

Ozone Effects on the Quality of Swiss Chard. Peri-Urban Crops a Case Study [†]

Susana Elvira ^{*}, Javier Sanz, Ignacio Gonzalez-Fernandez and Victoria Bermejo-Bermejo

Ecotoxicology of Air Pollution, Environmental Dept., Research Center for Energy, Environment and Technology (CIEMAT), 28040, Madrid, Spain; j.sanz@ciemat.es (J.S.); ignacio.gonzalez@ciemat.es (I.G.-F.); victoria.bermejo@ciemat.es (V.B.-B.)

^{*} Correspondence: Susana.elvira@ciemat.es

[†] Presented at the 2nd International Electronic Conference on Plant Sciences—10th Anniversary of Journal Plants, 1–15 December 2021. Available online: <https://iecps2021.sciforum.net/>.

Abstract: The results of this open-top chamber study show that the variety of chard cv. Fuenlabrada cultivated in the outskirts of Madrid can be considered moderately sensitive to ozone. A significant loss of marketable production, alteration of pigment concentration and alteration of nutritional quality was measured in the plants exposed to different ozone levels. Swiss chard has a high nutritional value due to its high macronutrient content and the presence of micronutrients essential for human health. The results of this study showed a significant reduction in the concentration of Mg and Ca in the commercial chard leaves of the local variety Fuenlabrada. These results suggest that ozone can induce a loss of nutritional quality in leafy crops with negative consequences for human health.

Keywords: ozone effects; global change; Swiss chard quality; nutrients; leafy crops

Citation: Elvira, S.; Sanz, J.; Gonzalez-Fernandez, I.; Bermejo-Bermejo, V. Ozone Effects on the Quality of Swiss Chard. Peri-Urban Crops a Case Study. *2021*, *1*, x. <https://doi.org/10.3390/xxxxx>

Academic Editor: Iker Aranjuelo

Published: 30 November 2021

Publisher's Note: MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.



Copyright: © 2021 by the authors. Submitted for possible open access publication under the terms and conditions of the Creative Commons Attribution (CC BY) license (<http://creativecommons.org/licenses/by/4.0/>).

1. Introduction

Tropospheric ozone (O₃) is an important greenhouse gas. It is considered one of the most damaging air pollutants to vegetation due to its phytotoxicity and prevalence at high concentrations over large areas globally [1–5]

The photochemical pollution in the Southern Europe is a subject of great environmental importance [6]. Despite the considerable improvements in air quality during the last decade in Europe, 80% of the urban areas were exposed to ozone levels exceeding the WHO guidelines. A recent study on ambient air quality in Spain shows a substantial improvement in the last 25 years, with an exception of tropospheric ozone [7]. The same trend has been observed in the Madrid air basin [8]. The ozone episodes in summer records high ozone concentrations in rural and urban stations [9,10].

The ozone impact assessments for crops have been mainly based on modelling conducted for changes on crop yield. The estimated global impact of O₃ on crop suggests a yield reduction in major crops by 3–7%, causing high economic losses [11–14]. However, most estimates of the impact of current O₃ levels on crop production do not include horticultural production. These crops cover small areas, but they are of great economic importance.

Horticultural crops have been identified as extremely sensitive to O₃ damage [1,15–18]. Ozone causes a wide variety of damage to agricultural crops, including visible damage, reduced photosynthesis, alterations in carbon allocation, and reduction in the quantity and quality of yield [1,17,19–21]. Visible leaf damage has been recorded in leafy crops, reaching in some cases a total loss of harvest in the field [15,22,23] The effects of chronic

exposure to O₃ on physiology or performance have been studied for species such as lettuce, spinach or palak [24–30]. However, little information is available about the risk of ozone effects on quality traits.

Swiss chard is consumed over the world, due to their organoleptic properties as well as their numerous benefits in human nutrition [31]. In addition, chard has high nutritional importance due to the content of fiber, vitamins ACEKB and minerals (calcium, iron, zinc phosphorus, magnesium, potassium, iron and manganese) [32–34].

Cumulative metrics for O₃ exposure have been used along with assumptions about minimum thresholds for damage to estimate crop yield losses due to O₃. Ozone threshold values (critical levels) have been established within the framework of the Convention on Long-Range Transboundary Air Pollution (CLRTAP, UNECE) for the protection of different vegetation types [35]. These critical levels have been the basis for defining the objective values for plant protection in the European Air Quality Directive (2008/50/EC) and are the main tools for O₃ risk assessment studies.

2. Material and Methods

2.1. Experimental Field Facilities and Plant Material

The ozone fumigation experiments were carried out in the open-top chamber (OTC) facility located in central Spain, Finca Experimental La Higuera (CSIC) (Santa Olalla, Toledo, 450 m.a.s.l.; 40°3' N, 4°26' W). Swiss chard plants were exposed to four O₃ treatments: charcoal filtered air (CFA), with over 50% filtration efficiency that kept O₃ levels below background concentrations; non-filtered air (NFA) reproducing current ambient levels; non-filtered air supplemented with 20 nl l⁻¹ O₃ (NFA+) and non-filtered air supplemented with 30 nl l⁻¹ O₃ (NFA++). Additional O₃ supply for NFA+ and NFA++ treatments was applied during 8 hours per day, 7 days a week using an O₃ generator system (Model 16, A2Z Ozone Systems Inc., Louisville, KY, USA) fed with pure oxygen. The experiment followed a random block design with four O₃ treatments, each replicated three times (three OTCs per O₃ treatment). More details about the facility are provided in Calvete-Sogo et al. (2014).

The study was carried out on a native variety of chard from Fuenlabrada. The seeds were grown according to greenhouse standards until the plants were large enough for transplantation. The plants were grown in pots and were exposed to different ozone treatments for 43 days (from 4 April to 25 May).

A risk assessment study of ambient ozone effects on a native variety of chard cv. Fuenlabrada was carried out at a peri-urban agricultural area, Parque Agrario de Fuenlabrada (Fuenlabrada, Madrid, 660 m.a.s.l., 40°17' N, 3°51' W). Commercial chard production was evaluated at end of the summer growing season.

In parallel, the impact of ambient ozone levels was investigated through quantification of visible lesions in the leaves of O₃ sensitive and tolerant varieties in an O₃ bioindicator garden. Environmental growing conditions and ozone levels were collected from the Air Quality Network of the Community of Madrid (Fuenlabrada station).

2.2. Physiological Measurements

2.2.1. Biomass

The biomass production was determined at the maximum development of the plant. All the leaves were collected and classified as commercial and non-commercial biomass. This classification was based on the absence or presence of lesions on the leaves. The fresh weight (FW) was determined for the total plant. A known FW sample from each category was dried in a ventilated oven at 65 °C for 48 h to obtain the dry weight (DW). Data were analyzed based on total biomass and commercial biomass per plant.

2.2.2. Foliar Pigment Content

The content of chlorophyll, polyphenols (anthocyanins, flavonols) and the nitrogen balance index (NBI®) were measured with DUALEX® (Force-A, Orsay, France) in fully developed leaves in the external position of the plant. Four plants were measured by OTC.

2.2.3. Nutrient Analysis

For nutrient analysis, oven-dried samples from the final harvest were ground in a stainless steel grinder. Elemental composition analysis was performed by an elemental analyzer (TruSpec CHN-S, LECO Corporation, St. Joseph, MI, USA). Nutrients composition (Mg, Ca, Na, K, Fe, Mn and Zn) was analyzed by an ICP-OES analyzer (5900 ICP-OES, Agilent, Santa Clara, CA, USA).

The analyses were carried out using 150 mg of commercial biomass per plant. Four plants per OTC. The values were expressed as mg/100 g DW.

2.2.4. Statistical Analysis

All statistical analysis was done by using Statistica (TIBCO, Palo Alto, CA, USA). Differences among O₃ treatments in physiological parameters were tested through one way analysis of variance (ANOVA). A probability level $p < 0.05$ was considered statistically significant and p values between 0.05 and 0.1 are discussed as significant trends.

3. Results

3.1. Growing Conditions and Ozone Concentration

The growing conditions in OTC were sunny and warm. The temperature range was (8–21 °C) and the relative humidity (39–80%) (Table 1), while the environmental conditions in the periurban crops were warmer and drier in the summer months. The ambient temperature was in the range 16–33 °C and the relative humidity 12–57% (Table 1).

The concentration of ambient ozone recorded in peri-urban crops exceeds the critical level of vegetation protection (3000 ppbh). Ozone peaks throughout the day were recorded between 2:00 p.m. and 6:00 p.m., coinciding with the highest values of temperature and lower relative humidity.

3.2. Visible Injury

The plants of Swiss chard var. Fuenlabrada showed an unspecific discoloration after 25 days of treatment in the OTC. These white spots were more intense in the NF++ treatment. At the end of the experiment, these spots in ozone treatments were less apparent.

Table 1. Environmental growing conditions and ozone exposure in OTC and peri-urban field.

Year	Ozone Exposure (Days)	[O ₃] ppb ^a -AOT 40 (ppb·h)				RH (%) ^a	T (°C) ^a
		FA	NFA	NFA+	NFA++		
OTC	25	[17]-0	[36]-1869	[42]-4084	[49.5]-7158	59	16
	43	[17]-0	[36]-3550	[42]-8075	[49.5]-13809		
Peri-Urban Field	Environmental O₃ Concentration						
	Summer (JJA)			[38]-10459		29	25
	43			[40]-5840		25	25.5

T air temperature; RH air relative humidity; [O₃] ppb Ozone concentration; AOT40 accumulated ozone concentration over 40 ppb during dayligh hours. ^a Averages 24 h.

In the O₃ bioindicator vegetable garden, no visible damage was detected in the chard varieties. Ozone damage was only identified in a variety of sensitive Tobacco (Bel W3).

3.3. Biomass

The total production of chard was similar between different treatments. When the marketable production was selected, a significant reduction in the harvest was detected in the ozone treatments ($p < 0.01$) and a significant increase in no-commercial biomass in NF++ treatment ($p < 0.05$). The harvest of chard cv. Fuenlabrada has been reduced in 30% in the NF++ and 17% in the NF+ treatment compared to the filtered air treatment harvest (Figure 1).

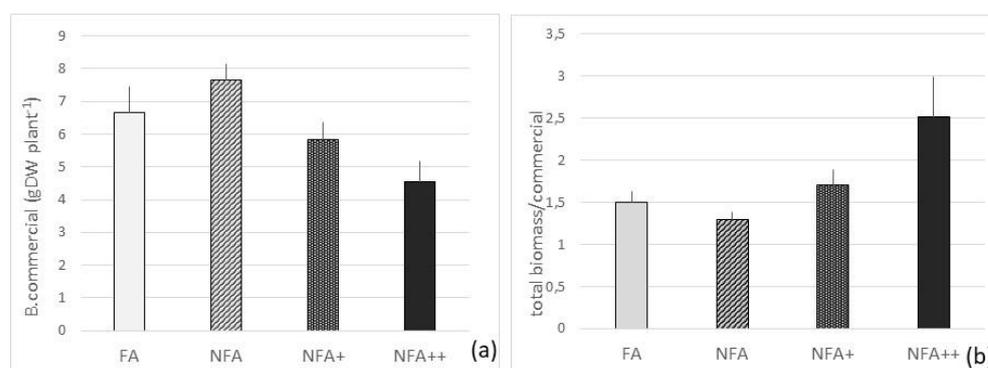


Figure 1. Swiss chard (a) commercial biomass (g dry weight plant⁻¹); (b) total biomass:commercial ratio. Values are means ± standard errors for ozone treatments. Charcoal filter air (FA), ambient (NFA), ambient plus 20 ppb ozone (NFA+) and ambient plus 40 ppb ozone (NFA+).

3.4. Pigments Concentrations

Pigment analysis at 25 days of the experiment showed a 15% reduction in chlorophylls in the NF++ treatment ($p < 0.01$) (data not show). At the end of the experiment (after 43 days of exposure to different concentrations of ozone), the leaves of plants in NF++ treatment showed a significant reduction of the chlorophyll concentration of 15% ($p < 0.001$). As well as a reduction of 15% in the nitrogen index (NIB) ($p < 0.01$) and an increase of 10% in the concentration of anthocyanins ($p < 0.001$) in plants exposed to a higher concentration of ozone (NF++), while no differences were obtained in flavonoids (Figure 2). Measurements with “Dualex” in peri-urban crops at different foliar ages showed a significant reduction in flavonoids ($p < 0.001$) and an increase in NBI in the youngest leaves ($p < 0.001$), located in the center of the plant.

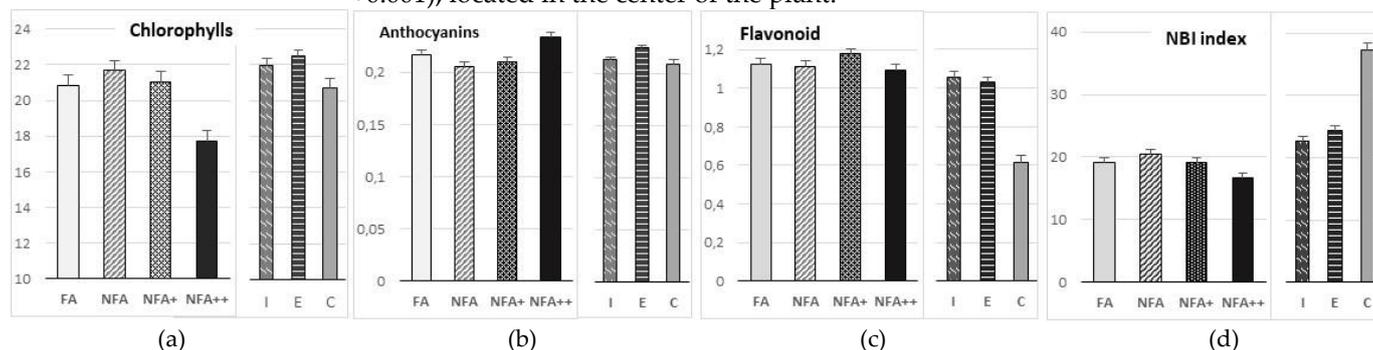


Figure 2. (a) The content of chlorophyll, (b) anthocyanins, (c) flavonoids and (d) the nitrogen balance index (NBI) was measured with a dualex device, inside the OTC and in the peri-urban field. Values are means ± standard errors for ozone treatments. The ozone treatments are: air with carbon filter (FA), ambient (NFA), ambient plus ozone 20 ppb (NFA+) and ambient plus ozone 40 ppb (NFA+). Those measured in the field were classified according to the age-position of the leaf. E = external, I = internal and C = center.

3.5. Nutrient

The main nutrients in the chard leaves grown in the OTC were K, Na, Mg and Ca (Table 2). At the end of the experiment, a significant reduction of Mg of 25% and Ca 30% ($p < 0.05$) was observed in the NF++ treatment plants. And a non-significant reduction of

Mn 10% and Zn 5% in the same treatment. The concentration of K was significantly higher in the plants of the NF++ treatment ($p < 0.001$) with respect to NF and NF+ but not with respect to FA. The concentration of other nutrients such as nitrogen (N) or phosphorus (P) increased slightly with increasing exposure to ozone (Table 2).

The variation of Mg with respect to the exposure to ozone showed a high correlation with the changes in the concentration of chlorophylls ($R^2 = 0.9$) measured after 43 days of exposure.

Table 2. P-values of ozone effects on nutrient levels and nutrient concentrations (mg/100 g DW) in commercial leaves after 43 days of exposition to different ozone treatments. Values are means \pm standard errors for ozone treatments. The letters denote differences between ozone treatments in the post-hoc tests. The ozone treatments are: air with carbon filter (FA), ambient (NFA), ambient plus ozone 20 ppb (NFA+) and ambient plus ozone 40 ppb (NFA+).

Factor	N	Mg	Ca	K	Na	P	Fe	Mn	Zn	Cu
O ₃	n.s.	0.02	0.03	0.01	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
FA	3482 \pm 160	1370 \pm 57 a	877 \pm 66 a	3690 \pm 137 ab	3500 \pm 135	256 \pm 17	21 \pm 2.4	17 \pm 0.9	9.16 \pm 0.39	2.59 \pm 0.20
NFA	3336 \pm 171	1229 \pm 105 b	770 \pm 91 a	3145 \pm 163 b	3636 \pm 164	240 \pm 7.6	18 \pm 1.8	18 \pm 1.1	9.77 \pm 0.66	2.44 \pm 0.22
NFA+	3354 \pm 190	1236 \pm 127 b	727 \pm 106 ab	3160 \pm 231 b	3727 \pm 201	253 \pm 21	19 \pm 1.3	16 \pm 1.5	8.63 \pm 0.91	2.37 \pm 0.19
NFA++	3836 \pm 200	960 \pm 60 b	541 \pm 38 b	3836 \pm 143 a	3240 \pm 93	287 \pm 15	17 \pm 0.9	15 \pm 1.2	8.45 \pm 0.53	2.75 \pm 0.17

4. Discussion

The results of this study in the OTC show that the variety of chard cv. Fuenlabrada cultivated in the outskirts of Madrid can be considered moderately sensitive to ozone. The alteration of concentration of pigments was detected earlier, with lower accumulated ozone exposure. As a result, a significant loss of marketable production and alteration of nutritional quality was measured at the end of the experiment in the plants exposed to high ozone levels.

Usually, leafy crops with visible damage are often not marketable; consequently, the production of leaf crops has been reduced where ambient O₃ concentration reaches high levels [15,18,36,37]. Yield quality traits have been studied in a limited number of crops.

The alteration of pigment concentrations has been recorded after ozone exposure in numerous studies [20]. The reduction of chlorophyll has been detected in leafy crops [27,28]. These changes were more evident in old leaves and were dependent on the variety studied [37]. Other studies showed a seasonal response [29]. The earlier chlorophyll breakdown could be related to a premature and accelerated senescence due to ozone [21,38]. Enhanced senescence will also reduce the photosynthesis and the length of time over which nutrients will be taken up from the soil [39]. The response of increased anthocyanin concentration measured in the plants exposed to high ozone leaves has been linked to the response to oxidative stress.

Chlorophyll reduction is not a specific effect of ozone. Low concentration of chlorophyll is also observed in young leaves. The values obtained of anthocyanins, flavonoids, or NBI index measured with a "Dualex device" could help to discern between the effect of ozone and the age of the leaf. Low values of flavonoid and high index NBI values (measured with Dualex) have been related to less leaf development. This same pattern was observed in tobacco plants grown in the bioindicator garden.

The chlorophyll content in chard leaves was highly correlated ($R^2 = 0.97$) with the concentration of Mg in these plants. Both traits showed a significant reduction in NF++ treatment. These measurements have been determined on green leaves without visible symptoms. A lower correlation value was recorded with Ca and Fe. However, an inverse relationship with nitrogen concentration has been detected. Plants with a lower concentration of chlorophylls have a higher concentration of nitrogen. However, the differences in N concentration were not significant. The increase in nitrogen with exposure to ozone has been reported in different studies, especially in wheat grains. This means that grain

quality is improved with respect to concentration, but the amount of accumulated proteins and minerals per unit area is reduced, which can have serious effects on human nutrition [39].

Chard has been traditionally used for its health benefits [31,32]. Regarding the mineral content, the abundance of macro elements such as Mg, Ca or K and the presence of the microelements Fe, Cu, Mn and Zn are some of the most interesting aspects of the use of this crop in human nutrition. Mineral deficiency is one of the causes of numerous chronic and degenerative disorders. The consumption of a plate (200 g) of fresh Swiss chards leaves could cover around 20% and 40% of Fe, 100% Mg and 15 % of Ca (RDA) (Recommended Dietary Allowance). Exposure to ozone reduces the concentration of Mg, Ca or Fe by 5–20%, causing a reduction in the RDA in the diet. Similar reduction of nutrients have been recorded in the Palak when the plants were exposed to ozone in winter but not differences were recorded in summer [29]. The reduction of Mg, Ca, Fe and Zn have been related to the increasing senescence of the exposure of the leaves to ozone [38].

The OTC results in chard cv Fuenlabrada showed that this cultivar was moderately sensitive to ozone. The first effect of ozone (chlorophyll reduction) has been detected with the accumulated ozone concentration in the range of 4000 ppb·h (AOT40). A reduction in marketable biomass and a moderate loss of quality has been detected with a cumulative ozone concentration of 13,800 ppb·h (AOT40).

Ozone effects were not clearly detected in the field. No visible symptoms or reductions in growth were detected, and pigment concentration values were in the range of healthy plants. Different results could be related to the meteorological conditions during the growth of the crop. In the OTC facilities, the conditions were milder than in the peri-urban field, where very low relative humidity values were recorded in the summer months, coinciding with the maximum ozone concentration. Drier conditions could reduce the absorption of pollutants [20,21].

The evaluation of O₃ pressure on global agriculture should include quality and nutritional aspects with direct consideration of the potential for adverse health effects and malnutrition associated with reduced production of proteins and a variety of minerals.

Author Contributions: Conceptualization, V.B.-B., S.E., I.G.-F., J.S.; methodology V.B.-B., S.E., I.G.-F., J.S.; writing—original draft preparation, S.E.; writing—review and editing, S.E.; funding acquisition, V.B.-B. All authors have read and agreed to the published version of the manuscript.

Funding: Funding was provided by Grupo Operativo OZOCAM (PDR 2014- 2020, funded by FEADER (EU), MAPA (Spain) and Comunidad de Madrid (CAM, Spain) through IMIDRA (CAM); and AGRISOST-CM (S2018/BAA-4330, CAM y Structural Funds 2014-2020 FEDER and FSE).

Institutional Review Board Statement: Not applicable

Informed Consent Statement: Not applicable.

Data Availability Statement: Please refer to suggested Data Availability Statements in section “MDPI Research Data Policies” at <https://www.mdpi.com/ethics> (accessed 2nd November 2021).

Acknowledgments: The authors express their gratitude to Jose M^a Camacho; Angel Gonzalez and Ricardo Marquez.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Ashmore, M.R. Assessing the future global impacts of ozone on vegetation. *Plant Cell Environ.* **2005**, *28*, 949–964.
2. Ainsworth, E.A.; Yendrek, C.R.; Sitch, S.; Collins, W.J.; Emberson, L.D. The Effects of Tropospheric Ozone on Net Primary Productivity and Implications for Climate Change. *Annu. Rev. Plant Biol.* **2012**, *63*, 637–661.
3. Cooper, O.R.; Parrish, D.D.; Ziemke, J.; Balashov, N.V.; Cupeiro, M.; Galbally, I.E.; Gilge, S.; Horowitz, L.; Jensen, N.R.; Lamarque, J.-F.; et al. Global distribution and trends of tropospheric ozone: An observation-based review Global distribution and trends of tropospheric ozone. *Elem. Sci. Anthr.* **2014**, *2*, 000029.

4. Monks, P.S.; Archibald, A.T.; Colette, A.; Cooper, O.; Coyle, M.; Derwent, R.; Fowler, D.; Granier, C.; Law, K.S.; Mills, G.E.; et al. Tropospheric ozone and its precursors from the urban to the global scale from air quality to short-lived climate forcer. *Atmos. Chem. Phys.* **2015**, *15*, 8889–8973.
5. DeLang, M.N.; Becker, J.S.; Chang, K.L.; Serre, M.L.; Cooper, O.R.; Schultz, M.G.; Schröder, S.; Lu, X.; Zhang, L.; Deushi, M.; et al. Mapping Yearly Fine Resolution Global Surface Ozone through the Bayesian Maximum Entropy Data Fusion of Observations and Model Output for 1990–2017. *Environ. Sci. Technol.* **2021**, *55*, 4389–4398.
6. European Environment Agency, *Air quality in Europe – 2019 report* Report No 10/2019; European Environment Agency: Copenhagen, Denmark, 2019
7. Borge, R.; Requia, W.J.; Yagüe, C.; Jhun, I.; Koutrakis, P. Impact of weather changes on air quality and related mortality in Spain over a 25 year period (1993–2017). *Environ. Int.* **2019**, *133*, 105272
8. Saiz-Lopez, A.; Borge, R.; Notario, A.; Adame, J.A.; De la Paz, D.; Querol, X.; Artinano, B.; Gómez-Moreno, F.J.; Cuevas, C.A. Unexpected increase in the oxidation capacity of the urban atmosphere of Madrid, Spain. *Sci. Rep.* **2017**, *7*, 45956.
9. Querol, X.; Alastuey, A.; Gangoi, G.; Perez, N.; Lee, H.K.; Eun, H.R.; Park, Y.; Mantilla, E.; Escudero, M.; Titos, G.; et al. Phenomenology of summer ozone episodes over the Madrid Metropolitan Area, central Spain. *Atmos. Chem. Phys. Discuss.* **2018**, *18*, 6511–6533.
10. Salvador, P.; Barreiro, M.; Gómez-Moreno, F.J.; Alonso-Blanco, E.; Artinano, B. Synoptic classification of meteorological patterns and their impact on air pollution episodes and new particle formation processes in a south European air basin. *Atmos. Environ.* **2021**, *245*, 118016.
11. Holland, M.; Mills, G.; Hayes, F.; Buse, A.; Emberson, L.; Cambridge, H.; Cinderby, S.; Terry, A.; Ashmore, M. Economic assessment of crop yield losses from ozone exposure. Report to UK Department of Environment, Food and Rural affairs under contract. **2002**, *1*, 170.
12. Wilkinson, S.; Mills, G.; Illidge, R.; Davies, W.J. How is ozone pollution reducing our food supply? *J. Exp. Bot.* **2012**, *63*, 527–536.
13. Mills, G.; Sharps, K.; Simpson, D.; Pleijel, H.; Broberg, M.; Uddling, J.; Jaramillo, F.; Davies, W.J.; Dentener, F.; Berg, M.V.D.; et al. Ozone pollution will compromise efforts to increase global wheat production. *Glob. Chang. Biol.* **2018**, *24*, 3560–3574.
14. Tai, A.P.; Sadiq, M.; Pang, J.; Yung, D.H.; Feng, Z. Impacts of Surface Ozone Pollution on Global Crop Yields: Comparing Different Ozone Exposure Metrics and Incorporating Co-effects of CO₂. *Front. Sustain. Food Syst.* **2021**, *5*, 63.
15. Fumagalli, I.; Gimeno, B.S.; Velissariou, D.; De Temmerman, L.; Mills, G. Evidence of ozone-induced adverse effects on crops in the Mediterranean region. *Atmos. Environ.* **2001**, *35*, 2583–2587.
16. Mills, G.; Buse, A.; Gimeno, B.; Bermejo, V.; Holland, M.; Emberson, L.; Pleijel, H. A synthesis of AOT40-based response functions and critical levels of ozone for agricultural and horticultural crops. *Atmos. Environ.* **2007**, *41*, 2630–2643.
17. Fagnano, M.; Maggio, A.; Fumagalli, I. Crops' responses to ozone in Mediterranean environments. *Environ. Pollut.* **2009**, *157*, 1438–1444.
18. Booker, F.; Muntifer, R.; McGrath, M.; Burkey, K.; Decoteau, D.; Fiscus, E.; Manning, W.; Krupa, S.; Chappelka, A.; Grantz, D. The ozone component of global change: potential effects on agricultural and horticultural plant yield, product quality and interactions with invasive species. *J. Integr. Plant Biol.* **2009**, *51*, 337–351.
19. Fuhrer, J.; Martin, M.V.; Mills, G.; Heald, C.L.; Harmens, H.; Hayes, F.; Sharps, K.; Bender, J.; Ashmore, M.R. Current and future ozone risks to global terrestrial biodiversity and ecosystem processes. *Ecol. Evol.* **2016**, *6*, 8785–8799.
20. Ainsworth, E.A. Understanding and improving global crop response to ozone pollution. *Plant J.* **2016**, *90*, 886–897.
21. Emberson, L.D.; Pleijel, H.; Ainsworth, E.A.; Berg, M.V.D.; Ren, W.; Osborne, S.; Mills, G.; Pandey, D.; Dentener, F.; Bueker, P.; et al. Ozone effects on crops and consideration in crop models. *Eur. J. Agron.* **2018**, *100*, 19–34.
22. Velissariou, D. *Toxic Effects and Losses of Commercial Value of Lettuce and Other Vegetables Due to Photochemical Air Pollution in Agricultural Areas of Attica, Greece. Critical Levels for Ozone—Level II*; Swiss Agency for Environment, Forest and Landscape: Bern, Switzerland, 1999; pp. 253–256.
23. Oshima, R.; Taylor, O.; Cardiff, E. Possible new toxicant indicated in severe air pollution episode in south coast basin. *Calif. Agric.* **1974**, *28*, 12–13.
24. Goumenaki, E.; Barnes, J. Impacts of tropospheric ozone on growth and photosynthesis of lettuce. *Acta Hort.* **2009**, *817*, 169–176.
25. Temple, P.J.; Jones, T.E.; Lennox, R.W. Yield loss assessments for cultivars of broccoli, lettuce, and onion exposed to ozone. *Environ. Pollut.* **1990**, *66*, 289–299.
26. Calatayud, A.; Iglesias, D.J.; Talón, M.; Barreno, E. Effects of 2-month ozone exposure in spinach leaves on photosynthesis, antioxidant systems and lipid peroxidation. *Plant Physiol. Biochem.* **2003**, *41*, 839–845.
27. Calatayud, A.; Barreno, E. Response to ozone in two lettuce varieties on chlorophyll a fluorescence, photosynthetic pigments and lipid peroxidation. *Plant Physiol. Biochem.* **2004**, *42*, 549–555.
28. Goumenaki, E.; Fernandez, I.G.; Papanikolaou, A.; Papadopoulou, D.; Askianakis, C.; Kouvarakis, G.; Barnes, J. Derivation of ozone flux-yield relationships for lettuce: a key horticultural crop. *Environ. Pollut.* **2007**, *146*, 699–706.
29. Tiwari, S.; Agrawal, M.; Marshall, F.M. Seasonal variations in adaptational strategies of *Beta vulgaris* L. plants in response to ambient air pollution: Biomass allocation, yield and nutritional quality. *Trop. Ecol.* **2010**, *51*, 353–363.
30. Kumari, S.; Agrawal, M.; Tiwari, S. Impact of elevated CO₂ and elevated O₃ on *Beta vulgaris* L.: pigments, metabolites, antioxidants, growth and yield. *Environ. Pollut.* **2013**, *174*, 279–288.

31. Gamba, M.; Raguindin, P.F.; Asllanaj, E.; Merlo, F.; Glisic, M.; Minder, B.; Bussler, W.; Metzger, B.; Kern, H.; Muka, T. Bioactive compounds and nutritional composition of Swiss chard (*Beta vulgaris* L. var. *cicla* and *flavescens*): a systematic review. *Crit. Rev. Food Sci. Nutr.* **2020**, *61*, 3465–3480.
32. Ninfali, P.; Angelino, D. Nutritional and functional potential of *Beta vulgaris* *cicla* and *rubra*. *Fitoterapia* **2013**, *89*, 188–199.
33. Sánchez-Mata, M.C.; Loera, R.D.C.; Morales, P.; Fernández-Ruiz, V.; Cámara, M.; Marqués, C.D.; Pardo-De-Santayana, M.; Tardío, J. Wild vegetables of the Mediterranean area as valuable sources of bioactive compounds. *Genet. Resour. Crop. Evol.* **2012**, *59*, 431–443.
34. Mzoughi, Z.; Chahdoura, H.; Chakroun, Y.; Cámara, M.; Fernández-Ruiz, V.; Morales, P.; Mosbah, H.; Flamini, G.; Snoussi, M.; Majdoub, H. Wild edible Swiss chard leaves (*Beta vulgaris* L. var. *cicla*): Nutritional, phytochemical composition and biological activities. *Food Res. Int.* **2019**, *119*, 612–621.
35. Mills, G., Harmens, H., Hayes, F., Pleijel, H., Bükér, P., Gonzalez-Fernandez, I., Eds. Mapping Critical Levels for Vegetation. In *Manual on Methodologies and Criteria for Modelling and Mapping Critical Loads and Levels and Air Pollution Effects, Risks and Trends*. Convention on Long-Range Transboundary Air Pollution; Umweltbundesamt, Berlin, Germany, 2017.
36. Marzuoli, R.; Finco, A.; Chiesa, M.; Gerosa, G. A dose-response relationship for marketable yield reduction of two lettuce (*Lactuca sativa* L.) cultivars exposed to tropospheric ozone in Southern Europe. *Environ. Sci. Pollut. Res.* **2017**, *24*, 26249–26258.
37. González-Fernández, I.; Elvira, S.; Calatayud, V.; Calvo, E.; Aparicio, P.; Sánchez, M.; Alonso, R.; Bermejo, V.B. Ozone effects on the physiology and marketable biomass of leafy vegetables under Mediterranean conditions: spinach (*Spinacia Oleracea* L.) and Swiss chard (*Beta Vulgaris* L. Var. *cycla*). *Agric. Ecosyst. Environ.* **2016**, *235*, 215–228.
38. Fangmeier, A.; De Temmerman, L.; Black, C.; Persson, K.; Vorne, V. Effects of elevated CO₂ and/or ozone on nutrient concentrations and nutrient uptake of potatoes. *Eur. J. Agron.* **2002**, *17*, 353–368.
39. Broberg, M.C.; Feng, Z.; Xin, Y.; Pleijel, H. Ozone effects on wheat grain quality—A summary. *Environ. Pollut.* **2015**, *197*, 203–213.