

**Constraints on photosynthesis and antioxidant
metabolism in winter and summer crops induced by
sulphur dioxide fumigation**

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*When you are privileged to view the Earth from afar, when you can hold out your thumb and cover it with your thumbnail, you realise that we are really, all of us around the world, crew members on the space station Earth. Of all the accomplishments of technology, perhaps the most significant one was the picture of Earth over the lunar horizon. If nothing else, it should impress our fellow man with the absolute fact that our environment is bounded, that **our resources are limited**, and that our life support system is a closed cycle. And, of course, when this space station is viewed from 240,000 miles away, only its beauty, its minuteness, and its isolation in the blackness of space, are apparent. A traveller from some far planet would not know that the size of the crew is already too large and threatening to expand, **that the breathing system is rapidly becoming polluted**, and that the water supply is in danger of contamination with everything from DDT to raw sewage. **The only real recourse is for each of us to realise that the elements we have are not inexhaustible.** We're all in the same spaceship.*

Astronaut Frank Borman -Commander of Apollo 8:

The first manned spacecraft to fly around the moon in 1968

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Abstract

Recently, major advances have been made in developed countries elucidating the effects of air pollutants on crop plants. In contrast, similar studies on the effects of elevated SO₂ concentrations on crops in developing countries such as South Africa are far less advanced. In South Africa, fossil fuel combustion is the main source of energy for most of the country. The tremendous increase in population size and consequential increase in energy demand has led to considerable increases in fossil fuel burning. This phenomenon has led to increases in tropospheric air pollutants such as SO₂, NO₂ and the secondary pollutant, O₃. These increases, combined with climatic variations are subject of much concern in agricultural sectors. Fortunately, through many research studies done in European and other developed countries, threshold values have been established for selected crops in an attempt to mitigate the damage done by SO₂ and other air pollutants. However, it is with due care that we apply these legislative thresholds since the environmental conditions in the Southern hemisphere differs greatly from that of the Northern hemisphere.

The main aims of this study was firstly to determine the physiological and biochemical basis of SO₂ induced inhibition in the C₃ and C₄ crops, *Brassica napus* and *Zea mays*, respectively, and secondly, to study their response with special reference to photosynthesis. The combination of different SO₂ levels and induced drought was also investigated. It was hypothesised that SO₂ will impair the photosynthetic capacity of both *Brassica napus* and *Zea mays* test plants, but that with the addition of drought as co-stress, partial stomatal closure would lead to a mitigation of the SO₂-effect on the photosynthetic apparatus of the mentioned crops.

Most of the research that has been done on air pollutants was short term studies, focused on generating dose-response data only over a few weeks of growth. Short term exposures do not answer questions on how initial constraints on photosynthesis could affect crops at a later stage, i.e. how and if these inhibitions affect the yield. In the present study, crops were grown for an entire growth season, from germination until harvest in open-top chambers (OTCs) in an attempt to link early photochemical inhibition to the reduction in yield. OTCs are internationally accepted as the best method to assess the effect of pollutant dosage on crops. Two crops, *Brassica napus* (C₃) and *Zea mays* (C₄) were cultivated and subjected to SO₂ enriched air (50,

100 and 200 ppb) for eight hours/day, seven days a week. Control plants only received carbon filtered (CF) air. An additional drought treatment was induced in half of the plants of each SO₂ treatment. Experiments specifically focussed on the detrimental effect of SO₂ on the photosynthetic capacity of the test plants. The photosynthetic capacity was evaluated using chlorophyll *a* fluorescence induction and photosynthetic gas exchange measurements in parallel, on a weekly basis. Analysis of the OJIP transients provided a number of parameters estimating the energy fluxes and ratios through photosystem II and the intersystem electron transport chain. Gas exchange parameters were deduced from CO₂ response curves (A:Ci curves). The ability of the antioxidant metabolism to detoxify reactive oxygen species (ROS) was determined by measuring the POD activity and comparing it to the H₂O₂ content for *Zea mays* leaves over a period of nine weeks. Ultimately the cumulative effect of SO₂ on the yield was evaluated by determining the shoot mass of *Brassica napus* and the cob mass of *Zea mays*.

Elevated SO₂ concentrations resulted in the partial destruction of chlorophyll pigments, leading to the formation of yellow chlorotic regions in both *Brassica napus* and *Zea mays* leaves. These visual effects appeared long after first changes occurred in photosystem II function or photosynthetic gas exchange. In addition to the visual damage, results revealed that elevated SO₂ concentrations lead to an impaired photosynthetic capacity in both *Brassica napus* and *Zea mays* plants, especially concerning PSII function. The decline in photosynthetic capacity was mainly due to a loss in stomatal functionality, indicated as a reduction in the stomatal conductance for both *Brassica napus* and *Zea mays* plants. This was true for well watered and drought stressed treatments in both C₃ and C₄ crops. The reduced photosynthetic capacity was due to stomatal limitation and to a greater extent, biochemical (mesophyll) limitation. Mesophyll limitation was evident by the decrease in Rubisco activity (*Brassica napus*: C₃) and PEPc activity (*Zea mays*: C₄), in well watered and drought stress treatments. The inability to effectively regenerate ribulosebiphosphate (*Brassica napus*: C₃) and phosphoenolpyruvate (*Zea mays*: C₄) in well watered and drought stressed plants was another mesophyll limitation that contributed to the decline in photosynthesis. By in depth analysis of the chlorophyll *a* fluorescence transients according to the JIP test, the sites of inhibition in the photosynthetic electron transport chain were elucidated. The changes in the fluorescence transients revealed that the inhibition of the primary processes of photochemistry was mainly due to uncoupling of the oxygen evolving complex in well watered *Zea mays* and drought stressed *Brassica napus* plants and to the

inhibition of the reduction of end electron acceptors beyond PSI in well watered and drought stressed *Brassica napus* plants and drought stressed *Zea mays* test plants. In *Zea mays* the source of the inhibition of the primary photochemistry through the decline in the reduction of end electron acceptors, was further investigated by in depth analysis of the I-P phase of the OJIP fluorescent transients, i.e. a segment only through photosystem I (PC→RE). The inhibition in well watered and drought stressed treatments were found, not only to be a result of the reduced pool size of electron acceptors, but was also due to a decline in the rate at which end electron acceptors were being reduced. These constraints on the functioning of the photosynthetic electron transport chain were reflected by the inhibition of CO₂ assimilation rate, the decline in Rubisco activity (C₃ plants) and PEPc activity (C₄ plants), and decline in the regeneration rate of ribulosebiphosphate (C₃ plants) and phosphoenolpyruvate (C₄ plants) both due to the decreasing production of reduction equivalents in the light phase. This means that although the fluorescence transients are measured within one second in the dark adapted state, they provide a reliable measure of the whole photosynthetic electron transport chain.

SO₂ affected both the stomatal function and photosynthetic capacities of *Zea mays* and *Brassica napus*. The SO₂-related stomatal closure resulted in a decrease in CO₂ influx into the leaf and thus a decline in CO₂ assimilation. This phenomenon was corroborated by the large decrease in water use efficiency in *Zea mays* and *Brassica napus*. A marked SO₂-concentration dependent decline in the shoot biomass in well watered and drought stressed *Brassica napus* was evident. Similarly, a reduction in yield occurred in *Zea mays* test plants, namely reductions in cob mass, in both well watered and drought stressed treatments. The data of the current investigation presented clearly indicate that marked impairment of photosynthesis and yield reduction in the crops, *Zea mays* and *Brassica napus*, occurred at SO₂ concentrations of 50 ppb. These findings proved the first hypothesis of this study to be true in that SO₂ adversely affects the photosynthetic capacity of crop plants. In *Zea mays*, more energy was expended towards growth than detoxification of sulphur metabolites. Due to this fact, *Zea mays* plants still grew to a considerable length with less energy available for cob formation. An increase in H₂O₂ content due to elevated SO₂ concentrations, lead to the degradation of chlorophyll molecules and inhibition of the photosystems which consequentially inhibited the photosynthetic capacity of well watered and drought stressed *Zea mays* plants. The effectiveness of the antioxidant metabolism to remove H₂O₂ from mesophyll cells was displayed by the overall decrease in H₂O₂

content for WW and DS treatments after 9 weeks fumigation. This was achieved by the increased scavenging enzyme activity (increased POD activity) that effectively removed the ROS from the mesophyll cells.

Ultimately the data showed that the C₃ plant, *Brassica napus*, was more adversely affected by elevated SO₂ concentrations, reducing the photosynthetic assimilation rate greatly. Drought stress however, ameliorated the damaging effect of SO₂ on the photosystems to some extent, proving the second hypothesis true for *Brassica napus* plants. *Zea mays* plants however showed greater sensitivity towards elevated SO₂ concentrations with the addition of drought as a co-stressor, while amelioration of the inhibitory effect through stomatal closure, proved not to be effective. These findings proved that the second hypothesis was thus only partially proven to be true, and only at low SO₂ concentrations for *Zea mays* crop plants.

Within natural environments there may be a magnitude of biotic and abiotic stresses being imposed on crops. The work done in this study is thus of great value to the agricultural sector in early determination of how multiple stressors (SO₂ and drought in this case) might affect yield. Management plans can be implemented accordingly. This fact emphasises the magnitude of the relevance and the importance of multiple stress-response studies done on crops, such as the present.

Opsomming

Daar is onlangs reuse vooruitgang gemaak in ons kennis van lugbesoedeling en die effek daarvan op gewasse in ontwikkelde lande. Hierteenoor is daar baie min studies gedoen wat die effek van toenemende SO₂-konsentrasies op gewasse in ontwikkelende lande soos Suid Afrika kwantifiseer. In Suid-Afrika is die hoofbron van elektriese energie die verbranding van fossielbrandstowwe. Enorme toenames in die populasie oor die afgelope paar jaar het gelei tot 'n toename in energie-aanvraag, wat op sy beurt gelei het tot die drastiese toename in fossielbrandstofverbranding. Die toename in troposferiese besoedelingsgasse soos SO₂, NO₂, asook die sekondêre besoedelingsgas, O₃, kan dus verwag word. Toenames in dié

besoedelingse, onder varieërende klimaatstoestande, wek kommer vir die sekuriteit van toekomstige hulpbronne, veral in landbousektore. Heelwat navorsing aangaande drumpelwaardes van besoedelstowwe is veral in Europese lande gedoen. Hierdie drumpelwaardes is daargestel in 'n poging om die effek wat SO₂ en ander besoedelingse op landbougewasse te verminder. Hierdie drumpelwaardes moet egter met sorg aangewend word in Suid Afrika, aangesien die omgewingstoestande in die Suidelike halfrond wesentlik verskil van dié in die noordelike halfrond. Hierdie verskille kan lei tot onvoorspelbare fluktuasies in data.

Die hooftemas van die huidige navorsingstudie is eerstens, die kwantifisering van die fisiologiese en biochemiese grondslag van SO₂-geïnduseerde remming op fotosintese van geselekteerde C₃ en C₄ gewasse. Tweedens is die effek van verskillende SO₂-vlakke gekombineer met droogtestres, op die gewasse bestudeer, met die klem op fotosintese. Daar is van die werkshiptese uitgegaan dat SO₂ die fotosintese kapasiteit van *Brassica napus* en *Zea mays* plante sal inhibeer, maar dat die toevoeging van droogte as 'n ko-stressor, die nadelige effek op die fotosintese-apparaat sal versag. Groot skaalse navorsing is reeds gefokus op korttermyn responsproewe, waar die toediening van besoedelingse en die kwantifisering van die effekte, oor 'n paar weke strek. Sulke korttermyn proewe se tekortkoming is dat dit nie die vraag beantwoord, nl. wat die effek van die remming van fotosintese vroeg in die groeistadium, op latere ontwikkeling en opbrengs sou hê nie. Belangrike bevindinge rakende die effek wat 'n bepaalde stressor op opbrengs mag hê word dus nie terdeë ontrafel nie. Met hierdie studie is die proefplante oor 'n volle groeiseisoen (tot volwassenheid) blootgestel aan verhoogde SO₂-vlakke/droogte in 'open-top'- groeikamers (OTCs). Hierdie metodiek is gebruik om vroeë remming van fotosintese in verband te bring met oesverliese. 'Open-top'-groeikamers word internasionaal aanvaar as die geskikste metode vir kwantifisering van die effekte van besoedelingse op gewasse. Twee gewasse, naamlik die C₃ gewas *Brassica napus*, en die C₄ gewas *Zea mays*, is gekweek en begas in OTCs vir agt ure per dag, sewe dae per week. Die proefplante is aan verskillende konsentrasies (0, 50, 100 en 200 dele per biljoen) SO₂ blootgestel, terwyl kontroleplante slegs koolstofgefiltreerde lug ontvang het. Bykomstig tot die SO₂ behandelings, is die helfte van die proefplante aan droogte blootgestel. Die eksperimentele metings wat uitgevoer is, het gefokus op die effek wat SO₂ mag hê op verskillende prosesse van fotosintese. Versillende aspekte van fotosintese is ge-evalueer deur weekliks, in parallel chlorofil *a* fluoressensieinduksie en fotosintetiese gaswissellings te meet. Die analise van die OJIP

fluoresensiestyging het verskeie parameters verskaf wat die energievloede en energieverhoudings deur fotosisteam II en die intersisteam elektrontransport ketting, kwantifiseer. Verskillende gaswisselingsparameters is afgelei vanaf die CO₂ responskromme (A:Ci krommes) wat verdere lig gewerp het op die grondslag van die remmende effekte op van die proefplante. Die vermoë van die antioksidantmetabolisme van die proefplante om reaktiewe suurstofmolekules te detoksifiseer, is bepaal deur die peroksidase-aktiwiteit as maatstaf. Die peroksidase-aktiwiteit en waterstofperoksiedkonsentrasie van *Zea mays* blare is drie maal oor 'n tydperk van nege weke gemeet. Die kumulatiewe effek van SO₂ op die proefplante is bepaal deur hul opbrengs te meet. In *Brassica napus* plante is die bogrondse biomassa bepaal, en in *Zea mays*, die lengte van die plante vanaf die basis tot by die vlagblaar, asook kopmassa.

Verhoogde SO₂ vlakke het gelei tot die degradering van die chlorofilpigmente. Die vorming van geel nekrotiese areas in beide *Brassica napus* en *Zea mays* blare, het ontstaan lank na enige inhibering van fotosisteam II funksie of van die gaswisseling waargeneem kon word. Benewens visuele skade het SO₂-inhibering van fotosintese in beide *Brassica napus* en *Zea mays* gewasse vroeg ingetree, veral wat betref PSII funksie. Die afname in fotosintese was grotendeels as gevolg van die verlies van stomatale funksie. Hierdie verskynsel het geblyk uit die afname in die stomatale geleiding van beide goed benatte en die droogtegestremde *Brassica napus* (C₃) en *Zea mays* (C₄) plante. Die remmende effek op fotosintese was egter nie alleenlik as gevolg van stomatale beperking nie, maar was veral ook te wyte aan mesofil (biochemiese) -beperking. Die mesofilbeperking het geblyk uit die afname in Rubisco-aktiwiteit (*Brassica napus*: C₃) en PEPc-aktiwiteit (*Zea mays*: C₄) vir goed benatte en droogte behandelde plante. Hierbenewens is die onvermoë om ribulosebifosfaat (*Brassica napus*: C₃) en fosfoënpirovaat (*Zea mays*: C₄), in beide goed benatte en droogte behandelde plante effektief te regenerereer, ook 'n aanduiding van die biochemiese remming van fotosintese.

Analise van die chlorofil *a* fluoresensie induksiekrommes met behulp van die JIP-toets, het gelei tot die ontrafeling van die posisie waar inhibering plaasvind in die elektrontransportketting. Veranderinge in die fluoresensiekrommes het daarop gedui dat die inhibering van die primêre prosesse van fotochemie te wyte is aan die ontkoppeling van die suurstofvrystellingskompleks (OEC) (in goed benatte *Zea mays* en droogte behandelde *Brassica napus* plante) sowel as inhibering van die reduksie van eindelektronontvangers, na fotosisteam I (in goed benatte en

droogte behandelde *Brassica napus* plante, en in *Zea mays* slegs die droogte behandelde plante). In *Zea mays* is daar verder toegespits op die oorsaak van die afname in die reduksie van eindelektronontvangers deur 'n in diepte analise van die I-P fase van die OJIP fluoressensiekromme. Dit is gedoen deur die segment van die fluoressensiekromme wat deur fotosisteam I verteenwoordig word (PC→RE), verder te analiseer. Inhibisie in goed benatte en droogte behandelings was as gevolg van 'n afname in die eindelektrononvangerpoel, sowel as 'n afname in die tempo waarteen die eindelektronontvangers gereduseer word. Die remmende effek van SO₂ op die elektrontransportketting het ook duidelik geblyk uit die afname in die CO₂-assimilerings tempo, afname in Rubisco-aktiwiteit (C₃ plante) en PEPc aktiwiteit (C₄ plante), asook die afname in die vermoë om ribulosebisfosfaat (C₃ plante) en fosfoënpirivaat (C₄ plante) te regeneer. Beide die inhibisies op ribulosebisfosfaat- en fosfoënpirivaatregenerering was die gevolg van die afname in produksie van reduksie-ekwivalente in die ligfase. Hierdie feit beklemtoon die waarde van die JIP toets om lig te werp op die fotosintetiese potensiaal van plante, nieteenstaande dat hierdie meting binne in 'n enkele sekond op donkeraangepaste plante gedoen word. Soortgelyke afnames in fotosintese, asook 'n afname in stomatale funksie in *Brassica napus* en *Zea mays* plante het voorgekom. Die SO₂-afhanklike reduksie in stomatale geleiding het gelei tot 'n afname in diffusie van CO₂ in die substomatale ruimte in, met 'n gevolglike afname in CO₂ assimilering. Hierdie verskynsel het ooreengestem met die groot afname in die water verbruikingseffektiwiteit van beide *Brassica napus* en *Zea mays* plante. 'n Merkbare SO₂-afhanklike afname het voorgekom in die bognondse biomassa in goed benatte en droogte behandelde *Brassica napus*. Netso was daar ook 'n afname in die kopmassa van goedbenatte en droogte behandelde *Zea mays* plante. Die data toon duidelik dat aansienlike inhibering van die fotosintetiese kapasiteit van beide *Zea mays* en *Brassica napus* plante by SO₂ konsentrasies van laer as 50 dpb plaasvind.

'n SO₂-geïnduseerde toename in die H₂O₂ konsentrasie het gelei tot degradering van chlorofil molekules en die inhibering van die fotosisteme. Hierdie inhibering het gevolglik gelei tot die remming van die fotosintese kapasiteit van goed benatte en droogte behandelde *Zea mays* plante. Die afname in die H₂O₂ konsentrasie in goed benatte en droogte behandelde plante na 9 weke se begassing met SO₂, weerspieël die doeltreffendheid van die antioksidant metabolisme om die H₂O₂ te detoksifiseer en te verwyder uit die mesofilselle. Hierdie detoksifisering en

verwydering van H_2O_2 is bemiddel deur die verhoging van die aktiwiteit van die antioksidantensiem, peroksidase (POD).

In hooftrekke toon die data dat die C_3 gewas, *Brassica napus*, meer geaffekteer is deur die verhoogde SO_2 konsentrasies as die C_4 gewas, *Zea mays*. Hierdie verskynsel is veroorsaak deur remming van verskeie aspekte van die fotosintetiese metabolisme. Die toevoeging van droogte, het wel tot 'n mate die effek van SO_2 versag, wat dus die tweede hipotese gestel, gedeeltelik korrek bewys. In teenstelling hiermee het die *Zea mays* plante 'n groter sensitiwiteit teenoor SO_2 vertoon, veral met gepaargaande droogte by hoë SO_2 vlakke (200 dpb). Die tweede hipotese is dus verkeerd bewys in die geval van *Zea mays* plante.

In die natuurlike omgewing mag daar 'n menigte biotiese en abiotiese stressors van gewasgroei wees. Die navorsing waarvan hier verslag gedoen word is van groot waarde vir die landbousektor. Vroeë identifisering van die effek van 'n kombinasie van stressors (in hierdie geval SO_2 en droogte) kan uitermatige oesverliese voorkom. Bestuursplanne moet dus dienooreenkomstig geïmplimenter word. Hierdie navorsing beklemtoon die relevansie en belangrikheid van kombinasie stress-respons studies op gewasse.

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List of abbreviations

A_{370}	CO ₂ assimilation rate at ambient CO ₂ concentrations (370 $\mu\text{mol}\cdot\text{mol}^{-1}$)
A_0	CO ₂ assimilation rate at an intercellular CO ₂ concentration of 370 $\mu\text{mol}\cdot\text{mol}^{-1}$, or above, where no stomatal limitation is present.
ABS/RC	The specific energy flux (per PSII reaction centre) for light absorption
C_a	Atmospheric CO ₂ concentration
CCI	Chlorophyll content index
CE	Carboxylation efficiency
CF	Carbon filtered
C_i	Intercellular CO ₂ concentration
DCMU	(3-(3,4-dichlorophenyl)-1,1-dimethylurea)
DW	Dry weight
DS	Drought stress
ET	Electron transport
ET_0/RC	Specific energy flux (per PSII reaction center) for electron transport
F_V/F_M	Quantum yield of primary photochemistry
F_W	Fresh weight
g_s	Stomatal conductance
J_{max}	Maximum CO ₂ assimilation rate at saturating CO ₂ concentrations
ℓ	Relative stomatal limitation of photosynthesis
OEC	Oxygen Evolving Complex
OTC	Open Top Chamber
PAD	Pollutant absorbed dose
PAR	Photosynthetic active radiation
PEA	Plant Efficiency Analyser
$PI_{\text{ABS}\cdot\text{total}}$	Photosynthetic performance index based on absorption basis
PLC	Photosynthetic leaf chamber

PM _{2.5}	Particulate matter with an aerodynamic diameter smaller than or equal to 2.5 μm
(PM ₁₀)	Particulate matter with an aerodynamic diameter equal to or less than 10 μm
Ppb	parts per billion
PQ	Plastoquinone
PSI	Photosystem I
PSII	Photosystem II
PVPP	Polyvinylpyrrolidone
δ	Probability for the formation of end electron acceptors
ϕ_{E0}	Quantum yield of electron transport
ϕ_{R0}	Quantum efficiency of the formation of reducing equivalents
ψ_0	Efficiency of converting a trapped exciton to electron transport further than Q_A^- into the electron transport chain
Q_A	Primary bound quinone
Q_A^-	Reduced primary bound quinone
Q_B	Secondary bound quinone
Q_B^-	Reduced secondary bound quinone
RC	Photosystem II reaction center
RC/ABS	The density
WW	Well watered
WUE	Water use efficiency

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