (*Triticum aestivum* L. cv. Nandu). Plants were grown in open-top chambers (OTCs) which were arranged in a 2 × 2 factorial design consisting of two O₃ levels (ambient and 1.5-times ambient) in combination with two CO₂ levels (ambient and ambient plus 320 μmol mol⁻¹ CO₂). Subplots in each OTC were subjected to two levels of watering, well-watered and restricted water supply. Total leaf conductance (g_L), growth and biochemical analyses were determined to assess plant responses. The relative reduction in g_L due to CO₂, soil water deficit and/or O₃ depended on plant growth stage. However, complex interactions between the experimentally controlled factors occurred on many days. Reduced water supply resulted in growth reduction but provided some protection against the loss in dry weight caused by O₃. Elevated CO₂ stimulated plant growth, but no interactive effects between CO₂, soil water deficit and/or O₃ were observed, suggesting that elevated CO₂ reduced the adverse effects of O₃ and/or soil water deficit in an additive manner. The complex nature of the multiple stress interactions involved was also illustrated by the responses of specific biochemical variables (carbohydrates, proline, pigments). © 1999 Inra/Éditions scientifiques et médicales Elsevier SAS

carbon dioxide / ozone / soil water deficit / multiple stress interaction / wheat

Résumé – Effets interactifs d'une élévation de la concentration en CO₂ et en O₃ et du déficit hydrique du sol sur le blé de printemps (*Triticum aestivum* L. cv. Nandu). Les plantes ont été cultivées dans des enceintes à toit ouvert (OTC) selon un plan factoriel 2 × 2 comprenant deux niveaux d'ozone (niveau ambiant (n.a.) et n.a. × 1,5) combinés à deux niveaux de CO₂ (n.a. et n.a. + 320 μL mol⁻¹ CO₂). Dans chaque OTC des sous-ensembles ont reçu soit un arrosage suffisant, soit une alimentation hydrique restreinte. La conductance totale des feuilles (g_L), des mesures de la croissance

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et des analyses biochimiques ont été effectuées de manière à estimer la réponse des plantes. La réduction relative de g_L due au CO_2 , au déficit hydrique du sol et/ou à l'ozone dépend du stade de croissance des plantes. Cependant, des interactions complexes entre les facteurs contrôlés expérimentalement se sont produites au cours de nombreuses journées. La réduction de l'alimentation en eau a entraîné une diminution de la croissance mais a fourni également une certaine protection contre la perte de poids de matière sèche causée par l'ozone. L'élévation de la concentration en CO_2 a stimulé la croissance des plantes, mais aucun effet interactif n'a été observé entre le CO_2 , le déficit hydrique du sol et/ou l'ozone, suggérant ainsi que la forte concentration en CO_2 réduit les effets négatifs de l'ozone et/ou du déficit hydrique de manière additive. La nature complexe des interactions des multiples contraintes étudiées a aussi été illustrée par la réponse de variables biochimiques spécifiques (hydrates de carbone, proline, pigments). © 1999 Inra/Éditions scientifiques et médicales Elsevier SAS

gaz carbonique / ozone / déficit hydrique du sol / interaction de contraintes multiples / blé

1. Introduction

Anthropogenic activities have changed the chemical composition of the global atmosphere since the 19th century. The atmospheric CO_2 concentration has increased steadily from a pre-industrial level of about $280 \mu\text{mol mol}^{-1}$ to its current level of about $360 \mu\text{mol mol}^{-1}$ and is expected to continue rising for the foreseeable future [24]. Numerous studies have demonstrated that elevated CO_2 concentrations stimulate the rate of photosynthesis, reduce the rate of photorespiration, and enhance plant growth and productivity. Exposure to elevated CO_2 generally reduces stomatal conductance and thus transpiration declines, resulting in improved water-use efficiency at the leaf level [11, 39]. The majority of studies on the responses of plants to elevated CO_2 have been conducted under favourable environmental conditions, and little information exists in relation to the way in which plants will respond to rising CO_2 in the field, where they are subjected to various stresses including low/high temperature, UV-B radiation, drought and air pollutants [4, 26]. In their review on the impacts of CO_2 enrichment Idso and Idso [23] provided evidence that the percentage increase in plant growth may be greatest when resources are limiting or when plants are subjected to environmental stresses. However, experimental evidence to support this view is still scarce and researchers have only recently begun to attempt to address the combined effects of elevated CO_2 and other environmental constraints on plants.

Tropospheric O_3 is currently regarded as the most pervasive air pollutant in many industrialized regions. Plant responses to O_3 include visible leaf damage, premature leaf senescence, impairment of photosynthesis and carbon allocation, and reduced growth and biomass. Current ambient levels of O_3 are known to be high enough to suppress the yield of sensitive crops in many parts of Europe, the USA and Asia [16, 19, 25, 29]. The importance of considering concomitant changes in the atmospheric CO_2 concentration and tropospheric O_3 in the prediction of crop responses to a changing climate was first emphasized by Allen [1]. He assumed that partial stomatal closure induced by high $[\text{CO}_2]$ may reduce the impacts of O_3 , and there is now some experimental evidence to support this view [5, 20, 21, 28, 31, 33, 37, 40]. Heagle et al. [20] have shown, however, that the protection afforded by elevated CO_2 can diminish after prolonged O_3 exposure. Conversely, O_3 has been reported to reduce the stimulation in growth or yield resulting from CO_2 enrichment in wheat [6, 47], although this effect seems to depend on the O_3 sensitivity of the respective crop plant and growth conditions [22, 47]. In the recent study of McKee et al. [28] elevated CO_2 was found to provide a complete protection against the detrimental effects of O_3 on wheat growth, but not on yield. In contrast, Barnes et al. [6] found no protection against O_3 in high CO_2 -grown wheat, and suggested that CO_2 enrichment might make plants more susceptible to O_3 damage at the cellular level. High concentrations of O_3 are mostly associated with hot sunny weather and therefore tend to coincide with periods of soil

water deficit. Similar to O₃ stress, elevated CO₂ has often been shown to afford protection against the negative effects of soil water deficit on plant growth. For wheat, several studies [10, 17, 41] have reported that the relative response of plants to atmospheric CO₂ is greater under water-limited conditions than well-watered conditions.

Overall, these few examples illustrate the need for an improved understanding of multiple stress interactions involved in the modification of plant responses to predicted changes in climate and atmosphere. In particular, little is known about the interactive effects of CO₂ enrichment, soil water deficit and tropospheric O₃ on crop growth and physiology. The object of our study was therefore to determine the effects of O₃ and soil water deficit on spring wheat grown under present and elevated CO₂ concentrations. This paper documents the effects of the various treatment combinations on plant growth and development including stomatal responses and biochemical indicators (chlorophyll, proline and carbohydrates).

2. Material and methods

2.1. Plant culture and experimental conditions

Spring wheat (*Triticum aestivum* L. cv. Nandu) was planted in 3.1 dm³ pots (10 cm diam. × 40 cm height) on the 8th June, 1994. Potting medium was a 3:1 mixture (by volume) of a sandy loam and commercial sand, respectively. The loam was excavated from a field located at FAL in Braunschweig, Germany. Plants were thinned to a final density of one plant per pot on the 20th June and then moved in to non-filtered open-top chambers (OTCs) two days later and exposed to CO₂ and/or O₃. Plants were fertilized on the 19th July and the 1st August with N:P:K fertilizer (100 kg N, 60 kg K and 40 kg P ha⁻¹, respectively) based on soil test results. On the 29th July, plants were sprayed with insecticide (Pirimicarb) and a cocktail of fungicide (Tebuconazol, Triadimenol, and Anilazin) to control an outbreak of aphids and powdery mildew that occurred in two OTCs.

Irrigation needs were based on soil moisture tension as determined by tensiometers installed at a depth of 20 cm. All plants were kept well watered until 36 DAP (days after planting). Subsequently, four plants in each chamber received sufficient water to maintain soil mois-

ture tension at 150 mb (or below). According to soil moisture release curves, these pots were maintained at or near, pot capacity (% water). The remaining four pots in each chamber received half the volume of water delivered to the well-watered plants. Water was applied to the soil surface. Uptake of water from beneath was prevented by a 10-cm styrofoam platform. Pot temperature was moderated by a sheath of aluminum foil which effectively restricted soil temperature increases under chamber conditions.

Eight open-top chambers (for details see Weigel et al. [46]) were arranged in a randomized complete block design with two blocks based on relative shading from adjacent trees. A split-plot design was employed with chambers considered as whole-plots and sub-plots comprising the two watering treatments within each chamber. Treatments were arranged in a 2 × 2 factorial design

Figure 1. Seasonal daily mean CO₂ and O₃ concentrations inside open-top chambers for non-filtered, ambient air and elevated CO₂ and/or O₃ treatments (averaged across all chambers independent of CO₂ and O₃ additions, respectively).

(two O₃ levels × two CO₂ levels). Ozone levels were non-filtered (NF) ambient air and about 1.5 times NF, while CO₂ levels were ambient air and ambient air plus 320 μmol mol⁻¹ CO₂. Ozone and CO₂ enrichment operated 24 hours per day throughout the entire experiment. The seasonal courses of the 24-h mean CO₂ and O₃ concentrations (averaged across all chambers with CO₂ and O₃ addition, respectively) are shown in *figure 1*. Variation in ambient wind velocity and dispensing adjustments resulted in some deviation of the target CO₂ concentrations at the beginning and the end of the experiment. Mean CO₂ concentrations over the period of the experiment were 395 μmol mol⁻¹ and 720 μmol mol⁻¹ in ambient and elevated treatments, respectively. The 24-h seasonal mean O₃ concentration was 28 nmol mol⁻¹ (NF) and 40 nmol mol⁻¹ (1.5 × NF), corresponding to 12-h daily means of 36 nmol mol⁻¹ (NF) and 53 nmol mol⁻¹ (1.5 × NF). Seasonal 24 h day⁻¹ mean concentrations of SO₂, NO and NO₂ were lower than 5 nmol mol⁻¹ in the various treatments.

2.2. Stomatal conductance

Beginning at 29 DAP stomatal diffusive resistance to water vapour (r_s) was measured separately on both surfaces of the youngest fully expanded leaf on the main stem [44] using a Li-1600 steady state porometer (Li-Cor, Lincoln, NE). Measurements were made on the mid-portion of the leaf lamina between ± 1 h of solar noon on each clear day (PAR > 800 μmol m⁻² s⁻¹). Blocks were sampled in numerical order. Chambers were randomly sampled within each block. On every measurement day, two or more replicate plants were measured in each subplot. Leaf conductance to water vapour (g_s) was calculated as the reciprocal of r_s . Results are presented as total leaf conductance (g_L , i.e., $g_{S \text{ abaxial}} + g_{S \text{ adaxial}}$).

2.3. Harvest

Eight plants per treatment in each chamber in block 1 were harvested 70 DAP, and in block 2 71 DAP (i.e., plants had completed anthesis [44]). Between 10:00 and 11:00 ECT, four flag leaves were removed from each plant, weighed and leaf area determined using a leaf area meter (Li-COR 3000). Each was then cut in to pieces, wrapped in aluminum foil, and immediately shock-frozen in liquid nitrogen. Samples were stored at -60 °C until they were ground in liquid nitrogen and later lyophilized for carbohydrate analysis. Sub-samples of frozen leaf tissue were used for chlorophyll and proline analyses.

The remainder of each plant was separated in to tillers, leaves, and ears. Leaf sheaths were considered part of the tiller fraction. After the number of tillers, leaves, and ears were counted and green leaf area determined, plant material was dried at 90 °C for two days and then weighed. Root dry weight was determined from four plants per treatment and chamber, respectively, after removing the soil column and washing the roots.

2.4. Carbohydrate, chlorophyll and proline analysis

Carbohydrate concentrations (starch, glucose, fructose) were determined enzymatically on approximately 100 mg of lyophilized leaf tissue using commercially available reaction kits (Boehringer GmbH, Mannheim, Germany). Total nonstructural carbohydrate content (TNC) was considered to represent the sum of starch, glucose, and fructose (incl. fructans) fractions.

For chlorophyll analysis, 100 mg fresh weight of flag leaf was homogenized in 10 mL 80 % acetone. After centrifugation (10 min at 10 000 × *g*) chlorophyll a and b concentrations were determined according to Ziegler and Egle [48]. Proline concentrations were determined according to the method of Bates et al. [7].

2.5. Statistical Analysis

For harvest data, significant effects were determined by analysis of variance using the General Linear Models (GLM) procedure of SAS (SAS Institute, Cary, NC). Whenever the split-plot and sub-sampling errors were found not to be significantly different for individual response variables, the two errors were pooled to test the effects of water supply and all interactions with water. To assess treatment effects on stomatal conductance, an analysis of variance was performed separately for each measurement day and for each developmental stage. To combine measurements across the growing season, data were subjected to repeated measures analysis of variance.

3. Results and discussion

3.1. Stomatal conductance

In general, daily measurements showed that total stomatal conductance tended to be decreased by

elevated CO₂ and soil water deficit. However, there was considerable ontogenetic and temporal variability (figure 2), and main effects were not consistent across the season (table 1). The CO₂ effect appeared to be predominant early in the study, while the effect of the soil water deficit was most apparent late in the season (table 1). Although O₃ reduced stomatal conductance significantly ($P < 0.05$) on only one measurement day (DAP 46, table 1), O₃-treated plants tended to exhibit lower conductance than NF plants throughout the study. This would be consistent with results from other studies where partial stomatal closure has been found in plants exposed to O₃, but usually at higher O₃ concentrations [12, 27]. However, the significant O₃-induced reduction in g_L occurred on a day where O₃ reached peak values. Significant ($P < 0.05$) CO₂ × O₃ interactions were detected 35 and 46 DAPs (table 1). In both instances, the relative reduction in stomatal conductance due to CO₂ was greater in NF than enhanced O₃. These observations suggest that the combined effects of CO₂ and O₃ on stomatal conductance may be not consistent over time and may not always result in simply additive responses. The time-dependent effect of CO₂ and O₃ on leaf conductance is similar to that reported previously for soybean [15] and spring wheat cv. Minaret [33], suggesting that the

Figure 2. Mean midday leaf conductance (g_L) from tillering through anthesis in well-watered and water-deficient wheat plants for each CO₂ and O₃ treatment (NFA: non-filtered (NF), ambient air, NF+: NF ambient air + CO₂, OZA: NF ambient air + O₃, OZ+: NF ambient air + CO₂ and O₃).

Table 1. Summary of statistical analysis¹ of leaf conductance (g_L) measured on the flag leaf of wheat.

DAP ²	27	33	35	38	42	43	44	45	46	48	49	50	56
DOY ³	186	192	194	197	201	202	203	204	205	207	208	209	215
DAWS ⁴				2	6	7	8	9	10	12	13	14	20
O ₃	ns	ns	ns	ns	ns	ns	ns	ns	*	ns	ns	ns	ns
CO ₂	*	*	*	*	ns	*	ns	ns	*	ns	ns	ns	ns
O ₃ × CO ₂	ns	ns	*	ns	ns	ns	ns	ns	*	ns	ns	ns	ns
W ⁵	ns	ns	ns	*	*	ns	ns	ns	*	*	*	*	*
W × O ₃	ns	ns	ns	*	*	ns	*	*	*	ns	ns	ns	ns
W × CO ₂	ns	ns	ns	ns	ns	ns	ns	ns	*	ns	ns	ns	ns
W × O ₃ × CO ₂	ns	ns	ns	ns	*	ns	ns	*	*	*	ns	ns	ns

¹ Results are reported as significant at the 0.05 (*) probability level; ns denotes non-significance

² DAP = Days after planting

³ DOY = Day of year

⁴ DAWS = Days after initiation of water treatment

⁵ W = water treatment

effects of gaseous treatment combinations on g_L vary with plant development. In the present study, however, O_3 levels also varied considerably throughout the season (*figure 1*), which may have also influenced the combined effects of the gases on g_L . A water $\times$ O_3 interaction occurred on five measurement days (DAPs 38, 42, 44, 45, and 46, *table I*). Under NF conditions, stomatal conductance was reduced by soil water deficit treatments, but a similar effect was not observed in plants exposed to elevated O_3 and restricted water supply. These findings are consistent with the reported changes in g_s in beech [13, 35] and tomato [18] under the combination of O_3 and soil water deficit. Although water deficit affected stomatal conductance considerably more than O_3 , our data show that elevated O_3 may have caused some reduction in stomatal closure in wheat under soil water deficit.

When conductance data were further analyzed separately for each growth stage as shown in *figure 2*, it was found that the main effect of water supply was significant ($P < 0.05$) through anthesis, but CO_2 was significant only through booting (data not shown). In addition, a water- CO_2 interaction and the three-way interaction (water- O_3 - CO_2) were present during booting, an important growth stage representing a shift from vegetative growth to reproductive growth. Finally, when all stomatal conductance measurements were combined, there was strong evidence that water deficit reduced stomatal conductance throughout the entire experiment ($P < 0.001$, not shown), but no evidence that CO_2 had a season-long effect.

3.2. Growth and development

The effects of the respective treatments on growth-related parameters are shown in *table II*. Most harvest variables showed a negative response to reduced water inputs. Tiller number, tiller dry weight, leaf dry weight, and ear number (per plant) were significantly reduced due to water treatment, but these effects were not consistent across O_3 levels. For example, in the NF chambers restricted water supply resulted in a 23 % reduction in tiller

dry weight, while the relative reduction that occurred in the O_3 chambers was only 6 %. This response is in agreement with previous reports demonstrating that drought stress confers a degree of protection from O_3 damage in plants [32, 43]. Pell et al. [36] also reported significant protection against water-stressed induced declines in root, leaf, leaf area, and total dry weight of radish in O_3 -treated plants compared to well-watered controls. In contrast, Fangmeier et al. [14] did not detect any water supply by O_3 interactions for growth and yield responses measured in spring wheat. The reduced O_3 sensitivity of drought-stressed plants has often been found to be associated with reduced g_s and changes in osmoregulation [8, 38, 42]. Our leaf conductance data (*figure 2*) concur with these observations in showing that the water deficit treatments caused a reduction in leaf conductance, thus restricting O_3 uptake, although the $O_3 \times$ water deficit interactions that occurred on some days (*table I*) indicate that O_3 by itself may cause at least a partial stomatal opening during drought periods. However, there was no evidence from the growth data that this type of interaction on g_L would make O_3 -exposed plants more susceptible to soil water deficit.

Plants exposed to O_3 under ambient CO_2 under well-watered conditions developed typical visible symptoms of O_3 injury, consisting of chlorotic mottling and necrotic patches on fully expanded leaves. Visual estimates indicated that the extent of these O_3 symptoms was reduced by simultaneous exposure to elevated CO_2 (data not shown). In addition, soil water deficit and elevated O_3 significantly accelerated leaf senescence recorded as a reduction in green leaf area, but no significant interactions were found (*table II*).

CO_2 -enrichment resulted in an increase in tillering, tiller dry weight, ear number, ear dry weight, plant height, leaf area, and the number of leaves produced per plant. However, no interactive effects of elevated CO_2 with soil water deficit and/or O_3 were detectable. These results support the view that elevated CO_2 reduces the negative effects of environmental constraints on plant growth [23, 45]. In contrast to other studies [26, 39], no significant effects of the various treatments on root dry weight

Table 2. Mean¹ values and statistical significance⁵ of harvest variables measured under well-watered and water-deficient conditions and under varying ozone² and CO₂³ levels.

Ozone ³ /CO ₂ ⁴ Treatment	Leaf	Tiller	Root	Ear	Leaf	Tiller	Ear	Leaf area ⁴	Plant height
	g plant ⁻¹			no plant ⁻¹				cm ² plant ⁻¹	cm
				Well-Watered					
NFA	3.2	6.6	2.2	5.5	45	8	8	518	72
NF+	3.1	9.7	2.8	8.6	48	9	9	599	87
OZA	2.8	5.1	1.5	4.2	38	7	7	334	73
OZ+	3.0	7.5	2.9	7.1	48	8	8	487	82
				Water-Deficient					
NFA	2.8	5.2	1.9	5.5	35	6	6	415	71
NF+	2.7	7.4	2.6	8.2	42	7	7	411	82
OZA	3.0	4.8	2.2	5.3	37	6	6	273	66
OZ+	3.0	7.6	2.5	7.7	40	7	7	341	82
Source	Leaf	Tiller	Root	Ear	Leaf	Tiller	Ear	Leaf area	Plant height
O ₃	ns	ns	ns	ns	ns	ns	ns	*	ns
CO ₂	ns	*	ns	*	*	*	*	ns	*
O ₃ × CO ₂	ns	ns	ns	ns	ns	ns	ns	ns	ns
W ⁶	ns	*	ns	ns	*	*	*	*	*
W × O ₃	*	*	ns	ns	ns	ns	ns	ns	ns
W × CO ₂	ns	ns	ns	ns	ns	ns	ns	ns	ns
W × O ₃ × CO ₂	ns	ns	ns	ns	ns	ns	ns	ns	ns

¹ Each value is a mean of eight plants. Plants were harvested 70 days after planting² NF= nonfiltered air, OZ = 1.5 × ambient air plus 320 µmol mol⁻¹CO₂³ A = ambient CO₂, + = ambient air plus 320 µmol mol⁻¹ CO₂⁴ Green leaf area⁵ Results are reported as significant at the 0.05 (*) probability level; ns denotes non-significance⁶ W = water treatment

were detected. However, the restricted sampling available for the determination of treatment effects on root growth restricted the capacity to rigorously investigate possible impacts.

3.3. Biochemical responses

Table III shows the effects of the various treatment combinations on chlorophyll, proline and non-structural carbohydrate concentrations in flag leaves. These measurements were conducted to

assess plant responses at the leaf level (chlorophyll as a measure of leaf injury and senescence, TNC and proline as indicators for the impact of CO₂ and drought stress, respectively). The most remarkable effect on these biochemical indicators was found in plants subjected to reduced water supply. Plants that experienced restricted water supply had significantly ($P < 0.05$) higher concentrations in TNC, which could be regarded as a form of osmoregulation [30]. However, there was a trend ($P < 0.10$, not shown) for a water × CO₂ interaction. CO₂ enrichment resulted in higher TNC content in well-

Table III. Mean values and statistical significance¹ of chlorophyll, proline, and total nonstructural carbohydrate (TNC)² concentrations measured under well-watered and water-deficient conditions and under varying ozone and CO₂ levels at 70 days of planting.

Ozone ³ /CO ₂ ⁴	Chlorophyll a μg g ⁻¹	Chlorophyll b μg g ⁻¹	Proline μmol g ⁻¹	TNC mg g ⁻¹
Well-Watered				
NFA	1712	646	1342	22.2
NF+	1576	539	122	46.3
OZA	1542	517	1032	21.7
OZ+	1489	525	1341	44.2
Water-deficient				
NFA	2170	660	3145	46.5
NF+	1928	627	1213	52.7
OZA	1657	558	3304	55.2
OZ+	1714	596	2750	47.8
Source	Chlorophyll a	Chlorophyll b	Proline	TNC
O ₃	*	ns	ns	ns
CO ₂	ns	ns	ns	ns
O ₃ × CO ₂	ns	ns	ns	ns
W ⁵	*	*	*	*
W × O ₃	*	ns	ns	ns
W × CO ₂	ns	ns	ns	ns
W × O ₃ × CO ₂	ns	ns	ns	ns

¹ Results are reported as significant at the 0.05 (*) probability level; ns denotes non-significance

² TNC = starch + glucose + fructose

³ NF = nonfiltered air, OZ = 1.5 × ambient ozone

⁴ A = ambient; CO₂ + = ambient ozone

⁵ W = water treatment

watered plants, but not in plants subjected to restricted water supply.

Flag leaf chlorophyll content was unaffected by CO₂, but O₃ exposure resulted in a decline in chlorophyll a content under both ambient and elevated CO₂, which supports our observations on visible O₃ injury and accelerated leaf senescence. Contrasting effects on the pigment content of flag leaves were found under conditions of soil water deficit. Although it was shown that drought stress resulted in significant reductions in total green leaf area (*table II*), pigment contents in flag leaves of plants with reduced water supply were found to be higher compared to well-watered plants. Parallel

studies indicated a faster degradation of chlorophyll during flag leaf senescence (post-anthesis) of drought-stressed wheat [2, 34]. However, this cannot be established from the present data. Nevertheless, the accumulation of proline in the flag leaves suggested that the leaves experienced drought stress. There is extensive literature indicating that drought stress induces an accumulation of proline (e.g., reviewed in [9]), suggesting that the amino acid may be a useful early indicator of drought stress effects, although there is evidence in the literature that proline accumulation seems to occur only when plant growth is already retarded by drought stress [3].

4. Conclusion

The results of the present study, which is one of the few investigations that attempts to address possible impacts of elevated CO₂, O₃, and soil water deficit on plants, demonstrates the complex nature of interactions that can occur between the experimentally controlled factors. The data suggest that elevated CO₂ may reduce the negative effects of O₃ and/or soil water deficit on growth. Stomatal responses showed a high ontogenetic variability to the various treatment combinations, however, interactive effects on g_L occurred on many days. Significant O₃ × water supply interactions indicate that elevated O₃ may have caused some reduction in stomatal closure under soil water deficit, which would imply that O₃ may enhance the sensitivity of plants to soil water deficit. However, this was not seen in the growth responses. The present results may be useful indicators of the types of multiple stress interactions that can occur under future changes in atmospheric compositions. However, extrapolation of the results to field conditions where interactions are likely to be much more complex must be viewed with caution. The results draw attention for the need of further research to understand the complexity of multiple stress interactions involved in the modification of plant responses to predicted changes in climate and atmosphere.

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