



The International Cooperative Programme on Effects of Air Pollution on Natural Vegetation and Crops  
& ARCHES-Conseils  
& International Union of Forest Research Organizations - Research Group 7.01

*present the*

# International Conference on Ozone and Plant Ecosystems

## 21-25 May, 2018

SANT'APOLLONIA AUDITORIUM  
FLORENCE, ITALY



*Ozone & Plants conference  
Florence, 21-25 May 2018*



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## 1. Rationale of the Conference

Ozone pollution continues to be a serious issue for terrestrial ecosystems and plant health. This conference allows all experts in the interactions between ozone and plant ecosystems to meet and discuss the state-of-the-art and the future strategies for decision-makers.

The main subjects of the conference are:

### 1. Monitoring, modelling and assessing the risk of ozone damage to plant ecosystems

Proofs of the impacts of ambient ozone on plant ecosystems are still elusive. New monitoring approaches and epidemiological studies are developing. Modelling of ozone is becoming more and more sophisticated at high resolution. Risk assessment is evaluating many different metrics for plant protection, with a focus on stomatal ozone flux. All these developments, with focus on the most modern techniques, are discussed in this session.

### 2. How plant ecosystems affect ozone concentration in the atmosphere

Ozone deposition is strongly affected by the type of vegetation. Exchanges of biogenic volatile organic compounds is known to contribute to ozone chemistry in the atmosphere. This session addresses mechanisms, seasonality, responses to ozone singly and in combination with other environmental factors, as well as selection of appropriate green infrastructure for urban greening.

### 3. How plant ecosystems respond to ozone exposure

This session includes food security and effects on forests and grasslands. A main aim is to evaluate strategies for maximizing yield, productivity and other environmental services of plant ecosystems under ozone stress.



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## 2. Opening Addresses

**Elena Paoletti**, IUFRO RG7.01 Coordinator

As coordinator of the IUFRO (International Union of Forest Research Organizations) Research Group 7.01 'Impacts of Air Pollution and Climate Change on Forest Ecosystems', I am pleased to welcome in Florence around a hundred international experts from 29 countries distributed in four continents. This is an index of the global significance of the ozone and plants subject. IUFRO is the largest network of forest research in the world, and works for strengthening the forest science community worldwide. Within Division 7 'Forest Health', RG 7.01 is a major forum for discussing the topical issues of climate and pollution impacts on forests. This conference, in particular, is supported by the IUFRO Working Parties 7.01.09 'Ground-level ozone', 7.01.02 'Genetic, biochemical and physiological processes' and 7.01.05 'Modelling and risk assessment', because assessing ozone risk to plant ecosystems requires an holistic approach. We are glad to host this second conference on ozone and plant ecosystems in Italy, which traditionally is a hot spot of ozone pollution. Control measures, however, are becoming effective and improvements are coming. We are also very glad to collaborate with ICP Vegetation in expanding our focus from forests to grasslands and crops. We hope that the results of this conference, with 51 talks and 47 poster presentations, will help improving our understanding on the relationships between ozone and plants, and expanding this knowledge worldwide. I would like to express my sincere appreciation to the members of the local organization and wish all participants all the best for a very fruitful conference.

**Harry Harmens**, Chair of ICP Vegetation

As Chair of the ICP Vegetation I would like to welcome you to the second 'Ozone & Plants' conference. The ICP Vegetation is an International Cooperative Programme (ICP) that reports on the effects of air pollutants on natural vegetation and crops. The ICP Vegetation is a subsidiary body of the Working Group on Effects (WGE) of the UNECE Convention on Long-range Transboundary Air Pollution (LRTAP). In particular, the ICP Vegetation focuses on the following air pollution problems: i) Quantifying the risks to vegetation posed by ozone pollution and collating field-based evidence of ozone impacts; ii) The atmospheric deposition of heavy metals, nitrogen and persistent organic pollutants (POPs) to vegetation. Field-based evidence and assessments of current and future risks of impacts on vegetation are used to guide air pollution abatement policies developed within the LRTAP Convention, for ozone in particular with respect to the 1999 Gothenburg Protocol to Abate Acidification, Eutrophication and Ground-level Ozone (amended in 2012). The ICP Vegetation also encourages outreach activities to other regions such as Asia, Africa and South America and has recently established an ICP Vegetation-Asia network. Ground-level has been shown to have negative impacts on crop yield (both quality and quantity), tree growth (above- and below-ground) and growth/flower number/seed number of other ozone-sensitive plant species. Hence, ozone adversely affects food production, terrestrial ecosystems and the services they provide. Although peak episodes of ozone pollution have declined in recent decades in many parts of the USA and Europe, peak concentrations have risen in developing regions, in particular in South-East Asia. This has contributed to rising background ozone concentrations elsewhere, hence, air pollution abatement measures are needed globally to combat ozone pollution impacts worldwide. I wish you all a successful meeting and I am looking forward to enhance the evidence and knowledge base of ozone impacts and its mechanisms on vegetation.





### 3. Schedule and Programme

#### Programme in short

TIME	Monday, 21	Tuesday, 22	Wednesday, 22	Thursday, 23	Friday, 25
08.00		<b>Registration</b>			<b>Move to Sesto Fiorentino</b>
08.30			<b>Session 2</b> Chair: Z. Feng	<b>Session 2</b> Chair: A. Giovannelli	
09.00		<b>Opening remarks</b>			
09.30		<b>Session 1</b> Chair: Pierre Sicard	<b>Conference Picture</b>		<b>Visiting the Ozone-Face</b>
10.00		<b>Coffee break</b>	<b>Coffee break and Poster session</b>	<b>Coffee break and Poster session</b>	
10.30					
11.00		<b>Session 1</b> Chair: P. Sicard	<b>Session 2</b> Chair: E. Paoletti	<b>Session 2</b> Chair: E. Agathokleus	<b>Move to Verrazzano</b>
11.30					<b>Visiting the winery</b>
12.00					<b>Wine tasting and Lunch</b>
12.30		<b>Lunch</b>	<b>Lunch</b>	<b>Lunch</b>	<b>Move to Siena</b>
13.00					
13.30					
14.00		<b>Session 1</b> Chair: A. De Marco	<b>Session 2</b> Chair: L. Zhang	<b>Session 2</b> Chair: M. Domingos	
14.30					
15.00		<b>Coffee break</b>		<b>Coffee break</b>	
15.30					
16.00		<b>Session 1</b> Chair: H. Harmens	<b>Coffee break</b>	<b>Session 3</b> Chair: Y. Hoshika	<b>Free time to visit Siena</b>
16.30					
17.00			<b>Session 2</b> Chair: E. Carrari	<b>Round table</b> Chair: H. Harmens	
17.30				<b>Closing remark by E. Paoletti</b>	<b>Back to Florence</b>
18.00		<b>Wine tasting and Poster session (18.10-20.00)</b>			
18.30			<b>Free time</b>		
19.00	<b>Welcome cocktail and registration (19.00-20.30)</b>				
19.30					
20.00			<b>Conference Dinner (20.00-23.00)</b>		
20.30					
21.00					
23.00					



## Programme

### Monday 21<sup>st</sup> May, 2018

19:00-20:30	Welcome cocktail, registration of participants, mounting of posters
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### Tuesday 22<sup>nd</sup> May, 2018

8:00	Registration of participants	
9:00	Opening remarks	
	Elena Paoletti (local organizer) Harry Harmens (ICP Vegetation) Zhaozhong Feng (IUFRO RG7.01.02) Alessandra De Marco (IUFRO RG7.01.05) Pierre Sicard (IUFRO RG7.01.09) Furio Forni (Regione Toscana)	
<b>Session 1</b>	<b>“Monitoring, modelling and assessing the risk of ozone damage to plant ecosystems”</b> <b>Chair: Pierre Sicard</b>	
9:30	<b>Keynote speaker- Pierre Dizengremel</b> (France). Historical approach to research on ozone and plants	<b>S1.01</b>
10:00	Coffee break	
10:30	<b>Harry Harmens</b> (UK). TOAR-Vegetation: Present day tropospheric ozone distribution and trends relevant to vegetation	<b>S1.02</b>
10:50	<b>Howard Neufeld</b> (USA). Ozone trends in Great Smoky Mountains National Park and their implications for plants	<b>S1.03</b>
11:10	<b>Valda Araminiene</b> (Lithuania). Meteorological conditions and ambient ozone effects on temperate forest vegetation	<b>S1.04</b>
11:30	<b>Iva Hůnová</b> (Czech Republic). Mapping changes in spatial patterns of ambient ozone levels	<b>S1.05</b>
11:50	<b>Matej Rupel</b> (Slovenia). Air pollutant ozone comparison between city areas and big forest land in Slovenia	<b>S1.06</b>
12:10	<b>Algirdas Augustaitis</b> (Lithuania). Ozone flux and its effect in relation to meteorology on ecophysiological reactions in hemi-boreal forest tree species	<b>S1.07</b>
12:30	Lunch	
<b>Session 1</b>	<b>“Monitoring, modelling and assessing the risk of ozone damage to plant ecosystems”</b> <b>Chair: Alessandra De Marco</b>	
14:00	<b>Alessandro Anav</b> (Italy). A multi-model framework for ozone risk assessment	<b>S1.08</b>

<b>14:20</b>	<b>Barbara B. Moura</b> (Brazil). Exposure- and flux-based assessment of ozone risk to sugarcane plants	<b>S1.09</b>
<b>14:40</b>	<b>Yasutomo Hoshika</b> (Italy). Development of stomatal conductance modeling for forest trees under elevated ozone	<b>S1.010</b>
<b>15:00</b>	<b>Auke Visser</b> (Netherlands). Multi-scale monitoring and modeling of ozone deposition to ecosystems	<b>S1.011</b>
<b>15:20</b>	<b>Ionel Popa</b> (Romania). Passive vs. active: Applicability of Loibl function in modeling hourly ozone concentrations from passive samplers in Romanian Intensive Monitoring Network	<b>S1.012</b>
<b>15:40</b>	<b>Ioannis Droutsas</b> (UK). A new crop modelling technique for dealing with ozone stress	<b>S1.013</b>
<b>16:00</b>	<b>Coffee break</b>	
<b>Session 1</b>	<b>“Monitoring, modelling and assessing the risk of ozone damage to plant ecosystems”</b> <b>Chair: Harry Harmens</b>	
<b>16:30</b>	<b>Harry Harmens</b> (UK). ICP Vegetation-Asia: A new policy-focused evidence collecting and risk assessment network for ozone	<b>S1.014</b>
<b>16:50</b>	<b>Ya Tang</b> (China). Research is highly required on the impacts of increasing surface ozone in China	<b>S1.015</b>
<b>17:10</b>	<b>Xue Qiao</b> (China). Vegetation exposure to ozone in China: model performance and regions needing further field investigations	<b>S1.016</b>
<b>17:30</b>	<b>Frode Stordal</b> (Norway). Ozone and climate stresses on sub-Arctic tundra vegetation: Modelling of stomatal fluxes in midnight sun	<b>S1.017</b>
<b>17:50</b>	<b>Bheki Maliba</b> (South Africa). Using OJIP transients to monitor the effect of elevated ozone on canola plants, South Africa	<b>S1.018</b>
<b>18:10</b>	<b>Wine tasting and poster session</b>	

**Wednesday 23<sup>rd</sup> May, 2018**

<b>Session 2</b>	<b>“How plant ecosystems respond to ozone exposure”</b> <b>Chair: Zhaozhong Feng</b>	
<b>08:30</b>	<b>Keynote speaker - Giacomo Lorenzini</b> (Italy). A mechanistic understanding of ozone impact on forest ecosystems	<b>S2.01</b>
<b>09:00</b>	<b>Keynote speaker - Youzhi Feng</b> (China). Elevated ground-level ozone modifies the microbiome	<b>S2.02</b>
<b>09:30</b>	<b>Conference picture</b>	
<b>10:00</b>	<b>Coffee break &amp; Poster Session</b>	
<b>Session 2</b>	<b>“How plant ecosystems respond to ozone exposure”</b> <b>Chair: Elena Paoletti</b>	
<b>11:00</b>	<b>Harry Harmens</b> (UK). Impacts of changing ground-level ozone profiles in Europe on vegetation in the current and future climate	<b>S2.03</b>
<b>11:20</b>	<b>Zhaozhong Feng</b> (China). The response of water use efficiency in poplar to elevated ozone	<b>S2.04</b>

<b>11:40</b>	<b>Ane Vollsnes</b> (Norway). Ozone stress on sub-Arctic tundra vegetation: ozone exposure experiments with daylength manipulation	<b>S2.05</b>
<b>12:00</b>	<b>Tsetan Dolker</b> (India). Grassland community response to elevated ozone exposure by assessment of soil dynamics, plant diversity and total biomass	<b>S2.06</b>
<b>12.20</b>	<b>Lunch</b>	
<b>Session 2 “How plant ecosystems respond to ozone exposure” Chairs: Lu Zhang</b>		
<b>13:40</b>	<b>Keynote speaker - Hakan Pleijel</b> (Sweden). Ozone impacts on crop yield and food security	<b>S2.07</b>
<b>14:10</b>	<b>Katrina Sharps</b> (UK). Global food security modelling: quantifying the threat to crop production from ozone pollution	<b>S2.08</b>
<b>14.30</b>	<b>Shashi B. Agrawal</b> (India). Understanding the ozone response in Indian crop plants	<b>S2.09</b>
<b>14.50</b>	<b>Elina Oksanen</b> (Finland). Sensitivity of Indian crop plants to ozone	<b>S2.010</b>
<b>15:10</b>	<b>Michael Frei</b> (Germany). Breeding of ozone tolerant cereal crops: progress and prospects	<b>S2.011</b>
<b>15.30</b>	<b>Felicity Hayes</b> (UK). Impact of ozone on physiology and yield of African crops	<b>S2.012</b>
<b>15.50</b>	<b>Juliette Leymarie</b> (France). Responses of crops to ozone exposure: study of physiological parameters	<b>S2.013</b>
<b>16:10</b>	<b>Daniel B. Ward</b> (Portugal). Assessing the risk of tropospheric ozone phytotoxic effect on Southern European Mediterranean environments: a review with emphasis on vineyards	<b>S2.014</b>
<b>16.30</b>	<b>Coffee break</b>	
<b>Session 2 “How plant ecosystems respond to ozone exposure” Chair: Elisa Carrari</b>		
<b>17:00</b>	<b>Laurence Dalstein-Richier</b> (France). Monitoring ozone, foliar deficit and ozone-specific foliar damage on national forest plots in France over several years	<b>S2.015</b>
<b>17:20</b>	<b>Sheng Xu</b> (China). Physiological responses of turf-type <i>Festuca arundinacea</i> to elevated O <sub>3</sub>	<b>S2.016</b>
<b>17:40</b>	<b>Anthony Gandin</b> (France). Multi-level responses to ozone in Euramerican poplars	<b>S2.017</b>
<b>18:00</b>	<b>Tetsuto Sugai</b> (Japan). Non-linear responses of two larch species exposed to four ozone levels	<b>S2.018</b>
<b>18:20</b>	<b>Maamar Benchohra</b> (Algeria). Effects of short term ozone fumigation on young trees of poplar <i>Populus nigra</i>	<b>S2.019</b>
<b>20:00</b>	<b>Conference dinner</b> (Palazzo Budini-Gattai Via De Servi 51, see the map at chapter 6)	

Thursday 24<sup>th</sup> May, 2018

<b>Session 2 “How plant ecosystems respond to ozone exposure”</b>		
<b>Chair: Alessio Giovannelli</b>		
<b>8:30</b>	<b>Md. Ashrafuzzaman</b> (Germany). Insights into the mode of action of ethylenediurea (EDU) as an antiozonant in rice ( <i>Oryza sativa</i> L.)	<b>S2.O20</b>
<b>8:50</b>	<b>Richa Rai</b> (India). Impact of elevated tropospheric O <sub>3</sub> and ambient temperature on early and late sown cultivars of wheat: an insight to biochemical mechanism and yield	<b>S2.O21</b>
<b>9:10</b>	<b>Makoto Watanabe</b> (Japan). Leaf internal process of photosynthesis in Siebold's beech seedlings under elevated ozone	<b>S2.O22</b>
<b>9:30</b>	<b>Marisa Domingos</b> (Brazil). The role of secretory glands in plant sensitivity to ozone: an alternative route for uptake?	<b>S2.O23</b>
<b>09.50</b>	<b>Takayoshi Koike</b> (Japan). Leaf beetle activities on Japanese white birch grown under elevated O <sub>3</sub>	<b>S2.O24</b>
<b>10:10</b>	<b>Coffee break &amp; Poster Session</b>	
<b>Session 2 “How plant ecosystems respond to ozone exposure”</b>		
<b>Chair: Evgenios Agathokleous</b>		
<b>11:00</b>	<b>Ignacio González-Fernández</b> (Spain). Tropospheric ozone interactions on nitrogen cycling in Mediterranean annual pastures	<b>S2.O25</b>
<b>11:20</b>	<b>Lucienne De Witte</b> (Switzerland). Ozone flux is negatively related to foliar N and P in Swiss beech and Norway spruce forest	<b>S2.O26</b>
<b>11:40</b>	<b>Alessandra Podda</b> (Italy). Nutrient fertilization mitigates the effects of ozone exposure on poplar plants	<b>S2.O27</b>
<b>12:00</b>	<b>Lorenzo Labrador</b> (Switzerland). The WMO's Global Atmosphere Watch: a general overview and services to ecosystems	<b>S2.O28</b>
<b>12:20</b>	<b>Lunch</b>	
<b>Session 2 “How plant ecosystems respond to ozone exposure”</b>		
<b>Chair: Marisa Domingos</b>		
<b>13:50</b>	<b>Nicolas Dusart</b> (France). Poplar submitted to a succession of ozone and drought stresses: dynamic of stomatal responses	<b>S2.O29</b>
<b>14:10</b>	<b>Marco Landi</b> (Italy). Influence of drought stress on the ozone-induced signalling mechanisms of two Mediterranean tree species	<b>S2.O30</b>
<b>14:30</b>	<b>Elisa Pellegrini</b> (Italy). Antioxidative responses of three oak species under ozone and water stress conditions	<b>S2.O31</b>
<b>14:50</b>	<b>Pin Li</b> (China). Effects of elevated O <sub>3</sub> on biomass allocation, non-structural carbohydrates and rhizosphere soil function in poplar by joint water and nitrogen limitation	<b>S2.O32</b>
<b>15:10</b>	<b>Coffee break</b>	

<b>Session 3 “How plant ecosystems affect ozone concentration in the atmosphere” Chair: Yasutomo Hoshika</b>		
<b>15:40</b>	<b>Keynote speaker - Silvano Fares</b> (Italy). Ozone and BVOC exchanges between biosphere and atmosphere	<b>S3.01</b>
<b>16:10</b>	<b>Gabriele Guidolotti</b> (Italy). Ozone fluxes from an urban park: the unique station of Bosco di Capodimonte in Naples	<b>S3.02</b>
<b>16:30</b>	<b>Rüdiger Grote</b> (Germany). Linking ozone susceptibility to induced emissions of biogenic volatile organic compounds	<b>S3.03</b>
<b>16:50</b>	<b>Xiangyang Yuan</b> (China). Spatial variability of isoprene emission in China under ambient conditions	<b>S3.04</b>
<b>17:10</b>	<b>Saxena Pallavi</b> (India). Ornamental plants used as tool for remediation of ozone pollution in indoor environment	<b>S3.05</b>
<b>17:30</b>	<b>Discussion: “The future of research on ozone impacts on plant ecosystems” Chair: Harry Harmens</b>	
<b>18:30</b>	<b>Closing remarks with delivery of the best poster award Elena Paoletti</b>	

**Friday 25<sup>th</sup> May, 2018: Scientific excursion**

<b>8:00-8:15</b>	Meeting point Piazza della Libertà, 3 (see map at chapter 6)
<b>8:15-8:45</b>	Transfer by bus from Firenze to Sesto Fiorentino
<b>09:00-11:15</b>	Visit of the O <sub>3</sub> FACE facility
<b>11:15-12:15</b>	Transfer by bus to Castello di Verrazzano
<b>12:15-13:30</b>	Visit of the winery
<b>13:30-14:30</b>	Wine tasting and lunch
<b>14:30-15:30</b>	Transfer by bus to Siena across the Chiantishire
<b>15:30-17:00</b>	Free time to visit Siena (see the map in the information material provided for the excursion)
<b>17:00 - 18:30</b>	Transfer by bus to Firenze

**Poster Session**

<b>Evgenios Agathokleous</b> (Japan). Elevated O <sub>3</sub> affects the decomposition process in different types of soil	P1
<b>Evgenios Agathokleous</b> (Japan). Japanese larch seedlings grown in containers, inoculated with ectomycorrhizal fungi, and exposed to ozone	P2
<b>Kirsti Ashworth</b> (UK). Ozone deposition in forest canopies.	P3
<b>Ingrida Augustaitienė</b> (Lithuania). Resiliency of Scots pine ( <i>Pinus sylvestris</i> L.) tree to acidifying compounds and surface ozone under the pressures of climate changes	P4
<b>Manuela Baumgarten</b> (Germany). Ozone Risk for Trees – dose response functions embedded in future climatic scenarios	P5

<b>Svetlana Bičárová</b> (Slovakia). Phytotoxic ozone effects on montane pines in the High Tatra Mts., Slovakia	P6
<b>Arnaud Carrara</b> (Spain). Ozone fluxes and GHG balance in a Spanish rice paddy field	P7
<b>Elisa Carrari</b> (Italy). MOTTLES: an innovative long term strategy for the definition of new critical levels to protect forest from ozone	P8
<b>Nivedita Chaudhary</b> (Israel). Effect of ozone on physiological response of Israeli wheat cultivars	P9
<b>Stan Cieslik</b> (Italy). Vine and ozone	P10
<b>Marisa Domingos</b> (Brazil). Anatomical responses of a tropical liana species ( <i>Passiflora edulis</i> ) to ozone stress	P11
<b>Nicolas Dusart</b> (France). Regulation of the ascorbate-glutathione cycle in leaves of poplar exposed to combined stresses (ozone and/or drought)	P12
<b>Marisa Domingos</b> (Brazil). Assessment of tolerance level of <i>Eugenia uniflora</i> L. to ozone under FACE fumigation	P13
<b>Anne Charlott Fitzky</b> (Austria). Ozone and biogenic volatile organic compound (BVOC) interactions on leaf surfaces of stressed urban trees: A project plan	P14
<b>Anthony Gandin</b> (France). PEPc contribution to CO <sub>2</sub> assimilation in ozone-treated poplars	P15
<b>Alessio Giovannelli</b> (Italy). Effects of long-term ambient ozone exposure on xylem morphology of O <sub>3</sub> sensitive poplar treated with ethylenediurea (EDU)	P16
<b>Felicity Hayes</b> (UK). Evidence of impacts of ozone on ecosystem services of grasslands	P17
<b>Yasutomo Hoshika</b> (Italy). Soil water availability affects ozone risk assessment in three European oaks	P18
<b>Takayoshi Koike</b> (Japan). Plant-insect interaction of elm seedlings treated with (NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> spray grown under free-air O <sub>3</sub> fumigation	P19
<b>Marco Landi</b> (Italy). Reversible photoinhibition in purple- and green-leafed sweet basil ( <i>Ocimum basilicum</i> ) exposed to ozone	P20
<b>Stefan Leca</b> (Romania). Radial growth response to ozone exposure and uptake of sessile oak ( <i>Quercus petraea</i> ) in Mihaesti Level II Forest Monitoring plot, Romania	P21
<b>Juliette Leymarie</b> (France). Physiological and ecological consequences of changes in volatile plant emissions induced by elevated atmospheric ozone and CO <sub>2</sub> concentrations	P22
<b>Jacopo Manzini</b> (Italy). Estimating the contribution of urban plants to the removal of atmospheric pollution: a species-specific case study in Italy	P23
<b>Teis N. Mikkelsen</b> (Denmark). Variable ozone episodes influence on yield and physiology in old and new wheat accessions under a climate change regime with elevated temperature and CO <sub>2</sub>	P24
<b>Tanja Mrak</b> (Slovenia). Effects of water deficiency and elevated ozone on root traits of three oak species	P25
<b>Elena Paoletti</b> (Italy). Complex responses of volatile organic compound emission to realistic levels of ozone and drought stress in three oak species	P26



<b>Elena Paoletti</b> (Italy). Isoprene response to increasing ozone concentrations in Oxford 'O <sub>3</sub> -sensitive' poplar clone grown in enhanced nitrogen and phosphorus soil content	P27
<b>Elena Parfenova</b> (Russia). Siberian pine and fir decline in the southern Siberian mountains: hypotheses and investigations	P28
<b>Elisa Pellegrini</b> (Italy). Potential roles of WRKY transcription factors in regulating oxidative protection and signalling in <i>Salvia officinalis</i> plants exposed to ozone	P29
<b>Alessandra Podda</b> (Italy). Photosynthetic performance, oxidative injury and growth of pomegranate plants under salt and ozone stress	P30
<b>Margarita Préndez</b> (Chile). What we can't see in the urban forest ecosystem: greenhouse gases or its precursors	P31
<b>Margarita Préndez</b> (Chile). Biomonitoring of anthropogenic volatile organic compounds in a urban arboreal species. Santiago de Chile	P32
<b>Chiara Proietti</b> (Italy). Ozone and climate change impacts on Southern European forests: MITIMPACT project concept	P33
<b>Chiara Proietti</b> (Italy). The importance of soil water availability on stomatal conductance regulation: implications for tropospheric ozone	P34
<b>Amélie Saunier</b> (Finland). Isoprene contribution to ozone production in a context of climate change in a <i>Quercus pubescens</i> forest	P35
<b>Bo Shang</b> (China). Ozone exposure- and flux-based response relationships with photosynthesis, leaf morphology and biomass in two poplar clones	P36
<b>Katrina Sharps</b> (UK). ICP Vegetation smart-phone App for recording incidences of ozone injury on vegetation	P37
<b>Tommaso Stella</b> (Germany). Modelling BVOC potential emission from energy crops – a case study in Brandenburg, Germany	P38
<b>Tetsuto Sugai</b> (Japan). Effects of ozone on two larch species treated with ammonium sulfate or salt loading	P39
<b>Haoye Tang</b> (China). Effects of elevated ozone concentration on CH <sub>4</sub> and N <sub>2</sub> O emission from paddy soil with two Chinese rice cultivars under fully open-air field conditions	P40
<b>Lei Tong</b> (China). Diurnal and phenological variations of O <sub>3</sub> and CO <sub>2</sub> fluxes of winter wheat canopy under short-term O <sub>3</sub> exposure	P41
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## Session 1

# **Monitoring, modelling and assessing the risk of ozone damage to plant ecosystems**



## Historical approach to research on ozone and plants

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Seventy years ago, Haagen-Smit was commissioned to determine the nature of the smog in Los Angeles, which caused haze, eye irritation and plant damage. Earlier work related that damages to crops were different from those observed in smog episodes in the eastern USA and Europe. Consequently, Haagen-Smit realized that the odor of smog resembled that of oxidized hydrocarbons. He tested the action of ozone and gasoline on crops and got symptoms of damage similar to those caused by smog. Getting similar results with hydrocarbons and nitrogen dioxide under sunlight, he concluded that the smog resulted from the photochemical reaction of hydrocarbons and nitrogen dioxide from car exhausts and fuel combustion, ozone being a secondary pollutant. Visible symptoms of ozone damage on pines were also identified in the mountains surrounding Los Angeles. This breakthrough in knowledge further led to the development of research programs in the USA. Concurrently, in the eighties in Europe, German scientists claimed that the conifers in Germany and France were declining. A fruitful cooperation started between the two countries, followed by European programs, allowing large improvement in knowledge about the physiology of crops and trees and their behavior when exposed to ozone. At the leaf level, the decreased photosynthesis and the increased respiration were the physiological symptoms of a lower growth of plants. The negative impact of ozone counteracting the possible positive effect of an increasing atmospheric CO<sub>2</sub> cannot be denied. To improve the indices of risk assessment, the SUM0 and AOT40 metrics were abandoned in favor of integrating the real quantity of ozone entering the leaf, leading to POD's. The challenge remains to better include the detoxification capacity of cells even though recent work has discussed this aspect. An important point concerns the differences between C<sub>3</sub> (including trees) and C<sub>4</sub> plants. At similar POD's, C<sub>4</sub> plants show a faster decline in metabolic activities under ozone. However, they resist better to ozone in the fields, thanks to their lower stomatal conductance. The study of the behavior of these two groups of plants exposed to ozone and associated stresses (drought, elevated CO<sub>2</sub>), and the upscaling to ecosystems, need to be improved.

**Keywords:** history, smog, ozone, physiology, C<sub>3</sub> and C<sub>4</sub> plants

## **TOAR-Vegetation: Present day tropospheric ozone distribution and trends relevant to vegetation**

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This paper summarises results from analysis of ozone vegetation metrics as part of the global Tropospheric Ozone Assessment Report (TOAR), submitted to Elementa as TOAR-Vegetation. It reports on the present day global distribution of ozone at over 3300 vegetated sites across the world and on long-term trends at nearly 1200 sites. TOAR-Vegetation focusses on 5 year means (2010-2014) and long-term trends (1995 to 2014 and 2000 to 2014) for M12, AOT40 and W126. These are presented for climatic-zone relevant three months periods for wheat and rice, and for hemisphere-specific six months periods for perennial vegetation. The highest values for these metrics are currently in mid-latitudes of the northern hemisphere, including S USA, the Mediterranean basin, N India, NW and central China, the Republic of Korea and Japan. Ozone concentrations are lowest in Australia, New Zealand, southern parts of S America and some northern parts of Europe, Canada and the USA. W126 and AOT40 were mainly decreasing in North American and European sites (1995 – 2014) whilst M12 values were increasing at many sites in these regions. AOT40 critical levels and W126 values of concern continue to be exceeded in many of these areas, indicating that vegetation is likely to be at risk of negative effects of ozone exposure. In East Asia, primarily represented by sites in Japan and the Republic of Korea, the mean AOT40 and W126 values were higher than those in North America and Europe for perennial vegetation and wheat time-periods. Trend analysis indicated that the ozone threat to vegetation has increased from 1995 to 2014 in Japan, and 2000 to 2014 in the Republic of Korea.

**Keywords:** Global, ozone metrics, trends, wheat, rice, perennial vegetation

## **S1.O2**

## Ozone trends in Great Smoky Mountains National Park and their implications for plants

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Great Smoky Mountains National Park (GRSM) is the most visited national park in the United States and has some of the highest levels of biodiversity of any park unit. It is topographically complex, with an elevational range of 1757 m, yet relatively small in size (~211,419 ha) with maximum width and length of 29 and 52 km, respectively. The Park has historically been subject to elevated levels of pollutants, including sulfur dioxide (SO<sub>2</sub>), ozone (O<sub>3</sub>), and nitrogen oxides (NO<sub>x</sub>), and as a result it is one of the most intensively monitored. This paper reports on [O<sub>3</sub>] trends from 1989 to 2016 for six monitoring sites in and adjacent to GRSM, ranging in elevation from 564 m to 2030 m. W126<sub>12-h</sub> exposure indices (Apr-Oct) were found to have increased from the 1980s until the late 1990s, before reversing and decreasing to the present. Spatially, [O<sub>3</sub>] are higher in the western portion and higher elevations of GRSM. The highest 1-h concentration ever recorded was 135 ppb in 1998, but since 2004, only 2 sites have experienced [O<sub>3</sub>] ≥ 100 ppb. Decreased exposures result primarily from a reduction in [O<sub>3</sub>] ≥ 60 ppb. Maximum W126<sub>12-h</sub> exposures have shifted almost exclusively to earlier in the season (Apr – Jun). Exposure episodes (3 or more consecutive hours with [O<sub>3</sub>] ≥ 60 ppb) are greatly reduced in recent years and respite periods (2 or more hours with [O<sub>3</sub>] < 60 ppb) are more frequent. Decreases in W126<sub>12-h</sub> are highly correlated with reduced NO<sub>x</sub> emissions from regional power plants and are coincident with those found over most of the eastern United States. The combination of shorter episodes and longer respite times, coupled with reduced peak [O<sub>3</sub>], suggests plants are at reduced risk from ozone exposure compared to those experienced 20-25 years ago.

**Keywords:** Smoky Mountains, ozone trends, W126 exposure, plants

## **Meteorological conditions and ambient ozone effects on temperate forest vegetation**

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Tropospheric ozone can cause damage on sensitive vegetation during the growing season. Higher concentrations of tropospheric ozone more often are fixed in the Northern than in the Southern Hemisphere. Ozone concentrations and emissions of ozone precursors vary in the Northern Hemisphere. As a fact, the emissions of ozone precursors are recently slightly decreasing in Europe and North America and increasing in Asia. Lithuania represents temperate forest zone. Despite relatively low mean ambient ozone concentration in Lithuania, its negative impact on vegetation has been recently registered. The local studies indicated that ozone concentration has induced by 5-10% decrease in crop yield. In Lithuania, visible ozone injuries on forest plant leaves are assessed since 2007. The study aim was to estimate interaction between meteorological conditions, ambient ozone level and the severity of visible ozone injuries on species representing temperate forest zone plants. The study was performed in nine Forest Monitoring Level II plots situated in Lithuania during 2007–2017–year period. Visible foliar injuries were assessed in the Light Exposed Sampling Sites (LESS); main meteorology parameters were provided from six meteorological stations located nearby Forest Monitoring plots. Ozone concentrations were measured in two Monitoring Stations, representing the Western and Eastern Lithuania. During the recent decade, mean tropospheric ozone concentration ranged between 25–34 ppb during April–September period in the Eastern part of Lithuania. Meanwhile, in the Western part of the country, the concentration of ozone ranged within 32–37 ppb or it was significantly higher than in the Eastern part. Mean annual ozone concentration slightly increased during last three years. There was found positive correlation between ozone concentration in August and number of plants with visible ozone injury on leaves. The species *Rubus idaeus*, *Frangula alnus*, *Corylus avellana* and *Fraxinus excelsior* were mostly damaged by ozone.

**Keywords:** Ozone, forest vegetation, Lithuania, defoliation, visible ozone injury, temperate forest

## **S1.04**



## Mapping changes in spatial patterns of ambient ozone levels

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Ambient ozone ( $O_3$ ) is a key atmospheric constituent. It belongs to green-house gases and plays a major role in atmospheric chemistry. It contributes substantially to the oxidation capacity of atmosphere supporting its self-cleaning capacity. On the other hand its toxic both for human health and ecosystems. The formation of  $O_3$  is highly dependent on meteorological conditions (such as solar radiation, air temperature, relative humidity, occurrence of precipitation events) and thus the year-to-year variability of  $O_3$  concentrations is high. In the Czech Republic (CR), ambient ozone concentrations have been observed within a monitoring network since 1993, currently by *ca* 50 measuring sites representing different environments. Our results reveal that,  $O_3$  levels are high enough to harm both human health and ecosystems (Hůnová, Schreiberová 2012; Hůnová et al. 2013, Hůnová et al. 2016). The aim of our contribution is to map the areas potentially at risk from high ambient ozone exposure. Specifically, we intend to indicate the hotspots, where effects of elevated ozone exposures are to be expected and where forests are endangered due to excessive  $O_3$  loads. We present the-year-to-year spatial variability in  $O_3$  concentrations for forested areas. The final output is a map showing risk areas in the Czech forests averaged over the period of 2000–2015, the risk being defined in relative terms.

### Reference:

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**Keywords:** ambient ozone, concentrations, risk areas, spatial variability

**S1.O5**

## **Air pollutant ozone comparison between city areas and big forest land in Slovenia**

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Ozone is important air pollutant, dangerous for plants, animals and human health. The Slovenian Forestry Institute (SFI) have 15 years experiences (2003 – 2017), assessing ozone visible injury on forest vegetation in Slovenia, observed as foliage damage on the forest trees and shrubs species. During these years the visible ozone damages were observing at 5 to 11 intensive monitoring plots (ICP Forests Level II) and some last years also in Ljubljana urban and peri-urban forests. In the same time span the SFI has been monitoring ozone concentrations in the air with passive samplers. In 2013, we expanded air monitoring to three other pollutant gases: sulphur dioxide, nitrogen dioxide and ammonia. We monitor air pollution with passive samplers also in the capital of Slovenia – Ljubljana. We perform measurements in urban areas and urban and peri-urban forests (city parks, nature park, forest covered hill, riparian forests). Identification of visible ozone or visible ozone like symptoms in small trees and shrubs in Ljubljana urban forests was performed. Visible foliar injury by ozone we surveyed on 3 *off-plots*, on light-exposed sampling sites at urban forest edge according to the ICP Forests manual. Our observations of negative impacts of ozone on forest trees, we compare between city areas in Ljubljana and big forest land Kočevska. Kočevska landscape is one of the most naturally preserved parts of Slovenia and Central Europe. 90% of the province is covered by forests, predominantly Dinaric fir and beech forests. We looked different forest trees and shrubs species. Different species show different sensibility to the same ozone dose. We also try to compare ozone visible damages vegetation and ozone concentrations values in the air with passive samplers.

## **S1.06**

## **Ozone flux and its effect in relation to meteorology on ecophysiological reactions in hemi-boreal forest tree species**

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In the presented study the causative relationships between environmental factors and tree ring width formation of the prevailing in Lithuania tree species as the main parameters of tree capacity to adapt to and mitigate the recent global changes at hourly and diurnal scales were investigated. The obtained data revealed that Norway spruce trees are better adapted to recent climatic conditions in temperate forest than Scots pine and birch trees. Even during the drought episode spruce stem increment exceeded increment of the rest of the considered tree species. Scots pine seems to be the most sensitive species to seasonal environmental changes. The highest reversible fluctuations in stem circumference on hourly scale resulting from meteorology and surface ozone became the main drivers of their gradually increasing growth intensity. Silver and Downy birch tree reactions indicated the lowest sensitivity of these tree species not only to unfavorable environmental factors but also to favorable factors which should stimulate tree growth intensity. This is why the growth intensity of these tree species recently has been gradually decreasing. The hypothesis that the coniferous species are more adaptive to recent climate changes and their capacity to mitigate the threats of global changes is higher than that of deciduous tree species was confirmed. The study is based on the results obtained conducting national project supported by Lithuanian Council of Research "FOREstRESS" (SIT- 3/2015).

**Keywords:** ozone flux, meteorology, stem circumference, integrated effect

## **A multi-model framework for ozone risk assessment**

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Plant ecosystems play a key role in regulating the concentration of air pollutants in the planetary boundary layer. Among common air pollutants, O<sub>3</sub> is probably the most damaging to forests and crops, frequently reaching high concentrations over large regions of the world and particularly in Mediterranean area. However, O<sub>3</sub> effects on vegetation depend not only on the atmospheric concentrations but also on O<sub>3</sub> uptake through the stomata. Therefore, the quantification of the amount of ozone removed from the atmosphere, and the consequent injuries to vegetation, are particularly relevant for climate, ecological and risk assessment studies. We developed a regional multi-model framework to be used for integrated risk assessment and for studies on potential risks caused by O<sub>3</sub> pollution on European forests. This framework relies on a mesoscale model that generates climate forcing used offline to run a Chemistry Transport Model (CTM). The O<sub>3</sub> concentrations computed through the CTM (CHIMERE) and the climatic variables computed by a regional weather forecast model (WRF) combined with a stomatal conductance model, allowed us to estimate the stomatal O<sub>3</sub> fluxes using different phenological models and assumptions on the water uptake by plants at different soil depths in the rooting zone and to evaluate the differences between an exposure-based index (i.e. AOT40) and an index based on the effective absorbed O<sub>3</sub> dose (i.e. POD<sub>Y</sub>).

**Keywords:** O<sub>3</sub>, AOT40, POD<sub>Y</sub>, DO<sub>3</sub>SE, Plant Phenology

## **S1.O8**

## Exposure- and flux-based assessment of ozone risk to sugarcane plants

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Ozone (O<sub>3</sub>) is a toxic oxidative air pollutant, with significant detrimental effects on crops. Sugarcane (*Saccharum* spp.) is an important crop with no O<sub>3</sub> risk assessment performed so far. This study aimed to assess O<sub>3</sub> risk to sugarcane plants by using exposure-based indices (AOT40 and W126) based on O<sub>3</sub> concentrations in the air, and the flux-based index (POD<sub>y</sub>, where y is a threshold of uptake) that considers leaf O<sub>3</sub> uptake and the influence of environmental conditions on stomatal conductance ( $g_{sto}$ ). Two sugarcane genotypes (IACSP94-2094 and IACSP95-5000) were subjected to a 90-day Free-Air Controlled Experiment (FACE) exposure at three levels of O<sub>3</sub> concentrations: ambient (Amb); Amb x1.2; and Amb x1.4. Total above-ground biomass (AGB), stalk biomass (SB) and leaf biomass (LB) were evaluated and the potential biomass production in a clean air was estimated by assuming a theoretical clean atmosphere at 10 ppb as 24 h O<sub>3</sub> average. The Jarvis-type multiplicative algorithm was used to parametrize  $g_{sto}$  including environmental factors such as air temperature, light intensity, air vapor pressure deficit, minimum night-time temperature. Ozone exposure caused a negative impact on AGB, SB and LB. The O<sub>3</sub> sensitivity of sugarcane may be related to its high  $g_{sto}$  (~535 mmol H<sub>2</sub>O m<sup>-2</sup> s<sup>-1</sup>). As sugarcane is adapted to hot climate conditions,  $g_{sto}$  was restricted when the current minimum air temperature ( $T_{min}$ ) was below ~14°C and the minimum night-time air temperature of the previous day ( $T_{nmin}$ ) was below ~7.5°C. The flux-based index (POD<sub>y</sub>) performed better than exposure-based indices in estimating O<sub>3</sub> effect on biomass losses. We recommend a y threshold of 2 nmol m<sup>-2</sup> s<sup>-1</sup> to incorporate O<sub>3</sub> effects on both AGB and SB and 1 nmol m<sup>-2</sup> s<sup>-1</sup> on LB. In order to not exceed 4% reduction in sugarcane growth, and considering the genotypes we recommend the following critical levels: 1.09 and 1.04 mmol m<sup>-2</sup> POD<sub>2</sub> for AGB, 0.91 and 0.96 mmol m<sup>-2</sup> POD<sub>2</sub> for SB, and 3.00 and 2.36 mmol m<sup>-2</sup> POD<sub>1</sub> for LB of IACSP95-5000 and IACSP94-2094 respectively.

**Keywords:** tropospheric ozone, POD<sub>y</sub>, stomatal conductance, ozone FACE, air pollution

## Development of stomatal conductance modeling under elevated ozone for forest trees

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Ozone enters leaves via stomata and causes a damage to leaves of trees. Modeling of stomatal conductance ( $g_s$ ) is considered as an essential factor to assess ozone impacts. In this presentation, recent developments for the modeling of  $g_s$  are summarized: 1) we reviewed 235 publications of field-observed  $g_s$  for the parameterization of Jarvis-type model in global woody plant functional types (PFTs). The relationships between stomatal parameters and climatic factors (MAT, mean annual air temperature; MAP, mean annual precipitation) were assessed, and 2) to discuss the effect of ozone on  $g_s$ , an analytical model was proposed based on the optimization theory for maximizing carbon gain while minimizing concurrent accompanying water loss and ozone influx. Regarding 1), we found that maximum stomatal conductance ( $g_{max}$ ) in global woody plants correlated with MAP rather than MAT. The optimal temperature of  $g_s$  and stomatal response to predawn water potential changed according to the growth conditions. Regarding 2), in the ozone FACE (Free-Air Controlled Exposure) experiments, the optimal stomatal model explained ozone-induced stomatal closure in early summer. This suggests that ozone-induced stomatal closure may reduce ozone influx, and allow maximum photosynthetic capacity to be reached. However, in late summer and autumn, the model did not explain the effects of ozone on stomatal conductance. Also an increase of y-intercept of photosynthesis-stomatal conductance relationship ( $g_{min}$ , minimum conductance) was found. This reflects the loss of closing response of stomata by ozone (i.e., stomatal sluggishness). In fact, in late summer, ozone caused: 1) slower dynamic stomatal response to light variation, 2) less sensitivity of stomata to vapour pressure deficit (VPD), 3) reduced sensitivity of stomata to abscisic acid (ABA) and 4) increased night-time stomatal conductance. As a result  $g_{min}$  increased with increasing cumulative ozone uptake among species.

**Keywords:** ozone, stomatal conductance, stomatal conductance model, maximum stomatal conductance, stomatal sluggishness

**S1.O10**

## **Multi-scale monitoring and modeling of ozone deposition to ecosystems**

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Ozone uptake through stomata damages plant tissue, thereby reducing plant photosynthetic capacity and thus, at a larger scale, inhibiting carbon sequestration by ecosystems. Assessments of such O<sub>3</sub>-CO<sub>2</sub> atmosphere-biosphere interactions rely on combined O<sub>3</sub> and CO<sub>2</sub> flux observations. Although essential for process studies focusing e.g. on in-canopy ozone loss mechanisms, O<sub>3</sub> flux data coverage is too sparse in space (only a few European flux measurement sites) and time (often only several years of data) to assess O<sub>3</sub> deposition impacts on ecosystem functioning at a continental scale. Our approach aims to gain a such a larger-scale insight into O<sub>3</sub>-CO<sub>2</sub> interactions by combining various modelling approaches with in situ and remote sensing (RS) observations to study ozone deposition impacts on ecosystem functioning in Europe. We use the regional atmospheric chemistry and transport model WRF-Chem to simulate O<sub>3</sub> and its precursor gases nitrogen oxides (NO<sub>x</sub>) and volatile organic compounds (VOCs) during a summer season. NO<sub>x</sub> emissions in WRF-Chem are constrained using satellite NO<sub>2</sub> column observations from the Ozone Monitoring Instrument. We expect that this will especially improve model-simulated peak O<sub>3</sub> concentrations and deposition fluxes. In addition, a chemical canopy-exchange model is applied for various O<sub>3</sub>-CO<sub>2</sub> flux measurement sites to simulate ecosystem NO<sub>x</sub>-VOC-O<sub>3</sub> interactions and to assess the partitioning between stomatal and non-stomatal O<sub>3</sub> flux components. The O<sub>3</sub>-induced impacts on ecosystem carbon sequestration will eventually be quantified in a multivariate statistical framework combining the highly-resolved O<sub>3</sub> flux simulations and RS-based vegetation productivity products.

**Keywords:** ozone deposition, air pollution modelling, remote sensing

**S1.O11**

## **Passive vs. active: Applicability of Loibl function in modeling hourly ozone concentrations from passive samplers in Romanian Intensive Monitoring Network**

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Ozone was monitored using Ogawa passive samplers during 2010 - 2016 growing seasons (biweekly) in four Romanian ICP-Forests Level II Intensive Monitoring plots: Stefanesti-stejar (88 m a.s.l. minimum on a 5 km radius 70 m a.s.l.), Mihaesti-gorun (405 m, 312 m), Fundata-fag (1119 m, 840 m) and Predeal-molid (1107 m, 780 m). Also, hourly ozone concentrations were measured some of the years in all plots. AOT40 was modeled from passive samplers using Loibl function and also calculated from hourly measurements where data were available. The differences between real and estimated AOT40 were significant in all cases, the index calculated based on passive samplers overestimating ones based on hourly ozone concentrations by 67% (2015) - 448% (2016) at Stefanesti-stejar plot, by 75% (2013) – 1485% (2014) at Mihaesti plot, by 173% in 2015 and 1515% in 2016 at Predeal-molid plot and by 102% in 2015 at Fundata-fag plot. In addition, when comparing seasonal mean ozone concentrations derived from active and passive monitoring, the differences are both negative and positive, ranging from -14% in 2015 at Stefanesti-stejar plot to 77% in 2012 at Mihaesti-gorun. These are preliminary results question the applicability of Loibl function for modeling hourly values from passive samplers in Romanian conditions. In the light of the new developed critical levels for ozone based on the pollutant uptake through stomata, the next step is modelling and comparing PODx values from active and passive measurements.

**Keywords:** passive samplers, Loibl function, AOT40, ICP-Forests level II

**S1.012**



## **A new crop modelling technique for dealing with ozone stress**

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Crop protection and preservation is a crucial aspect of food security in a world where food supply is becoming an even bigger challenge for the continuously increasing population. Plants are influenced by a wide range of biotic and abiotic factors which play an important role on their performance and yield. High air pollution levels can damage plant ecosystems while ozone is the most phytotoxic air pollutant at a global scale. Moreover, changes in climatic parameters such as water availability and temperature can be combined with high air pollution levels to impact on crop yield. Crop models are tools for quantifying the growth and development of crops and their responses to stresses. However, only a few crop models include an ozone stress parametrization. Here we introduce a significant modification to an existing crop model (GLAM) in order to apply an ozone dose-response function into the model. The new model version is called GLAM-ROC (GLAM-Relative Ozone Concentrations) and accounts for the ozone impact on wheat. Before the inclusion of the ozone effects, the wheat version of GLAM model was re-parametrized and a new modelling technique has been introduced into the model. This new approach is based on a simultaneous solution of the set of model equations instead of the prevailing step-by-step method used in most crop models. The model was made conceptually simpler by taking in integrated systems approach. The new structure improves the model skill under water stress conditions. GLAM-ROC is therefore suitable also in environments where ozone interacts with other stresses, such as drought.

**S1.O13**

## **ICP Vegetation-Asia: A new policy-focused evidence collecting and risk assessment network for ozone**

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Reporting to the United Nations Convention on Long-Range Transboundary Air Pollution, the ICP Vegetation involves over 250 scientists representing 50 countries. For ozone, the primary objectives are to (i) collate and review evidence of impacts on vegetation and (ii) to assess spatial patterns and temporal trends in the risks of damage from current and future ozone. As part of the Convention's outreach activities, the ICP Vegetation is developing a new Asian network, ICP Vegetation-Asia. The network is being coordinated by Prof. Kim Oanh of the Asian Institute of Technology, Thailand and will initially focus on food security impacts. Assisted by the ICP Vegetation Coordination Centre in the UK, the first phase of activities is on the theme of "seeing is believing", aiming to show policy makers that effects on crops should also be considered along with effects on health. Activities will include a review of evidence presented in the published and "grey" scientific literature and the establishment of a network of sites where sensitive ozone species (SOS) will be used to draw the attention of policy makers, agronomists and the public to the growing ozone problem in Asia. An initial pilot study will be conducted in 2018 at selected sites in S and SE Asian countries, involving ozone-sensitive and -resistant bean varieties grown together with local varieties. The distinctive visible symptoms on the beans will be used to provide "SOS" warnings of the pollutant's potential to damage food crops. Later activities will include expansion of the SOS network, the development of region-specific critical levels and flux-based risk assessments and organising knowledge exchange workshops. Policy makers are involved from the start to ensure the activities of ICP Vegetation are suitably focussed on policy needs. New groups from Asia are very welcome to join the network.

**Keywords:** ozone, crops, network, field evidence, visible injury, food security, Asia

**S1.O14**

## **Research is highly required on the impacts of increasing surface ozone in China**

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Environmental pollution has increased quickly in parallel with fast economic growth in the past three decades in China but has not received adequate attention until about two decades ago. In the past decade, air pollution, particularly PM<sub>2.5</sub> pollution has been the focus of the public and many research projects. Public awareness about the effects of PM<sub>2.5</sub> on human health has been an important driving force to initiation of many research projects in China. Unlike PM<sub>2.5</sub>, surface ozone pollution is known only to a very limited number of people despite its recent quick increasing trend in many Chinese cities and its effects on human health and ecosystems. Existing research on ozone in China is largely focused on the effects of ozone on crops and some plants and on characterizing ozone pollution in urban areas. As a result of increasing public awareness about environmental pollution, declining use of coal and fast increasing vehicle population, air pollution in China has been in the transition from that caused largely by coal burning to complex pollution caused by coal burning, use of natural gas and transport. Further increase in surface ozone concentrations is expected in many parts of China. To reduce the effects of elevated ozone in China, research and international collaboration are highly required not only on changes in ozone trend in the urban areas, but on ozone impacts on forests. As the effects of air pollution including ozone on forests are probably seen after decades, building on achievements of existing international knowledge, it would be greatly important to plan and implement integrated research framework on impacts of surface ozone pollution in China. Our surface ozone monitoring and field observations in a few remote forests in southwestern China corroborate the significance of international collaboration and the urgent need for research programs.

**S1.O15**

## **Vegetation exposure to ozone in China: model performance and regions needing further field investigations**

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As surface ozone concentrations have increased significantly in many regions of China, it is concerned that ozone exposure to forests across the country, particularly those in the national nature reserves and biodiversity hotspots, may have exceeded foliar injury threshold values. To quantitatively assess the impacts of ozone to forests, several most frequently used ozone exposure indices (M7, M24, SUM60, W126, and AOT40f) were calculated using simulated hourly from the Community Multiscale Air Quality (CMAQ) model for the year of 2013. While the annual average ozone concentrations (M7 and M24) were generally higher in the Qinghai-Tibetan Plateau (QTP) in western China due to higher background ozone, the other three ozone exposure indices (SUM60, W126, and AOT40f) were lower in most parts of QTP. In contrast, the North China Plain (NCP) and Yunnan Province had lower annual average concentrations but higher values of SUM60, W126, and AOT40f, as they had larger numbers of high ozone hours (i.e. hourly concentration > 100 ppb). The west rims of Sichuan Basin and some regions in the southern and eastern rims of QTP had relatively higher values of all the five exposure indices. Compared with critical loads reported in the literature, about 80% and 75% of forests had SUM60 and W126 higher than the critical load for natural ecosystems (12 ppm-h) for moderately sensitive plant species (23.8 ppm-h), respectively. In addition, about 90% of forests had AOT40f values higher than the critical load of 10 ppm-h. Based on the spatial distributions of ozone exposure, forests, and nature reserves, we suggest that future field observation studies of forest injuries due to air pollution should be prioritized for forested regions in the Yunnan Province, eastern and southern rims of QTP, and western rim of Sichuan Basin.

**S1.O16**

## Ozone and climate stresses on sub-Arctic tundra vegetation: Modelling of stomatal fluxes in midnight sun

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The coupling of biogeochemical and biogeophysical processes are the focus of an interdisciplinary project, OzoNorClim, on the northernmost parts of Fennoscandia, which is exposed to 24 hour daylight during summer, possibly inhibiting night-time repair of ozone-damaged cells and making plants more vulnerable to ozone damage (<http://www.mn.uio.no/ibv/english/research/sections/evogene/projects/ozonorclim/>).

With less sea ice in the Arctic Ocean, more ships are passing the coast of Northern Fennoscandia, causing increased emissions of ozone precursors. Warming at high latitudes has led to changes in the boreal and Arctic vegetation cover, "Arctic greening", which is observed across the circumpolar Arctic and boreal region. In particular increase of shrub vegetation cover in the Arctic biomes is a fast response to warming in high latitudes ecosystems. Emission of Biogenic Volatile Organic Compound (BVOC) from expanding shrubs is expected to yield ozone production. Further, oil exploration is being planned in the Barents Sea adjacent to the coastal areas, also yielding ozone precursor emissions. The combination of elevated ozone exposure and potentially higher than expected ozone sensitivity of tundra plants could lead to a much higher ozone effect risk, with negative impacts on the ecosystem (biodiversity) and local economies (livestock farmers). We have modelled stomatal ozone fluxes in a selection of tundra plants. The Deposition of Ozone for Stomatal Exchange (DO3SE) model coupled to the Weather Research and Forecasting model coupled with chemistry (WRF-Chem) has been used for this purpose, applying emissions from increased shipping in the Arctic, potential oil exploration in the Barents Sea, and the expanding shrub cover in the Arctic. Results are presented in terms of the 24 hour accumulative stomatal ozone flux above an hourly threshold  $Y$  (in  $\text{nmol m}^{-2}$ ); the Phytotoxic Ozone Dose ( $\text{POD}_Y$ ), e.g.  $\text{POD}_6$ . Concentration based metrics are also used, such as AOT40 and SUM06 (accumulated exposure when ozone exceeds 40 or 60 ppb).

**Keywords:** stomatal fluxes, plant stress, repair inhibition, ozone precursor emissions, shipping, oil exploration, shrub expansion

**S1.017**

## Using OJIP transients to monitor the effect of elevated ozone on canola plants, South Africa

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The effects of high ozone levels (80 and 120 ppb) on photosynthetic efficiency and growth of canola plants were studied in open-top chambers. The chlorophyll a polyphasic fluorescence rise kinetics OJIP, stomatal conductance, Chlorophyll Content Index (CCI) and biomass were measured after 15 and 30 days of ozone fumigation, as well as in control plants. Analysis of the OJIP kinetics by the JIP-test led to the calculation of several photosynthetic parameters and the total Performance Index ( $PI_{total}$ ). The observed decline of  $PI_{total}$  under the 80 ppb ozone treatment (about 10%, both after 15 and 30 days of fumigation) was due to a lower density of reaction centers (RC/ABS), while the notable decline under the 120 ppb (about 60% after 15 days and about 85% after 30 days) was found to be due to both a further decline of RC/ABS and the pronounced lowering of the efficiency with which an electron can move from the reduced intersystem electron acceptors to the PSI end acceptors ( $\delta_{R0}$ ). Stomatal conductance was affected by both treatments, already after 15 days of fumigation, with a decrease of about 45% that reached about 55% after 30 days under 120 ppb ozone. Biomass was found to be affected by ozone fumigation, decreasing by 40% at 80 ppb and by more than 70% under 120 ppb, *i.e.* under a two times and three times, respectively, higher ozone concentration than the limit (40 ppb) known to be tolerated by agricultural crops. Our findings indicate that biomass decline is due to both the lowering of CCI and the photosynthetic efficiency parameters. They thus suggest that two simple, non-invasive and rapid methods, namely the analysis of OJIP fluorescence transients and the measurement of CCI, can be used to screen the effect of elevated ozone on the growth rate of canola plants.

**Keywords:** canola; chlorophyll a fluorescence; JIP-test; open-top chamber; ozone

**S1.O18**

## Session 2

# **How plant ecosystems affect ozone concentration in the atmosphere**





## **A mechanistic understanding of ozone impact on forest ecosystems**

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Changes in air chemistry and subsequent physical changes in the environment have a profound effect on the classic “disease triangle”. Environmental factors alter ecosystem health and host susceptibility to biotic and abiotic factors. Plant response to various disease-causing stressors can be changed through introducing ozone (O<sub>3</sub>) into the system. These effects are variable, depending on the timing, intensity and order of the exposure. It is well known that O<sub>3</sub> may produce on a plant functional alterations even without, before the onset of, or in addition, to macroscopic effects. Plants are able to respond to O<sub>3</sub>: several potential mechanisms comprehending exclusion, tolerance, compensation and repair may work simultaneously to create a complex signaling network. These include changes in photosynthesis, cellular redox homeostasis, perception by apoplastic proteins, oxidative damage to membranes, hormonal regulation of the lesion formation, modulation of osmoprotectants and activation of enzymatic and non-enzymatic antioxidant systems. Within a single species, differences in structural, functional and transcriptional traits may play important roles in adaptation/acclimation to environments characterized by high oxidative pressure. Functional traits can predict plant behavior in its natural environment and have been correlated to the degree of tolerance to oxidative stress. The science to accurately describe how O<sub>3</sub> (alone and/or in combination with other biotic/abiotic stressors) affects plants and ecosystems in a changing climate is of paramount importance to guide political decision making. Diagnosis based on plant sampling and physiochemical analysis using traditional laboratory methods can be precise, but have a number of limitations as they are commonly time-consuming, destructive, and expensive. An alternative approach to monitoring ecosystem functions includes the development of new technologies, advancing computational capacity and improving methodological approaches to environmental monitoring.

**Keywords:** global change, abiotic stress, plant response, disease triangle, O<sub>3</sub>, oxidative burst, signaling network

## Elevated ground-level ozone modifies the microbiome

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Elevated ground-level ozone ( $eO_3$ ) imposes adverse influences on ecosystems. However, the information regarding responses of belowground, such as soil microorganisms, to  $eO_3$  as well as their feedbacks remains elusive. As the indispensable part of soil ecosystem, the knowledge on soil microorganisms is pivotal to comprehensive understanding of global change influencing ecosystem. To this end, a field experiment in China Ozone Free-Air Concentration Enrichment (FACE- $O_3$ ) facility on a rice-wheat rotation system was carried out to investigate paddy microbial responses to  $eO_3$ . Using real-time quantitative PCR, DNA-based fingerprinting and high throughput sequencing approaches in combined with culture-reliant method, we evaluated two paddy anoxic microorganisms, anoxygenic phototrophic purple bacteria (AnPPB) and methanogenic archaea, and total bacterial community in response to  $eO_3$  as well as the differences between different  $O_3$ -sensitive rice cultivars. It is found that  $eO_3$  reduces AnPPB and methanogenic archaeal abundances in flooded rice soils via decreasing their genotypic diversity and metabolic capability. Concomitantly, their community compositions changes under  $eO_3$ . For total bacterial community, the similar phenomena are observed. Furthermore, when comparing two different  $O_3$ -sensitive rice cultivars, it is found that crop cultivar is important in determining the responses of soil biota to  $eO_3$ . The contrasting responses of soil bacterial and methanogenic archaeal communities in two rice cultivars are observed to  $eO_3$ . Although more adverse influences on  $O_3$ -sensitive cultivar, several keystone bacterial guilds are consistently negatively affected by  $O_3$  pollution in two rice cultivars. Collectively, the abovementioned findings indicated that continuously  $eO_3$  would negatively influence paddy microorganisms and their critical ecological functions and more attention should be focused on the responses of soil microorganisms in crop cultivars when evaluating the effect of climate change on agroecosystems. These findings will contribute to a comprehensive understanding of the responses and feedbacks of paddy ecosystems to global climate change.

**Keywords:** Elevated ground-level  $O_3$ , Soil microorganisms, Diversity, Paddy soil, Rice cultivars

## S2.O2

## Impacts of changing ground-level ozone profiles (peak vs background) in Europe on vegetation in the current and future climate

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Recent decades have seen a changing profile of ground-level ozone (O<sub>3</sub>) in Europe. While peaks in O<sub>3</sub> concentrations during summer months have been declining in amplitude, background concentrations have gradually increased as a result of the hemispheric transport. Two case studies were conducted to assess the implications for impacts on 1) crop yield and 2) stomatal O<sub>3</sub> fluxes in (semi-)natural vegetation and tree species in future climates: 1) A modern wheat cultivar (Skyfall) was exposed to eight different realistic O<sub>3</sub> profiles repeated weekly: four profiles with increasing background O<sub>3</sub> concentrations (ca. 30 – 60 ppb) and four profiles with increasing O<sub>3</sub> peak concentrations (ca. 35 – 110 ppb). Both wheat yield and 1000-grain weight declined linearly with increasing stomatal O<sub>3</sub> flux. The slope of the flux-effect relationship was not affected significantly by the O<sub>3</sub> profile. Hence, flux-effect relationships developed for wheat based on exposure to enhanced peak O<sub>3</sub> concentrations are also valid for the changing European O<sub>3</sub> profile with higher background and lower peak concentrations. In addition, the modern wheat cultivar Skyfall is more sensitive to O<sub>3</sub> than older wheat varieties. 2) A modelling study using measured site-specific data showed that increases in stomatal O<sub>3</sub> uptake in future climates were larger in northern and mid-Europe than in southern Europe, and showed a strong relationship with latitude. O<sub>3</sub> concentrations as low as 10 ppb can contribute to stomatal O<sub>3</sub> uptake that can be above the Y threshold for accumulation of the Phytotoxic Ozone Dose (POD<sub>Y</sub>) of 1 nmol O<sub>3</sub> m<sup>-2</sup> s<sup>-1</sup> for trees and (semi-)natural vegetation. Therefore, impacts of O<sub>3</sub> pollution could occur at any time of year when plants are actively growing and not just during periods of peak O<sub>3</sub> concentrations. Contrasting impacts of climate change (+2°C, -20% rainfall) on stomatal O<sub>3</sub> fluxes in northern and southern Europe will be discussed.

**Keywords:** Background ozone; ozone episodes; climate change; wheat; *Betula pendula*; *Dactylis glomerata*; *Leontodon hispidus*

## The response of water use efficiency in poplar to elevated ozone

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Ground-level ozone ( $O_3$ ) negatively influences carbon and water balance by both photosynthesis and stomatal control of plant transpiration and decline in water use efficiency (WUE). We combined stable dual isotopic signatures  $\delta^{13}C$  and  $\delta^{18}O$  of leaves as well as instantaneous gas exchange performance to determine the effects of both  $O_3$  and leaf aging on light-saturated photosynthetic rate ( $A_{sat}$ ) and stomatal conductance ( $g_s$ ) in poplar clones '546', and poplar clones '107' exposed to five  $O_3$  levels in open-top chambers for one growing season. Intrinsic WUE decreased with increasing  $O_3$  concentration for all species, and it was more affected in mature than young leaves. The tight coupling of  $A_{sat}$  and  $g_s$  occurred at ambient  $O_3$  condition, but uncoupled under higher  $O_3$  treatment, as  $A_{sat}$  decreased while  $g_s$  did not response or increase. Ozone exposure increased stomatal sluggishness, and caused progressive loss in stomatal control over transpiration during the growing season. Furthermore, Ozone-induced reductions of  $g_m$  and  $V_{cmax}$  may play key roles in limiting  $A_{sat}$  and thus decreasing WUE. Mild-drought increased WUE, while  $O_3$  decreased it. But no significant interactions were detected. Our results suggest that  $O_3$ -induced loss of stomatal control could have an aggravating effect on carbon assimilation and water use efficiency of forest trees under future  $O_3$ -enriched air.

**Keywords:**  $\delta^{13}C$ ,  $\delta^{18}O$ , ozone, photosynthesis, stomatal conductance, mesophyll conductance, water use efficiency

## S2.04

## Ozone stress on sub-Arctic tundra vegetation: ozone exposure experiments with daylength manipulation

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The coupling of biogeochemical and biogeophysical processes are the focus of an interdisciplinary study on the northernmost parts of Fennoscandia, which is exposed to 24 hour daylight during summer, possibly inhibiting night-time repair of ozone-damaged cells and making plants more vulnerable to ozone damage (<http://www.mn.uio.no/ibv/english/research/sections/evogene/projects/ozonorclim/>).

The sub-Arctic tundra has its western limit in Northern Norway. Many of the dominant plant species found there are also important in other circumpolar tundra areas. Therefore, increased knowledge of these species' reaction to increased tropospheric ozone concentration is of great importance to climate modelling. Further, farmers in these areas grow grass and clovers to feed livestock, using cultivars adapted to short summer season and frost. Hence, there is also an economic interest in understanding the impact of ozone and climate change on plants of the northern latitudes. Several *Trifolium* species have shown increased ozone sensitivity in response to long as compared to short daylength. The effect was not due to differences in ozone doses or physiological status of the plants. We hypothesize that the lack of night-time repair in darkness may, at least partly, contribute to the reported increased ozone sensitivity. Although one may assume that the local vegetation is adapted to long days in northern latitudes, the ability to repair damages due to oxidative stress may be lower than in areas with dark periods during night, as seen in a *Trifolium pratense* cultivar developed in Sweden (65°N). Thus, plants growing in northern areas may be more sensitive to ozone than expected. We have grown plants from two sources: species native to the tundra, including expanding bushes, and frequently used grass and clover species in meadows North of 67°N for ozone exposure experiments. The daylength dependency of the ozone sensitivity and impacts on visible injuries, stomatal conductance, photosynthesis rates and night-time respiration were studied.

**Keywords:** tundra vegetation, ozone sensitivity, stomatal conductance, visible injuries, night-time repair

## **Grassland community response to elevated ozone exposure by assessment of soil dynamics, plant diversity and total biomass**

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Tropospheric ozone (O<sub>3</sub>) is a serious threat to global food security as well as to natural vegetation. The phytotoxic effects of O<sub>3</sub> on agricultural plants are well known, but its effects on natural grassland community and their belowground soil health are yet to be ascertained in developing countries. Considering these facts, plant composition and soil physiochemical properties of grassland (semi-natural community) were assessed in response to ambient (48ppb) and elevated (Ambient+20ppb) O<sub>3</sub> treatments in open top chambers installed directly on semi-natural grassland community. Elevated ozone induced alterations in soil properties with significant reductions in soil microbial biomass, total nitrogen, organic carbon, available phosphorus, β-glucosidase activities and polyphenol oxidase enzyme activities, while phosphatase enzyme activity was significantly increased under elevated O<sub>3</sub> concentration. O<sub>3</sub> treatment also showed significant decline in most of the soil nutrient contents (Na, Mg, K, Zn, Fe and Cu) compared to ambient, suggesting influence of O<sub>3</sub> in altering soil nutrient availability. Morphological characterization of dominant grass species such as of *Digitaria sanguinalis* and *Brachiaria* sp. showed significant reductions in shoot and root height, number of leaves, dry weights of shoot and leaves, whereas in case of *Cynodon dactylon*, a positive effect of elevated O<sub>3</sub> on plant biomass was recorded. The root dry weight of *Brachiaria* sp increased but of *Digitaria sanguinalis* decreased under elevated O<sub>3</sub> concentration. The total above ground biomass measurement of community showed increase in grass contribution whereas forbs biomass declined significantly under elevated O<sub>3</sub> compared to ambient. The variabilities in most of the soil parameters and differential sensitivity of two functional groups clearly suggest that elevated ozone may cause shift in community pattern due to changes in soil properties.

**Keywords:** Ozone; grass; soil properties; semi-natural grassland community; soil microbial biomass; soil enzymes

## **S2.O6**

## **Ozone impacts on crop yield and food security**

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Tropospheric ozone is generally considered to be the globally most important air pollutant with respect to effects on crops. Today, elevated levels of ozone are known to occur over large areas of the world's important agricultural production areas in Asia, Europe and North America. In other geographical regions the degree of ozone pollution and its effects on agriculture are less well known, but are likely to be significant. When assessing and communicating the importance of ozone for crop production and food security the evidence of effects from current ozone exposure is of particular importance. Although plant response to experimentally elevated levels of ozone is important for risk assessment and the derivation of response functions, effects of present ozone are highly relevant from a policy perspective, to evaluate the gains from reducing current air pollution, rather than to estimate the damage by potential future elevated ozone levels. The evidence of effects from present ozone comes mainly from bioindication, protection of plants by use of the ozone protectant chemical EDU and (meta-)analysis of the effects of reducing ozone by air filtration in chamber experiments. Examples of these three types of evidence will be given as well as of response functions based on stomatal ozone uptake including elevated ozone treatments. There is strong evidence that the variation in ozone sensitivity varies among crop species. A corresponding variation has also been established for different cultivars or varieties of certain crop species. The magnitude, statistical evidence and potential exploration, e.g. in decisions on the choice of crop and in plant breeding, of this variation will be described and exemplified. Also the causes for variation in sensitivity, depending on stomatal ozone uptake, antioxidant defense and crop life history, will be discussed. An important matter is the reasons why the threats of ozone to food security is often neglected in policy and agronomic contexts. Potential explanations for this will be outlined along with suggestions of ways to remedy this problem. Highlighting experimental evidence of ozone effects on crops from interaction experiments where ozone exposure was administered in combination with other environmental factors, like temperature, irrigation/water availability, fertilization and elevated CO<sub>2</sub>, which are familiar to agronomists and/or plant physiologists, is one example. The direct comparison of ozone effects with those from other factors could facilitate the communication of the importance of ozone effects. Another benefit from this approach is that it will promote an integrated analysis of different environmental drivers on food security, including ozone, which will be necessary in any case to understand the net effect of the multitude of aspects of global change. Finally, scenarios for the future development of ozone effects on food security will be given. Obviously, the long-term development will depend on a range of decisions related to emissions of ozone precursors including methane, climate change and adaptation of crops and agriculture. Current trends for ozone exposure of crops in different parts of the world will be described as well as projections of surface ozone development over the present century. To conclude, a reflection will be made over how international policy making can best handle the threats from ozone to food security.

**S2.07**

## **Global food security modelling: quantifying the threat to crop production from ozone pollution**

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At the global scale, ozone pollution has been predicted to pose as big a threat to food security as climate change by 2030. Several of the world's most important crops such as wheat, soybean, maize and rice respond to ozone pollution by decreasing vegetative growth, seed production and root growth, leading to reductions in both quantity and quality of yield. We have developed response functions relating crop yield and associated key crop processes to the cumulative stomatal uptake ("flux") of ozone modelled from plant, soil and climate factors and applied these models on a global scale to predict impacts of ozone on food security. Evidence from European chamber and field studies shows that the uptake of ozone by stomata (flux) is a superior predictor of ozone damage, compared to more conventional exceedance of ozone threshold concentrations. This analysis presents a major step forwards from previous predictions of risk based on ozone concentration as it includes the modifying effects of climate and soil moisture (including irrigation) on instantaneous ozone uptake and subsequent effect. We show that ozone is currently reducing wheat yield by 9% globally and causes a total of 49 million tonnes (Tg) of lost grain in the five biggest producing countries (China, India, USA, Russia and France). Within these countries, effects are highest where irrigation is already in use or in areas where rainfall is sufficient to ensure soil moisture is non-limiting to stomatal uptake. We also show the regions of the world where soybean, maize and rice are being impacted by ozone, and discuss other possible factors contributing to yield loss. This work highlights the spatial variability of global impacts of ozone pollution on crop production and demonstrates the need to consider ozone pollution as a modifying factor in global crop production and food security modelling.

**Keywords:** food security; global mapping; stomatal flux; yield loss; wheat

## **S2.O8**



## Understanding the ozone response in Indian crop plants

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Tropospheric ozone ( $O_3$ ), a secondary air pollutant and a major greenhouse gas, has already been recognized as a prime component of predicted global climate change. Numerous studies have confirmed the negative impact of  $O_3$  on agricultural productivity throughout the world. Ozone, a strong oxidant, enters plants through the stomata where it can be dissolved in the apoplastic fluid. Ozone can directly react with the plasma membrane through “ozonolysis” or it can be converted into reactive oxygen species (ROS), which can alter the major cellular functions causing cell death, premature senescence, and also the up- or down-regulation of specific genes. Like other tropical countries, India is also under the severe threat of  $O_3$  pollution. Our studies with major crops, *i.e.* rice, wheat, soybean, linseed etc., clearly established the damaging effect of  $O_3$  on Indian scenario. Yield of all the crops were significantly decreased under ambient levels of  $O_3$ ; however, the reduction was increased by many fold with elevation of  $O_3$  levels. Distinct foliar injuries were also observed in  $O_3$ -exposed plants. Different growth parameters, like – shoot height, root height, total number of leaves, leaf area, NAR, RGR, etc. were also affected. Plants, generally possess a vast array of anti-oxidants to cope up with prevailing ROS; however, under  $O_3$  stress, the amount and activity of those anti-oxidant molecules were induced by many times, irrespective of nature of plants. Even the reproductive structures like viable floral composition, viability and germination of pollen grains were also affected under  $O_3$  stress. RAPD analysis demonstrated that  $O_3$  severely affected the ‘genome template stability’ (GTS) in all the studied plants, and acted as a potent mutagen. In-depth proteomics analysis; through 1-DGE, 2-DGE coupled with protein sequencing, and immuno-blotting; again revealed that major photosynthetic proteins, like – RuBisCO, RuBisCO activase,  $O_2$  evolving protein; primary metabolism related proteins were highly affected under  $O_3$  stress; whereas, defense /stress related proteins were induced. Physiological traits; especially stomatal conductance in plants, act as a very important parameter under  $O_3$  exposure and controls the overall gaseous exchange; hence decide the sensitivity of crop cultivar. Results obtained would definitely help in selecting suitable resistant cultivars of crop plants to get optimum growth, and yield and also in strengthen the knowledge of molecular mechanism of action of ozone.

**Keywords:** Tropospheric ozone, Reactive oxygen species (ROS), proteomics, Indian crops, growth, yield, quality parameters

**S2.O9**

## Sensitivity of Indian crop plants to ozone

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Increasing anthropogenic and biogenic emissions of precursor compounds have led to high tropospheric ozone concentrations in India particularly in Indo-Gangetic Plains (IGP), which is the most fertile and cultivated area of this rapidly developing country. Current ozone risk models, based on European and North American data, provide inaccurate estimations for crop losses in India. Therefore, we have conducted several experiments with the main Indian crop species to estimate the magnitude of ozone risk on agriculture, and to screen the variation in ozone sensitivity/tolerance among the local cultivars. Field experiments in India, Lucknow area, were conducted with rice, wheat and mustard using EDU (ethylenediurea) as a chemical protectant against the adverse effects of ozone. The field experiments were complemented with chamber studies in Finland, simulating the prevailing ozone and climatic conditions in India. The plants were assessed for growth, gas-exchange (photosynthesis, stomatal conductance), antioxidants (SOD, CAT, GSH, GSSG), malondialdehyde (MDA) and biomass at the vegetative and the flowering phases. Yield parameters were measured at the final harvest phase. All study species showed sensitivity to prevailing ozone concentrations as indicated by yield losses and activation of defence systems. Seven out of the 18 rice cultivars and only three cultivars of the 11 wheat cultivars tested showed the good adaptability to high-ozone environment in terms of grain yield. Therefore, most of the Indian rice and wheat cultivars selected for the study showed high sensitivity to ozone. Both mustard cultivars under study were sensitive to ozone, but showed different strategies against ozone stress. Our results highlight the need for extensive screening of ozone sensitivity/tolerance of the cultivars of the main crops in order to maintain and improve the production rates and food security in this highly populated and polluted area of India.

**Keywords:** India, ozone, wheat, rice, mustard, EDU

## S2.O10

## **Breeding of ozone tolerant cereal crops: progress and prospects**

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Tropospheric ozone poses a major threat to global cereal production, especially in Asia, where air pollution has been rising steeply in recent years. As farmers have almost no management options to control the atmosphere which their crops are exposed to, the breeding of novel, ozone tolerant cereal varieties constitutes a promising approach to enhancing global food security. Therefore, we have screened for natural genetic diversity in adaptation to high ozone concentrations in rice, the main staple crop in Asia. We have identified substantial variability in ozone adaptation in terms of biomass and yield losses as well as ozone symptom formation. This phenotypic diversity was genetically dissected by mapping of quantitative trait loci (QTL) using bi-parental mapping populations or genome wide association study (GWAS) using a diversity panel. The QTL *OzT8* (affecting photosynthetic performance) and *OzT9* (affecting symptom formation) were demonstrated to alleviate adverse ozone effects on crop yield, grain and straw quality in rice plants grown in high ozone conditions. A candidate gene, *OsORAP1* (*OZONE RESPONSIVE APOPLASTIC PROTEIN1*) underlying the locus *OzT9* was characterized by reverse genetics, heterologous expression, and subcellular localization analysis. These analyses revealed that *OsORAP1* is an apoplastic protein, which is highly expressed in ozone stress and belongs to a subclade of ascorbate oxidases, which does not oxidize ascorbate *in planta* but is involved in programmed cell death. Naturally occurring polymorphisms in the promoter and 5'-UTR regions of *OsORAP1* in rice were highly correlated with mRNA expression and symptom formation, further highlighting the importance of this gene in ozone response of rice. In ongoing experiments, we are investigating the transcriptional responses to ozone and sequence polymorphisms of *OsORAP1* orthologues in other cereal crops including wheat, barley and maize. These investigations will contribute to the breeding of ozone tolerant cereal crops in the future.

**Keywords:** air pollution, barley, cereals, maize, molecular breeding, rice, wheat

**S2.O11**

## **Impact of ozone on physiology and yield of African crops**

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Ozone pollution is a growing problem in Africa. There is much evidence of adverse effects of ozone on crop yield and crop quality for European crops, but not for crops grown in Africa, or for African varieties of crops also grown elsewhere. We performed two experiments to investigate a) the impact of ozone on African crops and varieties, and b) whether irrigation strategies could be used to reduce the impact of ozone on wheat. African varieties of wheat (5 varieties), pearl millet (4 varieties) and finger millet (6 varieties), together with 6 varieties of dry (shelling) beans were exposed to ozone in ambient temperature (wheat) or heated (finger and pearl millet, beans) solar domes, with peak ozone concentrations of approximately 30 to 110 ppb. Ozone-induced visible leaf injury was observed on all crops and varieties tested, with a range of ozone-sensitivity between the different varieties. Large reductions in total yield and 1000 seed weight were found for wheat and bean in the highest ozone treatment. The ozone sensitivity of African wheat compared to European varieties based on Phytotoxic ozone dose will be shown, together with flux-based dose response relationships for the other crops. In a follow-up experiment, one of the ozone-sensitive varieties of wheat ('Kenya Korongo') was exposed to control (30 ppb) or elevated ozone (peaks of 80 ppb) for 3 weeks following anthesis, together with one of three irrigation regimes – well watered, frequent deficit or infrequent deficit irrigation. Reduced visible leaf injury with the deficit irrigation regimes and effects on yield will also be presented.

**Keywords:** crop yield, wheat, millet, beans, deficit irrigation, Phytotoxic ozone dose

**S2.012**

## Responses of crops to ozone exposure: study of physiological parameters

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In the context of increasing population and decreasing acreages, the reduction in crop yields induced by tropospheric ozone should be better evaluated. Therefore, ozone-resistant and -sensitive tobacco (cv. Bel-B and Bel-W3), barley and rapeseed plants were submitted to ozone-enriched air, during pre-flowering period. Six ozone concentrations (ranging from 30 to 130 ppb for six hours during the photoperiod) and two treatment durations (1 or 2 weeks) were applied. These treatments correspond to low, moderate and high exposure levels that occurs in France. Several leaf parameters were measured: necrosis development, chlorophyll content, chlorophyll fluorescence (Fv/Fm,  $\Phi$ PSII), gas exchange (carbon assimilation and leaf conductance), seed yield, and fatty acid composition in cell membranes. The fatty acid composition was used to calculate the "Omega-3 index", a biomarker based on stress-induced changes in leaf 18:3 content, developed to assess soil quality. To estimate the oxidative load in leaf tissues, quantification of targets of oxidative stress (such as intracellular proteins carbonylation) was rather used instead poorly reproducible ROS quantification. Results show that ozone treatments decrease chlorophyll content and omega-3 index. The extent of the declines and the threshold levels of ozone exposure triggering the declines observed in the different plant species studied will be discussed. Direct continuation of this study, will involve a water stress applied to the plants subsequent to the ozone treatments. The same physiological approach will then be used to characterize the plant responses to the combined stress treatments, to assess their resilience to drought after an ozone pollution episode.

**Keywords:** elevated CO<sub>2</sub>, tropospheric ozone, barley, tobacco, rapeseed, oxidative stress, omega-3 index

## **Assessing the risk of tropospheric ozone phytotoxic effect on Southern European Mediterranean environments: a review with emphasis on vineyards**

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Tropospheric ozone in Southern Europe has an increasing tendency in association with a greater incidence of warm summers and heatwaves. As there is already much evidence of the negative effects that current ambient ozone has on vegetation, there is a need for consistent risk assessment methods. Ozone plant exposure-based parameters have been used extensively to support decision-making. However, these parameters have been also criticised, as they do not relate with the actual dose of ozone entering the plant. Moreover, in Mediterranean environments, they often overestimate the risk as thresholds are exceeded without corresponding evidence of damaging effects. To overcome these limitations, dose-based approaches were developed. These approaches have a stronger biological basis as they are based on estimates of the amount of ozone molecules that diffuse into the leaf cells through the stomata. However, they have also limitations, as detoxification processes or non-stomatal uptake are not often taken into consideration. This work presents a review regarding ambient ozone effects on vegetation and the indices used to assess phytotoxic risk in southern European Mediterranean plant communities and crops. Emphasis is given to the grapevine as three southern European countries (Spain, Italy and Portugal) are major wine producers concentrating more than 20% of the area under grapevines globally. These countries hold a long winemaking tradition associated to renowned denominations of origin (DOs). Therefore, there is concern regarding climate change as a potential threat to wine typicity in these areas, most of the work focusing on atmospheric variables, bioclimatic and climate change indices only. Results from the DOUROZONE project are presented with the aim to analyse the implications climate change can have in a significant Portuguese wine region such as the Douro Demarcated Region (DDR) including ozone-related indices as a novelty among other more frequently used bioclimatic and climate change indices.

**Keywords:** tropospheric ozone, climate change, grapevine, phytotoxic risk assessment, Portuguese Douro Demarcated Region

## **S2.O14**

## **Monitoring ozone, foliar deficit and ozone-specific foliar damage on national forest plots in France over several years**

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Since 2000, the GIEFS (Groupe International d'Etudes des Forêts Sud-européennes) and the National Forestry Office (ONF) have joined forces to monitor ozone concentrations and their effects on forest plots belonging to the National Long-Term Monitoring Network of Forest ecosystems (RENECOFOR). The RENECOFOR plots are distributed throughout the French national territory. The measurements of ozone concentration, foliar deficit and ozone injury were carried out on a selection of fifteen conifer and deciduous plots. The studied conifer species were the fir (*Abies alba*), the Scots pine (*Pinus sylvestris*) and the Norway spruce (*Picea abies*). The hardwoods studied were the beech (*Fagus sylvatica*), the sessile oak (*Quercus petraea*) and pedunculate oak (*Quercus robur*). Leaf deficit data from 2000 to 2015 and a follow-up of ozone foliar symptoms during the period 2013 to 2015 were analyzed in this study. The Mann Kendall test has been used to identify possible leaf deficit trends for each specie over the past 15 years. The Spearman statistical test was applied to establish correlations between leaf deficit, atmospheric ozone concentration and weather parameters (humidity, precipitation, temperature) observed *in situ*. The percentage of ozone injury has been related to meteorological parameters and ozone concentration each year. Observations were also made on clearings (LESS) located near the plots in order to detect the damage specifically induced by ozone on the vegetation outside the forest. Species sensitivity to ozone and the importance of topographical situation are also presented in this study.

**Keywords:** Forest, ozone, monitoring network, trend, defoliation, visible injury

**S2.O15**

## **Physiological responses of turf-type *Festuca arundinacea* to elevated O<sub>3</sub>**

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Taking ambient environmental O<sub>3</sub> (40 ppb) as control, this experiment was conducted to assess the impacts of elevated O<sub>3</sub> (80 and 160 ppb) on growth, subcellular structure and reactive oxygen metabolism of turf-type *Festuca arundinacea* by using open top chambers (OTCs). The results showed that after 14-day fumigation, the height and leaf width of *F. arundinacea* decreased significantly, and total biomass decreased by 43.7%, and some fully expanded leaves yellowed under 80 ppb O<sub>3</sub>. Some visible injury symptoms, brown spots and necrosis appeared in leaves, the total biomass decreased by 46.2%, and plasma membrane became loose from the cell wall and convoluted, chloroplast and mitochondria were damaged under 160 ppb O<sub>3</sub> exposure. Compared with control, the rate of superoxide anion (O<sub>2</sub><sup>-</sup>) production, hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) content, malonaldehyde (MDA) content and the activity of antioxidant enzyme were higher under the increasing O<sub>3</sub> concentrations (80 and 160 ppb). Total phenolics content and the antioxidant capacity increased at first and then decreased with the rise of O<sub>3</sub> concentration. It indicated that elevated O<sub>3</sub> (80 ppb) inhibited growth of *F. arundinacea* and antioxidative metabolism before visible injury symptom appeared in leaves. *F. arundinacea* had an adaptive response to elevated O<sub>3</sub> in early stage of development, but it could not protect itself from excessive O<sub>3</sub> or long-term O<sub>3</sub> exposure.

**Keywords:** antioxidant capacity, biomass, *Festuca arundinacea*, turfgrass, ultrastructure

**S2.O16**



## Multi-level responses to ozone in Euramerican poplars

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Tropospheric ozone ( $O_3$ ) is the main secondary pollutant and considered to be the most damaging for plant growth and productivity. In addition to be the third strongest greenhouse gas  $O_3$  has the capacity to significantly alter forest carbon sink strength.  $O_3$  is well known to induce oxidative stress and ROS accumulation in leaf tissues. Therefore, several works have focused on tree abilities to cope with ozone exposure, either by closing stomata to limit ozone fluxes into the leaf, by modifying anatomy to limit  $O_3$  diffusion through leaf tissues or by activating antioxidant defenses to limit ROS accumulation. However, the relative contribution of these defense mechanisms to  $O_3$  tolerance is still unclear in trees. Here, we investigated the responses of these barriers in nine Euramerican poplar clones (*Populus deltoides* × *P. nigra*) exposed to 120 ppb of  $O_3$  for 3 weeks. Main drivers of tolerance and sensibility to  $O_3$  were isolated to explain the lesser and greater losses of biomass, respectively. It appeared that ascorbate peroxidase and ascorbate regeneration process are the main determinants of ozone tolerance in poplar, in protecting photosynthesis capacity from ozone damage and therefore, maintaining growth and productivity. Furthermore, closing stomata appeared very harmful for sensitive clones, suggesting that avoidance strategy may be even worth than coping with excessive accumulation of ROS. Finally, changes in gas diffusion properties of leaf tissues are currently studied in order to improve flux-based functions and highlight adaptative mechanisms restricting  $O_3$  influxes through leaf tissues.

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**Keywords:** antioxidant defense, ascorbate, ozone flux, poplar

**S2.O17**

## Non-linear responses of two larches species exposed to four ozone levels

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The ground-level ozone (O<sub>3</sub>) concentration has been increasing especially in Northeast Asian region. In several tree species, elevated O<sub>3</sub> inhibits growth and alters several physiological processes including stomatal reactions, photosynthesis, and nutrient translocation or storage. Larches (*Larix*) are representative trees in boreal forest and widely and continuously distribute at Eurasian continent. Japanese larch (*L. kaempferi*) has been major species in artificial forests in Asian countries due to high growth rate. However, it has been suffering from some biotic stresses, especially grazing by voles. To overcome difficulties, hybrid larch F<sub>1</sub> (*L. gmelinii* var. *japonica* x *L. kaempferi*) has been developed. Nowadays, relatively high O<sub>3</sub> is detected in Japan. Therefore to conserve these larch forests, we should reveal the effects of O<sub>3</sub> on the physiological responses and growth. In this study, Japanese larch and its hybrid were exposed to four O<sub>3</sub> levels [ranging from 10 to 60 nmol O<sub>3</sub> mol<sup>-1</sup>] in open-top chambers (OTCs) for two consecutive growing seasons. We found a significant hormetic response in net photosynthetic rate at 1700 μmol (CO<sub>2</sub>) mol<sup>-1</sup> (A<sub>max</sub>), maximum rates of carboxylation (V<sub>Cmax</sub>) and electron transport (J<sub>max</sub>) in both larches. Hormesis indicates that low levels of exposure to an agent stimulate the performance of an organism but high levels of exposure inhibit its performance. Despite the stimulation by low O<sub>3</sub> levels in physiological endpoints, hybrid larch had suppressed production of dry matter. These findings suggest that stimulation of physiological functions by low O<sub>3</sub> exposures may have negative consequences for larch afforestation.

**Keywords:** Hormesis, Species difference, Larch, Ground level O<sub>3</sub>

## S2.018

## **Effects of short term ozone fumigation on young trees of poplar *Populus nigra***

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During a period of 30 days, young trees of *Populus nigra* were fumigated with a single dose of ozone, 30 ppb for 4 hours, under open top chamber. Typical adaxial leaf brozing appeared since the 5th day and lesions gradually enlarged until the end of experiment. The relationship between necrosis and days was shown to be linear ( $p < 0.0000$ ). Some physiological parameters were investigated; ozone exposure caused a decrease of the amount of total chlorophylls, chlorophylls a and b, whereas membrane integrity, soluble sugars and stomatal conductance increased significantly after fumigation.

**Keywords:** Ozone, *Populus nigra*, Open Top Chamber, Physiological parameters, Tiaret

## **Insights into the mode of action of ethylenediurea (EDU) as an antiozonant in rice (*Oryza sativa* L.)**

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Ethylenediurea (EDU) has been widely used to suppress detrimental effects of ozone on crops. However, the protection mechanisms against ozone injury still remain elusive and constitutive effects of EDU on crops have barely been studied. Therefore, we performed a series of agronomic and physiological experiments exposing rice to four different treatments, (i) control, (ii) control+EDU, (iii) ozone (average 77 to 108 ppb for daily 7 h), and ozone+EDU. Our first experiment in a controlled soil-based open top chamber system confirmed the lack of constitutive effects of EDU on ozone-free control plants, while it significantly offset the negative ozone effects in sensitive genotypes on foliar symptoms, lipid peroxidation, stomatal conductance, SPAD value, spectral reflectance vegetation indices (NDVI, SR and PRI and ARI), panicle number, spikelet sterility and grain yields. Subsequently, we conducted in depth physiological analyses including transcriptomics using the ozone sensitive and EDU responsive genotype BR28. Transcriptome analysis using Illumina HiSeq 4000 revealed no differentially regulated genes (0.1 FDR) between control and control+EDU, further confirming the absence of constitutive effects of EDU. However, 3182 and 3367 (0.1 FDR) differentially expressed genes were found between control vs ozone and control+EDU vs ozone out of a total 23208 expressed rice genes, indicating significant effects of ozone on rice gene expression pattern. In contrast, only 7 differential genes (0.1 FDR) were found for the ozone and EDU interaction. Despite the obvious lack of the direct effects of EDU on rice metabolism, its application significantly ameliorated the foliar symptoms, tiller number, shoot length, carbon assimilation rate,  $V_{cmax}$ ,  $J_{max}$  and lipid peroxidation. These results indicated that EDU does not have any direct effect on plants under ozone stress, and rather provides an indirect damage prevention mechanism due to abiotic interaction with ozone on the leaf surface that restricted the entry of ozone into the leaf.

**Keywords:** Air pollution, Molecular breeding, Global change, Food security, Gene expression, Phenotyping

## **S2.O20**

## **Impact of elevated tropospheric O<sub>3</sub> and ambient temperature on early and late sown cultivars of wheat: an insight to biochemical mechanism and yield**

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Tropospheric ozone (O<sub>3</sub>) is a highly phytotoxic air pollutant and its higher concentrations during crop growing season may lead to substantial reductions in crop production. The present study was undertaken with the hypothesis that early sown cultivars will perform better than late sown cultivars in response to elevated O<sub>3</sub> and temperature variations in between early and late sown wheat cultivars with respect to their defence capacity and yield losses under elevated O<sub>3</sub> and ambient climatic variables. Experiments were conducted in open top chambers receiving ambient and elevated O<sub>3</sub> concentrations (ambient+20 ppb). Two wheat cultivars of early sown (HD 3086 and HUW 468) and late sown (HUW 234 and HD 3118) cultivars were chosen for the experiments. Eight hourly O<sub>3</sub> concentrations and meteorological parameters were measured throughout the experiment. Superoxide and H<sub>2</sub>O<sub>2</sub> content and its localization, lipid peroxidation, superoxide dismutase (SOD), ascorbate peroxidase (APX), glutathione reductase (GR) activities, ascorbic acid content and yield (wt. of grains plant<sup>-1</sup>) were measured. Mean (8h) ambient and elevated O<sub>3</sub> concentrations were 52.7 to 76.5 ppb for early sown cultivars (November, 2016 – March, 2017) and 52.6 to 75.2 ppb for late sown cultivars (December- March, 2017) in ambient and elevated O<sub>3</sub> chambers. Mean maximum temperatures were 26.7 and 27.8°C and minimum temperatures were 13.3 and 14.3°C during growth period of early and late sown cultivars, while SOD activity was higher in early sown cultivars. Ascorbic acid content was significantly higher in early sown than late sown cultivars. Yield measured as weight of grains plant<sup>-1</sup> showed higher reductions in early sown compared to late sown cultivars. The present study concludes that higher minimum temperature along with high O<sub>3</sub> concentration might have affected reproductive processes of early sown more than late sown cultivars, which led to higher yield reduction in early sown cultivars.

**Keywords:** wheat cultivars, tropospheric O<sub>3</sub>, temperature, ROS, antioxidative enzymes, yield

**S2.O21**

## Leaf internal photosynthetic activity of Siebold's beech seedlings under elevated ozone

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Ozone is a phytotoxic gaseous air pollutant. It is well known leaf photosynthetic activity decreased by ozone. Main factor of ozone-induced reduction in photosynthetic activity is considered as biochemical limitation at chloroplast (e.g. maximum carboxylation rate,  $V_{\text{cmax}}$ ). However, previous studies did not consider a conductivity of  $\text{CO}_2$  transfer from intercellular air space to chloroplast (i.e. mesophyll conductance,  $G_m$ ). In the present study, we analyzed leaf internal processes of photosynthesis including  $G_m$  in leaves of Siebold's beech (*Fagus crenata*) seedlings under elevated ozone. Seedlings of Siebold's beech were grown in the three gas treatments (charcoal-filtered air or ozone at 1.0- or 1.5-times ambient concentration) for two growing season in 2016-2017. Leaf gas exchange and chlorophyll fluorescence were simultaneously measured in July and September of the second growing season. We determined light saturated net photosynthetic rate ( $A$ ), stomatal conductance to water vapor ( $G_s$ ) and  $G_m$ , and analyzed the responses of  $A$  to intercellular  $\text{CO}_2$  concentration ( $C_i$ ) ( $A$ - $C_i$  curve) and to chloroplast  $\text{CO}_2$  concentration ( $C_c$ ) ( $A$ - $C_c$  curve) for calculating  $V_{\text{cmax}}$  ( $V_{\text{cmax}C_i}$  and  $V_{\text{cmax}C_c}$ , respectively). We also determined the contents of Rubisco and chlorophyll. There was no significant effect of ozone on any photosynthetic parameter in July. In September, ozone significantly decreased  $V_{\text{cmax}C_i}$ . At the same time, a reduction of  $G_m$  was found, whereas there was no significant reduction in  $V_{\text{cmax}C_c}$  and contents of Rubisco and chlorophyll in leaves. These results indicate that apparent reduction in  $V_{\text{cmax}}$  (i.e.  $V_{\text{cmax}C_i}$ ) does not mean a reduction of carboxylation capacity. Although  $G_m$  was decreased by ozone,  $C_c$  did not decreased. This is because of significant increase of  $C_i$  due to the insufficient stomatal closure. As a result of no change in  $C_c$  and  $V_{\text{cmax}C_c}$ , the  $A$  was not affected by ozone. We conclude that  $G_m$  is important factor explaining ozone effects on photosynthesis of Siebold's beech.

**Keywords:**  $A$ - $C_c$  curve, chloroplast  $\text{CO}_2$  concentration, maximum carboxylation rate, mesophyll conductance, Siebold's beech

## The role of secretory glands in plant sensitivity to ozone: an alternative route for uptake?

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Ozone (O<sub>3</sub>) dissociation leading to ROS formation occurs precisely in the aqueous medium, generally in the leaf apoplast. However, secretory glands located at the external surface of the leaf may also produce and reabsorb aqueous exudates (nectar and guttation droplets) that may be suitable places to ROS dissociation and direct uptake. Although the relevance of morphological structures in conferring tolerance to O<sub>3</sub> effects is often mentioned, the effective role of O<sub>3</sub> on secretory glands functioning and consequences to the plant is still unknown. Here we tested the hypothesis that aqueous secretory glands are direct routes of O<sub>3</sub>-dissociated ROS entrance into leaf tissues, resulting in higher vulnerability of the plant to O<sub>3</sub> effects. To test it, we worked on extrafloral nectaries of tropical species [*Astronium graveolens* (Anacardiaceae), *Piptadenia gonoacantha* (Fabaceae), *Passiflora edulis* (Passifloraceae)], and hydathode pores of sugarcane (Poaceae). Young plants were submitted to an acute O<sub>3</sub> fumigation inside chambers, and routes of nectar and guttation droplets reabsorption were identified by the application of Lucifer Yellow (LYCH) tracer over the exuded droplets. This methodology was also performed in a FACE experiment to verify if the results obtained in chambers are reflected in the real environment. In both chamber and FACE situations, LYCH tracer experiments showed that nectar or guttation droplets reabsorption (i.e. direct ROS entrance considering the O<sub>3</sub> dissociation in the exuded nectar/guttation droplets) occurred through extrafloral nectaries and hydathode pores previous to the rising of specific O<sub>3</sub>-symptoms. The reabsorption of ROS-dissociated nectar or guttation droplets is a route of direct ROS entrance into inner leaf tissues, thus increasing the bearing plant sensitivity to O<sub>3</sub>. This study highlights the relevance of plant surface structures as mechanisms of O<sub>3</sub> resistance or susceptibility. An alternative model for ozone uptake and plant sensitivity based on glands is proposed.

**Keywords:** *Astronium graveolens*, extrafloral nectaries, FACE system, fumigation chambers, hydathodes, *Passiflora edulis*, *Piptadenia gonoacantha*, sugarcane

**S2.O23**

## **Leaf beetle activities on Japanese white birch grown under elevated O<sub>3</sub>**

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Ground level ozone concentration (O<sub>3</sub>) has been increasing. Elevated O<sub>3</sub> reduces photosynthetic activities and usually leaf area. Grazing damages on Japanese white birch saplings by leaf beetle were frequently found in the center part of Sapporo city, northern Japan (Lat 43.01, Lon 141.35) whereas the damages were rare in suburb. Based on our free-air O<sub>3</sub> fumigation system, grazing damages caused by leaf beetles were smaller at ambient O<sub>3</sub> condition (about 40 ppb) than those at elevated O<sub>3</sub> (about 70 ppb). Although concentration of leaf defense chemicals (total phenolics and condensed tannin) was lower in elevated O<sub>3</sub>, adult leaf beetles avoided leaf grazing at elevated O<sub>3</sub>. To reveal the reason why grazing damages were smaller at O<sub>3</sub> site, we determined BVOC (Biological volatile organic compound) with a gas chromatography. There were no statistical differences between ambient and elevated O<sub>3</sub> sites in the composition of BVOC (i.e. monoterpene and sesquiterpene) emitted from birch leaves. However, some kinds of aldehyde were slightly larger at elevated O<sub>3</sub>. We further studied the preference traits of adult leaf beetles prior to oviposition in June and August. We could not distinguish female and male of leaf beetle by the size so we collected adults of leaf beetles of around 80-90 individuals as one cohort. And we used them randomly for the following preference experiments. By using the Y-shape tube test, we found that no preference of adults was found at O<sub>3</sub> concentration at less than 40 ppb, however, adults did not prefer BVOC with elevated O<sub>3</sub> (80 and 120 ppb). We may conclude behavior of grazing and making oviposition of leaf beetles is attributed to BVOC emission under elevated O<sub>3</sub>.

**Keywords:** Leaf phenology, Japanese white birch, leaf beetle, Y-shape tube, BVOC

## **S2.O24**



## **Tropospheric ozone interactions on nitrogen cycling in Mediterranean annual pastures**

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Annual herbaceous species are widely present in Mediterranean landscapes either forming pastures, dehesas or the understory of Mediterranean forests, and usually represent an important fraction of the biodiversity of Mediterranean ecosystems. Among them, dehesas, sparsely wooded grasslands commonly found in the Iberian peninsula, have been protected under the Habitats Directive (92/43/EEC) of the EU. Annual Mediterranean pastures are chronically exposed to tropospheric ozone levels above current European air quality regulations for the protection of vegetation and in some areas they are also subject to atmospheric nitrogen deposition levels that exceed empirical critical loads established for the protection of European grassland habitats. Recent studies on the ozone sensitivity of annual Mediterranean pasture species have shown that besides direct effects on plant growth, forage quality and reproductive ability, indirect effects on the nitrogen cycling may also be operating, changing the ecosystem functioning. Ozone has been described to reduce the fertilization effect of nitrogen and to increase soil nitrous oxide emissions of an annual Mediterranean pasture under experimental conditions (Calvete-Sogo et al., 2014, *AtmosEnv.* 95; Sánchez-Martín et al., 2017, *AtmosEnv.* 165). An open-top chamber experiment on the interaction of ozone and nitrogen in a simplified Mediterranean annual pasture community has been used to gain insights on the effects of ozone on nitrogen cycling and how these two pollutants interact at the plant and soil compartments. Ozone effects on the nitrogen use efficiency of component species and on the nitrogen balance of the pasture will be presented. Potential interactions between tropospheric ozone and nitrogen in will be put into context with the most important, present and foreseen, environmental driver of annual Mediterranean pastures: drought. Understanding interactions among air pollutants and how these interactions are modified by climate is one of the main current challenges for Mediterranean ecosystems.

**Keywords:** Mediterranean annual pasture; phytotoxic ozone dose; nitrogen use efficiency; nitrogen balance; soil nitrous oxide emissions; drought

**S2.O25**

## Ozone flux is negatively related to foliar N and P in Swiss beech and Norway spruce forest

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Observations in a network of Swiss forest monitoring plots show a decrease in foliar concentrations of nitrogen (N) and phosphorus (P) in European beech (*Fagus sylvatica*), Norway spruce (*Picea abies*) and oaks (*Quercus* sp.) during the last decades. In beech, also foliar potassium (K) and magnesium (Mg) concentrations decreased. The potential drivers for these changes were evaluated with the help of multivariable regression models. Among other drivers such as nitrogen deposition and soil acidification, ozone flux (POD1) was negatively related to foliar N and P in beech and Norway spruce. Meanwhile, ozone was found to be a significant predictor for the community composition of ectomycorrhiza in 15 beech plots of the same forest monitoring network. These results suggest a possible pathway for the reduced foliar nutrient concentrations. Decreased carbon allocation to roots is a known impact of ozone and mycorrhizal symbiosis plays a key role in nutrient uptake of trees.

**Keywords:** ozone, foliar nutrients, phosphorus, European beech, Norway spruce, ectomycorrhiza

**S2.O26**

## Nutrient fertilization mitigates the effects of ozone exposure on poplar plants

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During the last century, concentrations of tropospheric ozone (O<sub>3</sub>) have risen substantially on a global scale as a result of increasing anthropogenic emissions. Nutrient availability in the soil, especially nitrogen (N) and phosphorus (P), is considered as a pivotal modifier of plant response to O<sub>3</sub>. In this work, the interactions of three levels of O<sub>3</sub> (1.0, 1.5 and 2.0 times the ambient O<sub>3</sub> concentration; AA, 1.5 × AA and 2.0 × AA) and six combinations of nutrient treatment [two levels of N (0 and 80 kg N ha<sup>-1</sup>; N0 and N80) and three P concentrations (0, 40 and 80 kg P ha<sup>-1</sup>; P0, P40 and P80)] on biochemical traits were investigated in an O<sub>3</sub> sensitive poplar clone (rooted cuttings of *Populus maximoviczii* Henry × *P. berolinensis* Dippel) in an O<sub>3</sub> free air controlled exposure facility (FACE). Plants exposed to increasing O<sub>3</sub> concentrations (alone and in combination with N/P fertilization) developed visible stipples of browning tissue localized in the interveinal adaxial leaf area. This macroscopic effect was twinned with the alteration of membrane integrity and the impairment of PSII photochemistry: plants exposed to 2.0 × AA displayed 2-fold higher values of malondialdehyde and total carotenoids/chlorophylls ratio than plants exposed to AA. N fertilization had some “mitigating effects” against O<sub>3</sub>-induced oxidative burst by activating different detoxification mechanisms, i.e. accumulation of proline (+12, +67 and +18% in comparison to AA, 1.5 × AA and 2.0 × AA) and reduced ascorbate (+10, +5 and +8%, respectively). P fertilization partially balanced the reactive oxygen species production as confirmed by the significant decrease of the hydrogen peroxide content, but did not prevent foliar damage. Nutrient fertilization induced qualitative change of the carbohydrate pool suggesting a possible effect on the photosynthetic process and allocation of resources.

**Keywords:** climate change, oxidative stress, nutrients availability, membrane integrity, reactive oxygen species, proline, carbohydrates, non-enzymatic antioxidants

**S2.O27**

## **The WMO's Global Atmosphere Watch: a general overview and services to ecosystems**

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The Global Atmosphere Watch (GAW) is the World Meteorological Organization's research programme tasked with monitoring and keeping track of tendencies in the composition of the Earth's atmosphere. The GAW programme achieves this via an international network of high-quality atmospheric observations stations across local to global scales as well as teams of scientists providing expert advice on a range of atmospheric composition-related areas such as aerosols, reactive and greenhouse gases, urban air quality, total atmosphere deposition, etc. The programme's aim is to drive high quality and impact science while co-producing a new generation of research-enabled products and services. GAW's Scientific Advisory Group for Total Atmospheric Deposition (SAG-TAD) has a mandate to produce global maps of wet, dry and total atmospheric deposition for important atmospheric chemicals to enable research into biogeochemical cycles and assessment of ecosystem and human health effects. The most suitable scientific approach for this activity is the emerging technique of measurement-model fusion for total atmospheric deposition. This technique requires global-scale measurements of atmospheric trace gases, particles, precipitation composition and precipitation depth, as well as predictions of the same from global/regional chemical transport models.

**S2.O28**

## **Poplar submitted to a succession of ozone and drought stresses: dynamic of stomatal responses**

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Nowadays, plants are facing stronger environmental stresses due to anthropogenic activities (atmospheric pollution, climate changes). To protect forest health, we need to improve risk assessment, taking into account interacting stresses. For example, the impact of ozone (O<sub>3</sub>) events pollution on drought response needs a special attention. Indeed, it is very likely to have an O<sub>3</sub> spring episode before summer drought. O<sub>3</sub> could affect the stomatal dynamic under drought through stomatal sluggishness. We intend to decipher the response of stomatal closure/aperture mechanisms relative to light or vapor pressure deficit variation in condition of O<sub>3</sub> stresses and for drought stress (with or without previous O<sub>3</sub> stress). Two genotypes of *Populus nigra x deltoides* were exposed to various treatments during 21 days. Trees were exposed to 80 ppb/day O<sub>3</sub> for 13<sup>th</sup> day then to a moderate drought. The objectives of this work: - Are there differences of stomatal movement dynamics linked to environmental variables (light, VPD) among poplar genotypes and between hydric regimes or O<sub>3</sub>? - Does O<sub>3</sub> treatment modify the drought-induced stomatal closure? - How do those differences, studied at the leaf scale, contribute to WUE at the whole plant scale?

**Keywords:** poplar, ozone, drought, light, VPD, stomatal closure

**S2.O29**

## **Influence of drought stress on the ozone-induced signalling mechanisms of two Mediterranean tree species**

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The aim of the study was to investigate the influence of drought stress on the responses of two Mediterranean tree species, characterized by different drought-sensitivity, to a single pulse of ozone (O<sub>3</sub>). The behaviour of *Quercus cerris* (drought-sensitive) and *Q. pubescens* (mild-drought-sensitive) was evaluated in terms of ecophysiology and cross-talk responses among signalling molecules. Control plants maintained in well-watered and under filtered air conditions were compared with individuals subjected to drought (20% of the effective daily evapotranspiration, for 15 days) and later exposed to a single episode of O<sub>3</sub> (200 ppb, 5 h day<sup>-1</sup>). Both stressors, applied alone or combined, affected ecophysiological responses. However, differential behaviour was observed between the species: under drought, photosynthetic capacity significantly decreased (-69% in comparison to controls), due to both stomatal and mesophyll limitations only in *Q. pubescens*; the concomitant presence of drought and O<sub>3</sub> induced an ameliorative effect on photosystem II (PSII) performance during the fumigation in *Q. cerris*, whereas photochemical efficiency of PSII in light conditions of *Q. pubescens* was highly impacted by O<sub>3</sub> treatment (-75%). These differences were confirmed by the different oxidative load showed by the species. Different trends and consequently different role of phytohormones was observed in relation to drought and O<sub>3</sub>. Under O<sub>3</sub> alone, similar spatial and functional correlations among these molecules were shown by the two species: ethylene emission values peaked at the end of the treatment (4- and 8-fold higher than controls, in *Q. cerris* and *Q. pubescens*). In drought stressed-plants, phytohormones were differently involved in O<sub>3</sub>-induced signalling mechanisms: abscisic acid concentrations progressively increased starting to 2 and 5 h from the beginning of the exposure in *Q. cerris* and *Q. pubescens*. Our results highlight a differential response of plants suffering from water withholding to a single O<sub>3</sub> episode compared to well-watered plants.

**Keywords:** climate change, oak species, phytohormones, oxidative stress, photo-assimilation, ethylene, abscisic acid

## **S2.O30**

## Antioxidative responses of three oak species under ozone and water stress conditions

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The Mediterranean basin is considered a global biodiversity hotspot but Mediterranean plants are threatened by natural and anthropogenic factors (e.g., tropospheric ozone, O<sub>3</sub>) which are expected to be harsher in the near future. The objective of this work was to give a thorough description of the detoxification mechanisms at the basis of the high plasticity of Mediterranean oak species. Two-year-old seedlings of *Quercus ilex*, *Q. pubescens* and *Q. robur* were grown under the combination of three levels of O<sub>3</sub> (1.0, 1.2 and 1.4 times the ambient O<sub>3</sub> concentration; AA, 1.2×AA and 1.4×AA) and water irrigation [1.2, 0.6 and 0.12 l day<sup>-1</sup>; well-watered (WW), moderate drought (MD) and severe drought (SD)] from June to October 2015. In *Q. ilex*, no symptoms due to O<sub>3</sub> and/or drought were observed. This was confirmed by the minor propagation of reactive oxygen species (ROS) and the subsequent reduced cellular oxidative burst. *Q. pubescens* exposed to 1.4×AA and subjected to SD developed O<sub>3</sub> injuries, consisting in minute roundish browning necrosis scattered among the leaf veins of both surfaces of mature leaves. An induction of the phenylpropanoid biosynthetic pathway by both stressors led to an increase of phenolic acids (+61% in comparison to AA). This enhanced antiradical ability of cell wall components partially balanced the ROS production but did not prevent foliar damage. In *Q. robur*, O<sub>3</sub> symptoms were induced by both 1.2×AA and 1.4×AA concentrations under WW or MD conditions. Increasing O<sub>3</sub> levels induced a significant rise of total flavonoids (+43 and +16% in 1.2×AA and 1.4×AA). This species also displayed higher constitutive level of ascorbate. These mechanisms did not preserve the PSII photochemistry from impairment, as confirmed by the decrease of total chlorophylls under O<sub>3</sub>. A different species-specific degree of tolerance to single and combined stress was observed.

**Keywords:** global climate change, mediterranean plant species, drought, atmospheric pollution, reactive oxygen species, carotenoids, phenols, flavonoids, ascorbate

**S2.O31**

## Effects of elevated O<sub>3</sub> on biomass allocation, nonstructural carbohydrates and rhizosphere soil function in poplar by joint water and nitrogen limitation

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Ground-level ozone (O<sub>3</sub>) pollution has affected carbon metabolism and allocation in tree species, which is limited joint by water and nutrient availability. How variation in soil nutrients and water may act together to influence O<sub>3</sub> effect on plant and soil is unresolved. Here we report plant biomass allocation, major nonstructural carbohydrates in leaves and roots and rhizosphere edaphic functions from 1-year-old seedlings of poplar clones 546 (*Populus deltoides* cv. '55/56' × *P. deltoides* cv. 'Imperial') under two contrasting levels of O<sub>3</sub> concentration (CF, charcoal-filtered air, and E-O<sub>3</sub>, non-filtered air + 40 ppb), soil nitrogen treatments (N0, 0 kg N ha<sup>-1</sup> year<sup>-1</sup>, and N50, 50 kg N ha<sup>-1</sup> year<sup>-1</sup>) and watering regimes (well-watered, and reduced watering, 40-50% irrigation) for one growing season. We find that the O<sub>3</sub> deleterious effects depends on the amount of available nitrogen and water. Specifically, elevated O<sub>3</sub> levels led to decreases in root biomass and root-to-shoot ratio when well-watered, no nitrogen supply, or both; but these negative effects have alleviated when reduced watering and nitrogen supply. O<sub>3</sub> induced much greater changes in the amounts of carbohydrates in roots than in leaves when both water and nitrogen were at their lower level. We conclude that given widespread, simultaneous limitation by water and nutrients, large stimulation of biomass by increasing O<sub>3</sub> pollution may not be ubiquitous.

**Keywords:** Ozone; allocation; nonstructural carbohydrate (NSC) concentrations; nutrient availability; water limitation; poplar; rhizosphere soil



## Session 3

# **How plant ecosystems respond to ozone exposure**



## **Ozone and BVOC exchanges between biosphere and atmosphere**

**Silvano Fares**

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Ozone is the main oxidant stressor in regions with warm climates, where high temperatures and large emission of precursors (Nitrogen oxides and hydrocarbons) from anthropic sources lead to the formation of this secondary pollutant. Plants can remove ozone from the atmosphere through stomatal absorption (i.e. ozone penetrates stomata and reacts with leaf tissues) and various non-stomatal deposition processes. Many vascular plants can produce and emit into the atmosphere large amount of Biogenic Volatile Organic Compounds (BVOC), largely represented by isoprenoids. BVOC emission is particularly stimulated by high temperatures, similar conditions promoting high levels of tropospheric ozone. One of the reason why plants invest energy to produce BVOC is that these molecules can play as antioxidant, thus defending leaf tissues from the adverse effects of oxidant molecules. Chemical lifetime of isoprenoids such as sesquiterpenes is very short due to fast reactions with ozone and other reactive molecules. In order to fully explore plant-atmosphere interactions under environmental stress, bi-directional exchanges of BVOC, CO<sub>2</sub>, water, and ozone should be investigated simultaneously. Gas exchange studies through laboratory experiments using branch enclosures and through field measurements at canopy level with the Eddy Covariance technique help to understand possible links between BVOC emission and ozone removal inside leaves and in the gas phase in the sub-canopy region. In my talk, I will introduce examples of such studies, mostly performed in agricultural and forest ecosystems located in ozone stressed Mediterranean areas. Mediterranean forest ecosystems are indeed exposed to high loads of anthropogenic pollutants and are among the most threatened ecosystems on Earth by climate changes. Advantages and disadvantages from using state of the art instrumentation for fast detection of ozone and BVOC will be analysed, as well as the most advanced modelling approaches currently adopted to predict BVOC emission, and ozone sequestration in the soil-plant-atmosphere continuum. Future research needs will emerge after deep evaluation of what has been discovered so far and what is highly needed to better understand the link between BVOC emission and ozone removal by plants.

**S3.O1**

## Ozone fluxes from an urban park: the unique station of Bosco di Capodimonte in Naples

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The air quality of urban areas, and consequently the health of the citizens, is related mainly to the concentration of primary and secondary pollutants such as particulate matter (PM), nitrogen Oxides (NO<sub>x</sub>), ozone (O<sub>3</sub>). Particularly in Mediterranean environments, there is a growing interest to understand O<sub>3</sub> flux in cities and its removal by urban trees. Beyond the chemical reactions, air O<sub>3</sub> removal mainly occur by tree leaves through both stomatal and nonstomatal mechanisms. Moreover, oxidation of biogenic volatile organic compounds (VOC) by OH, in the presence of nitric oxide (NO), produces molecules of O<sub>3</sub>. Thus, in urban environment, plants can contribute to both O<sub>3</sub> removal and formation. For the elevated number of interacting factors, several models have been developed to estimate O<sub>3</sub> fluxes by urban forest (UF), rarely compared with field measurements, thus producing uncertain estimates. The eddy covariance (EC) technique is a reliable method to directly measure exchange of gases between biosphere and atmosphere. Here, we present the results of an innovative EC station in an UF, where fluxes of CO<sub>2</sub> and H<sub>2</sub>O along with several other trace gases including O<sub>3</sub> are simultaneously measured. The station was established in the “Real Bosco di Capodimonte”, a large urban park, dominated by *Quercus ilex*, within the large city of Naples in Italy. The high radiative force and temperature, coupled with the elevated urbanization level in Naples, offer a unique opportunity to study the interaction between vegetation and trace gases, and consequently the contribution of vegetation on O<sub>3</sub> concentration, through the balance between formation and uptake. Results demonstrate that O<sub>3</sub> fluxes are influenced by park spatial variability, especially because of the gradient between meadow and forest, and by the seasonality highlighting the role of physiological status of the vegetation and of the ambient O<sub>3</sub> concentrations.

**Keywords:** Urban forest, Eddy covariance, Ozone, *Quercus ilex*, Traces gas fluxes

## S3.O2

## **Linking ozone susceptibility to induced emissions of biogenic volatile organic compounds**

**Rüdiger Grote**

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Ozone damage is usually linked to ozone concentration and/ or uptake but the large variety of sensitivities is either neglected or linked to a single species-specific property. However, sensitivity to ozone stress differs not only by species but also by season, pre-disposition to other stresses (e.g. drought) and environmental conditions such as temperature. Therefore, estimates of damages due to ozone under future climates are highly uncertain. Sensitivity depends on at least four processes that are either related to stomatal control or to biochemical defense reactions. The latter are generally assumed to be constitutive but also have an inducible component. It has been noted that this inducible component is linked to the capacity of plants to form biogenic volatile organic compounds (BVOCs) such as isoprene. The current work describes the link as it has been put into a physiological model. Since the BVOC emission depends on photosynthesis activity, photosynthesis can be damaged by ozone uptake, but the damage depends on BVOC emission, and a full feedback cycle has been implemented. The model offers the possibility to hypothesize about future plant responses to air pollutants as well as about the effect of emission changes that in turn might affect ozone formation.

**Keywords:** ozone damage, BVOC emission, induced defense, modelling

## **Spatial variability of isoprene emission in China under the ambient O<sub>3</sub> concentration using MEGAN and PS\_BVOCs model**

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Due to a significant interaction between biogenic volatile organic compounds (BVOCs) and global climate change, improving understanding of regional isoprene emissions under the present and future elevated O<sub>3</sub> conditions is imperative. Past estimations of BVOC emissions in China were based on conventional algorithms and models, few take into account the impacts of O<sub>3</sub>. With an increasing ambient O<sub>3</sub> concentration in most parts of China, previous models will lead to large inaccuracies in the estimated results. To refine isoprene emission estimation for China and to further explore the role of O<sub>3</sub> impact on isoprene emission, the latest algorithms of MEGAN (Model of Emissions of Gases and Aerosols from Nature) and PS\_BVOCs model with the parameterization of O<sub>3</sub> were used to estimate the biogenic emissions of isoprene (C<sub>5</sub>H<sub>8</sub>) in China. The combined local-survey and experiment-based data were used to characterize plant functional types, and base emission factors of isoprene. Real-time MODIS (Moderate Resolution Imaging Spectroradiometer) data were introduced to update the land-use parameters and high-resolution meteorological outputs from the MM5 model to determine the influence of leaf area index (LAI) and leaf age deviation from standard conditions. Because of the considerable regional disparity in plant distributions and meteorological conditions across China, isoprene emission presented significant spatial and temporal variations in both two models. Compared with isoprene emission data from MEGAN model, the effects of O<sub>3</sub> was obvious especial in eastern region of China. The results also indicate high uncertainties in isoprene emission estimated. We present an improved estimation of isoprene emissions, which provides important information for further exploration of the role of global climate change on BVOCs emission.

**Key words:** Isoprene emission, Tropospheric O<sub>3</sub>, MEGAN model, PS\_BVOCs model

## **S3.O4**

## **Ornamental plants used as tool for remediation of ozone pollution in indoor environment**

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Indoor air pollution is more severe than outdoor air pollution especially in context with human health. A number of studies have been reported on outdoor environments which indicate that vegetation act as sink for outdoor ozone but very less data available for indoor environment taken ozone as a pollutant with the help of indoor ornamental plants. This study evaluate ozone deposition velocities ( $v_d$ ) for three common indoor ornamental plants (*Dracaena deremensis*, *Tagetes erecta* and *Lilium candidum*).  $V_d$  was calculated by using measured leaf areas of each plant and give exposures for two diurnal cycles where ozone concentrations in chamber tests were elevated for 8 h followed by 16 h in the absence of ozone. Estimates of  $v_d$  after the exposures ranged from 5.6 m/h for *Dracaena deremensis* to 0.9 m/h for *Lilium candidum*. Values of  $v_d$  were approximately 50% and 66% lower at the end of a second exposure and third exposure, respectively. For deposition velocities measured in this study, the ozone removal effectiveness ranges from 0.9% to 9% for leaf surface area to room volume ratio of 0.06/m when accounting for values for air exchange and background loss typical of a residential environment.





# 5.

## Abstracts of poster presentations

Posters are sorted in alphabetical order. The poster session is in the open cloister of the Auditorium. Poster slots are marked by Px in alphabetical order (see below). There are three poster sessions: on 22<sup>nd</sup> (18.00-19.00), 23<sup>rd</sup> (10.00-11.00) and 24<sup>th</sup> (10.00-11.00) regardless session number. Corresponding authors are required to attend the poster session in order to reply to questions.

All posters have the opportunity to receive the **Best Poster Award for Ozone and Plants 2018**. To apply for the Award, authors need to include the specific mark in the upper right corner of their poster.

During the poster sessions, participants will evaluate posters based on the level of research, quality of the poster and clarity of the presentation. The award consists in a certificate and will be assigned during the closing ceremony.





## Elevated O<sub>3</sub> affects the decomposition process in different types of soil

**Evgenios Agathokleous**<sup>(1,2)</sup>, Alessandra De Marco<sup>(3)</sup>, Mitsutoshi Kitao<sup>(1)</sup>, Takayoshi Koike<sup>(2)</sup>, William J. Manning<sup>(4)</sup>, Georgia Ntatsi<sup>(5)</sup>, Elena Paoletti<sup>(6)</sup>, Costas J. Saitanis<sup>(5)</sup>, Judith Sarneel<sup>(7)</sup>, Pierre Sicard<sup>(8)</sup>, Marcello Vitale<sup>(9)</sup>

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Ozone (O<sub>3</sub>) pollution remains a global challenge for the scientific community and stakeholders. Ample scientific evidence shows that elevated O<sub>3</sub> levels negatively affect trees with further potential ecological consequences in the long term. It remains however poorly understood whether and how elevated O<sub>3</sub> alters the core process of litter decomposition in forest ecosystems and how the type of soil modifies the impact of O<sub>3</sub>. We will present our novel project for studying the decomposition process in communities of native to Northeast Asia deciduous trees. Trees of these communities are growing in three different types of soil. These trees have been previously (during the years 2014-2016) exposed to ambient or elevated O<sub>3</sub> levels, in a novel and globally unique experimental station located at the Northern island of Japan, Hokkaido. Based on plant-level data, according to which O<sub>3</sub> affected nutrient re-translocation during the seasons 2014-2016, we hypothesized indirect effects of O<sub>3</sub> on the decomposition process in soils, independently from O<sub>3</sub> injury on plants and the leaf litter *per se*. To investigate the impacts on decomposition process, we employed the standardized Tea Bag Index (TBI) method. Accordingly, we implanted tea bags of two types of tea in the soil of each experimental condition in Autumn 2016, which we retrieve periodically. In addition, we installed field chambers from which we periodically receive samples to analyze the major greenhouse gases emitted from soil. This project will provide critical information which can be served as the basis for modeling too.

**Keywords:** nutrient cycling, decomposition, greenhouse gases, ozone, tea bag index

## **Japanese larch seedlings grown in containers, inoculated with ectomycorrhizal fungi, and exposed to ozone**

**Evgenios Agathokleous<sup>(1)</sup>, Mitsutoshi Kitao<sup>(1)</sup>, Hideyuki Saito<sup>(2)</sup>, Hisanori Harayama<sup>(1)</sup>, Akira Uemura<sup>(1)</sup>, Takayoshi Koike<sup>(2)</sup>**

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Ozone (O<sub>3</sub>) levels in wide areas of Asia already exceed O<sub>3</sub> air quality standards for protecting plants. Trees are exposed to chronic elevated O<sub>3</sub> levels throughout their life and may have degraded production in forestry practice. Hence, future timber production may be threatened. Many trees depend on root symbionts for their survival. Successful symbiosis of trees with ectomycorrhizae fungi may promote seedling tolerance to O<sub>3</sub>. However, successful symbiosis may also promote seedling tolerance to drought through an increase in water uptake. Such an improvement of water status may cause higher O<sub>3</sub> uptake via stomatal openness, leading to higher sensitivity to O<sub>3</sub>. In the framework of low-cost silviculture practice, container grown seedlings are widely used. Container-grown seedlings with high or low fertilization were irrigated with inoculated or non-inoculated water and exposed to ambient or elevated O<sub>3</sub> conditions to examine whether ectomycorrhizae may influence plant tolerance to O<sub>3</sub>. We will discuss our recent findings which will contribute in understanding tree responses to changing environment and promoting tree tolerance to O<sub>3</sub> in Asia.

**Keywords:** drought, ectomycorrhizae, fertilization, forestry practice, ozone, stress, tolerance

## Ozone deposition in forest canopies

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Dry deposition is the dominant sink for tropospheric ozone and removal rates by vegetated surfaces are several times higher than bare ground. However, ozone is a powerful phytotoxin and uptake through the stomata leads to oxidative stress and damage to plant cells. This impairs many physiological processes, reducing photosynthesis and carbon assimilation, inducing early senescence and disrupting stomatal functioning. However, the net effect of these various impacts on plant health and growth, and on ozone deposition rates and atmospheric concentrations is not well understood. Here we apply the FORCAST (FORest Canopy-Atmosphere Transfer) one-dimensional canopy model to investigate the underlying processes driving ozone deposition and uptake. We incorporate three photosynthesis and stomatal conductance models and evaluate the ability of the model to capture observed ozone concentrations and fluxes in a Holm oak forest at Castelporziano Estate, Rome. We perform sensitivity tests with each physiological mechanism to investigate stomatal and non-stomatal ozone deposition fluxes under a range of background ozone concentrations and environmental conditions. We determine the impact of ozone damage to physiological processes on atmospheric fluxes and concentrations and ozone reactivity within the canopy space. We identify the key processes governing the rate of ozone uptake in each case.

**Keywords:** ozone deposition, stomatal conductance, forest canopy, biosphere-atmosphere exchange, ozone reactivity

## **Resiliency of Scots pine (*Pinus sylvestris* L.) tree to acidifying compounds and surface ozone under the pressures of climate changes**

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The aim of the study was to quantify the integrated effect of meteorology and air pollutants on stem basal area increment and crown defoliation of Scots pine trees located in the north-eastern part of Lithuania. Air concentrations of sulphur species and ammonium as well as their deposition were the main drivers resulting in changes in mean defoliation of Scots pine trees in Lithuania. Meteorological factors stimulated the recovery of pine tree crown condition, while ozone concentrations were close to the level of insignificance. Contrary to this, meteorology was a key factor resulting in variation in stem basal area. During vegetation nitrate deposition as fertilizing compound stimulated the pine tree stem BAI formation while ammonium compounds reduced it on both diurnal and annual scale. Negative ozone effect on pine growth intensity was significant only on mineral soil FS. Higher moisture regime significantly increased the tolerance of pine trees to the negative effect of air concentration of acidifying compounds, their wet deposition and surface ozone. The study is based on the results obtained conducting national project supported by Lithuanian Council of Research "FOREstRESS" (SIT-3/2015).

**Keywords:** Scots pine, meteorology, acidifying compounds, ozone, defoliation, increment

## Ozone risk for trees – dose response functions embedded in future climatic scenarios

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Validation of ozone doses and adaption of model parametrization are crucial to ensure a state-of-the-art basis for future ozone risk assessments for forest trees. The main objective of our new research project starting in 2019 is to advance ozone risk assessment and modelling for forest trees including future climate scenarios based on the recently established representative concentration pathways (RCP, IPCC 2013) considering tropospheric O<sub>3</sub> and CO<sub>2</sub>-concentrations. Fundamental for evaluation of ozone doses is the determination of stomatal ozone fluxes and several gas exchange parameters. Besides biomass and tree allometric data, N- and C-allocation, fine root development and changes in ecto-mycorrhizal diversity will be assessed. The experiment will be conducted in newly established phytotrones with 5-8 year-old beech and spruce from natural regeneration, growing in their natural soil monolith. Two experimental approaches will be applied (1) simulation of an O<sub>3</sub>-gradient under standard climate/CO<sub>2</sub> -conditions and (2) a sophisticated simulation of a future climate and O<sub>3</sub>/CO<sub>2</sub>-gradient: (I) In order to develop ozone dose-response-functions adjusted to present conditions and to derive realistic target values for spruce and beech, four different O<sub>3</sub>-concentrations (pre-industrial, ambient, moderately elevated and high) will be applied for two vegetation periods. (II) In order to evaluate effects of a changing climate and emission scenarios in the future, daily cycles will be simulated for preindustrial conditions, the present, and future scenarios RCP2.6 as well as RCP8.5 (2100). The simulation of the corresponding O<sub>3</sub>-concentrations and the combination of ozone and CO<sub>2</sub> emission will on the one hand explain the CO<sub>2</sub>-influence and on the other hand indicate combined effects, which have to be implemented in future growth modelling. A low mountain range region is serving as a model forest region for Germany. All simulation data will be acquired at a hourly resolution as it is crucial to generate scenarios as "realistic" as possible, especially concerning the concordance of meteorological values with O<sub>3</sub>-concentration values within a hourly resolution. This represents the first simulation database in experimental climate impact research. Our experimental concept is open to further collaboration from interested international groups.

**Keywords:** stomatal ozone uptake, ozone dose-response functions, RCP future scenarios, ozone and CO<sub>2</sub> interactions, beech, spruce

## Phytotoxic ozone effects on montane pines in the High Tatra Mts., Slovakia

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Ozone (O<sub>3</sub>) concentrations in European mountains are high enough to cause injury to sensitive trees such as Swiss stone pine (*Pinus cembra*) and dwarf mountain pine (*Pinus mugo*). The aim of this work was to assess Phytotoxic Ozone Dose metric without threshold limitation (POD<sub>0</sub>) obtained for the temperate mountain forests in the High Tatra Mts in Slovakia. Field measurement of O<sub>3</sub> concentration and meteorological data were incorporated into the DO<sub>3</sub>SE deposition model. The model parameterization included the recommendations from available literature and the preset for coniferous forests (CF) parameter values built into model. Maximum level of stomatal conductance (G<sub>max</sub>) as a key parameter for calculation of stomatal O<sub>3</sub> flux has not been specified neither for dwarf mountain pine nor for Swiss stone pine. We carried out field measurements of stomatal conductance using LI-6400 photosynthesis system (Li-Cor, Inc., Lincoln, NE) on both pine species. Based on these measurements, maximum level of stomatal conductance for O<sub>3</sub> was determined as G<sub>max</sub> = 110 mmol O<sub>3</sub> m<sup>-2</sup> PLA s<sup>-1</sup> for both studied montane pines. Model results of POD<sub>0</sub> ranged from 12.5 to 22.4 mmol m<sup>-2</sup> PLA for Swiss stone pine and 11.4 to 19.3 mmol m<sup>-2</sup> PLA for dwarf mountain pine during the 2016 growing season. POD<sub>0</sub> exceeded critical level for highly O<sub>3</sub> sensitive conifers (CL<sub>ef</sub> = 19 mmol m<sup>-2</sup> PLA) such as Swiss stone pine only at sites with favourable sunshine exposure. The concentration of O<sub>3</sub> and sufficient amount of soil water in the submontane and alpine zones contributed to higher level of POD<sub>0</sub> (16.9–22.4 mmol m<sup>-2</sup> PLA) for Swiss stone pine compared with dwarf mountain pine (12.2–19.3 mmol m<sup>-2</sup> PLA). These differences between the two species may be associated with their different canopy height and root depth. Swiss stone pine and dwarf mountain pine branches showed an obvious visible O<sub>3</sub> injury at higher altitudes. More pronounced visual symptoms were observed for dwarf mountain pine than for Swiss stone pine plots. The oldest needles of both species were more frequently damaged by O<sub>3</sub> injury at all plots.

**Keywords:** O<sub>3</sub> concentration, montane forest, stomatal O<sub>3</sub> flux, soil water, visible O<sub>3</sub> injury



## Ozone fluxes and GHG balance in a Spanish rice paddy field

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Project GEISPAIN (CGL2014-52838-C2-2-R, MINECO, Spain) has been launched with the general objective of quantifying the GHG balance of different relevant Spanish ecosystems. Measurements of O<sub>3</sub> fluxes with a fast O<sub>3</sub> analyzer (FOS, Sextant) in combination with eddy covariance technique were carried out in a rice paddy in Sueca (eastern Spain) during all the vegetative period of the crop. During rice cropping period, ozone fluxes were highly correlated with CO<sub>2</sub> fluxes and largely driven by stomatal conductance, while during non-cropping period, the fluxes were lower and driven by non-stomatal surface resistance. Complementarily, gas exchange leaf measurements under ambient conditions were carried out with a LICOR-6400 in order to parameterize the DO<sub>3</sub>SE model and to model stomatal O<sub>3</sub> fluxes in rice. The proposed parameterization for “Japonica” varieties exhibited about double maximum stomatal conductance values ( $g_{max}$ ) than the Asian “Indica” varieties. We additionally measured carbon dioxide (CO<sub>2</sub>) and methane (CH<sub>4</sub>) fluxes with eddy covariance in order to obtain a full carbon balance. Methane (CH<sub>4</sub>) were also determined at different stages of rice cultivation using the static chambers technique to estimate greenhouse gas (GHG) budget. The ecosystem was a net carbon source during non-cropping period, reaching high respiratory rates ( $> 5 \mu\text{mol m}^{-2} \text{s}^{-1}$ ) during pre-seedling and post-harvest periods and nearly null CO<sub>2</sub> fluxes during autumn-winter flooding period. During rice cropping period (June-July), the ecosystem was a strong carbon sink with maximum CO<sub>2</sub> sequestration rates up to ca.  $50 \mu\text{mol m}^{-2} \text{s}^{-1}$ . Overall, the studied paddy rice field presented a net ecosystem productivity (NEP) of ca.  $650 \text{ g C m}^{-2} \text{ y}^{-1}$  and a Net Ecosystem Carbon Balance (NECB = NEP – C harvested) of ca.  $250 \text{ g C m}^{-2} \text{ y}^{-1}$ . The rice paddy field behaved as a CH<sub>4</sub> source most of the year, with higher emissions rates during the rice cropping period.

**Keywords:** GHG, ozone fluxes, eddy covariance

## **MOTTLES: an innovative long term strategy for the definition of new critical levels to protect forest from ozone**

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Current European directives for forest protection from ozone are based on the atmospheric concentrations (AOT40). Even if ozone levels regularly exceed the EU standards calculated for forests, very often no significant correlations are found in the epidemiological analyses combining concentration and ozone indicators for vegetation (e.g. visible leaf injury, crown defoliation and radial growth). It is widely demonstrated that ozone effects on forests depend not only on air concentration but also on O<sub>3</sub> uptake through stomata. Hence, the idea that EU Directives should adopt indexes based on stomatal fluxes, such as the PODY (Phytotoxic Ozone Dose above a threshold Y of uptake) is largely accepted. In this context, MOTTLES (LIFE15 ENV / IT / 183) establish a permanent new-generation monitoring system in areas at highest and medium risk of ozone injury (France, Italy and Romania) to define new standards based on PODY. Thanks to continuous measurements of ozone concentrations and meteorological parameters with hourly temporal resolution and annual in-field surveys, fluxes are modelled and combined with the results of epidemiological analyses. The derived flux based critical levels (CLef) set new levels of damaging ozone for forests and will be proposed as legislative standards to European political institutions in order to define new European standards biologically relevant. MOTTLES provide also a support for forest managers and planners, indicating the best strategies for ozone-oriented forestry and climate change.

## Effect of ozone on physiological response of Israeli wheat cultivars

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Tropospheric ozone (O<sub>3</sub>) is globally recognized as a phytotoxic atmospheric pollutant become a potential threat to agriculture. Numerous studies on effect of O<sub>3</sub> on crop plants revealed wheat as one of the sensitive crop additionally the effect varies with species, cultivars and environmental factors. Wheat is a major crop in Israel and the present study is the first attempt to investigate the effect of O<sub>3</sub> on commercial Israeli wheat (*Triticum aestivum* L.) cultivars selected according to phenological variability. Yuval, Zahir and Amit are very early; Galil, Negev and Ruta are late, while Gedera, Binyamin and Bar-Nir are intermediate maturing phenotypes. Study was undertaken to determine the effect of chronic O<sub>3</sub> exposure on plants using open top chambers and exposed with ~10 %, above ambient O<sub>3</sub> from flag leaf emergence till the maturity of the plants. Gas exchange measurements accomplished on randomly selected fully expanded flag leaf from emergence, heading and flowering stages of plants with maintained microclimatic condition inside the leaf chamber of photosynthetic system. Statistically significant reduction in photosynthetic rate (*P*<sub>s</sub>) was observed between O<sub>3</sub> and control treatments; however, across cultivar variations were insignificant. Amongst test cultivars BarNir showed maximum reduction in O<sub>3</sub> exposed plants as comparison to control. Stomatal conductance (*g*<sub>s</sub>) reduced in Amit and BarNir (significant) and Gedera, however, increased in others. Intercellular CO<sub>2</sub> also followed the similar trend of variation. An acute O<sub>3</sub> exposure experiment was also carried out in the greenhouse; cultivars were exposed continuously above 100 ppb O<sub>3</sub> for four days at the flowering stage resulted significant variation across treatment in *P*<sub>s</sub> and within cultivars in *g*<sub>s</sub>, besides O<sub>3</sub> induced foliar injury symptoms were also observed under elevated O<sub>3</sub> treatment. Study suggests that O<sub>3</sub> is the threat for wheat cultivation and pave the way for further investigation for differential cultivar responses with altering environmental factors and comparison with the present study results.

**Keywords:** Wheat; Ozone, Physiology, Open top chamber; Ozone injury

## Vine and ozone

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Grape vine (*Vitis vinifera*, L.) has since long been recognized as an ozone-sensitive crop. When the concentration of ozone in the atmosphere is high due to air pollution, important quantities of this substance penetrate the vine leaf tissues, causing cell damage and interfering with photosynthetic mechanisms, with subsequent slowing down of plant growth and premature leaf senescence. Secondary effects are changes in biochemical processes which affect the chemical composition of the must and are likely to cause alteration of the quality of the wine. An experiment was conducted during two growing seasons of grapevine in 2010 and 2011 to gain knowledge on the effect of high ozone levels on the yield and several biochemical characteristics of the plant, that could influence the quality of the final product, which is of economic importance for the agricultural production in Italy. The method used was a fumigation facility consisting of Open Top Chambers operated at a vineyard in Angera (northern Italy). Fumigation permitted to study the effect of different ozone levels. After the end of the experiment, the grapes were weighted and chemical analyses were carried out in order to gain understanding on the effect of ozone on the level of several chemical substances in the grapes, which influence the quality of the wine: degree Brix, pH, tartaric and malic acids; assimilable nitrogen, resveratrol, polyphenols. The parameters characterizing the different ozone levels were expressed in terms of exposure (AOT40) and dose (POD). High ozone levels affect grape weight and thus the grapevine yield. In addition, the quality of wine is affected by a reduction of malic acid and polyphenols, which cause a more aggressive taste of the wine.

**P10**

## **Anatomical responses of a tropical liana species (*Passiflora edulis*) to ozone stress**

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*Passiflora edulis* (Passifloreaceae) is a wide-world distributed Brazilian native liana species. It has a high economic value due to its fruits and pharmacological properties. Most *Passiflora* species are identified and collected in the border of fragmented vegetation. Indeed, lianas compete with trees, interfering in their growth and mortality rate. Due to these effects over trees, lianas are considered as plagues, compromising the ecosystem homeostasis. The effects of ozone over liana species are, however, almost completely unknown. Thus, we aimed to identify microscopic changes in *P. edulis* leaves maintained in a FACE (Free-Air Controlled Exposure) ozone system installed at the Institute of Sustainable Plant Protection/National Council of Research (IPSP/CNR). Plants were submitted to a high ozone exposure (ambient O<sub>3</sub> x 2.0) and compared to ambient air concentrations. Samples from asymptomatic and symptomatic leaves (predominantly chloroses) were collected at the beginning and after 94 days of exposure and detailed microscopic analyses were performed. Preliminary results indicated following changes in leaf structure of plants from high free air ozone exposure: epidermal cells with sinuous walls, and alterations in the palisade parenchyma tissue, such as a fraction of chloroplasts with plastoglobuli accumulation and fewer starch grains, nuclei deformation and plasmolysis of some cell groups. All of them are indicative of plant sensitivity to ozone. In addition, macroscopic symptoms and changes in leaf architecture were observed. We are now evaluating physiological and volatile data also collected during the fumigation exposure period to compare with anatomical data obtained so far.

**Keywords:** FACE system, leaf anatomy, microscopic markers, oxidative stress, passion fruit.

## **Regulation of the ascorbate-glutathione cycle in leaves of poplar exposed to combined stresses (ozone and/or drought)**

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Nowadays, due to anthropogenic atmospheric pollution, trees are facing stronger environmental stresses leading to an increase of oxidative stress. To protect forest health, improvements in risk assessment are needed, taking into account detoxification mechanisms and interacting stresses. For example, the impact of drought events on the effect of ozone (O<sub>3</sub>) pollution needs a special attention. In this work, we intended to decipher the response of detoxification mechanisms relative to Halliwell-Asada-Foyer cycle (HAF) in condition of combined stresses (drought + O<sub>3</sub>). Genotypes of *Populus nigra* x *P. deltoides* were exposed during 17 days to various treatments: i) a mild drought, ii) an exposure to 120 ppb O<sub>3</sub> and iii) a combination of both treatments. Ascorbate and glutathione pools (reduced and oxidized), enzyme activity and expression of the different gene isoforms coding for HAF proteins were studied. Depending the isoforms, and probably linked with the putative localization of ROS production in response to drought and ozone, respectively, different expression profiles were observed. Finally, some enzymes of ascorbate-glutathione cycle displayed an intermediate regulation (gene expression, activity level) for combined treatment compared to drought and ozone separately applied.

**Keywords:** poplar, ozone, drought, detoxification, ascorbate, glutathione

## Assessment of tolerance level of *Eugenia uniflora* L. to ozone under FACE fumigation

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*Eugenia uniflora* L. (Myrtaceae) is a native Brazilian tree species with wide distribution in different Biomes, like those regionally known as Caatinga, Cerrado and Floresta Atlântica. Although this species is commonly found in the city of São Paulo, growing and fruiting in urban locations highly contaminated by ozone, its tolerance level to this pollutant is still unknown. Thus, the objective of the present work was to establish the tolerance level of this tropical tree species, by measuring physiological leaf traits – enzymatic and non-enzymatic antioxidants, reactive oxygen species and relative growth rates (TGR) – in seedlings exposed to ozone for 70 days in a Free-Air Controlled Exposure (FACE) installed at the Institute of Sustainable Plant Protection/National Council of Research (IPSP/CNR). Two treatments were adopted: ambient O<sub>3</sub> concentrations (AA) and doubled ambient O<sub>3</sub> concentrations (O<sub>3</sub>x2.0). The first results indicated a significant increase in the leaf concentrations of hydroxyl radical and superoxide ion, as well as of compounds indicators of lipid peroxidation - hydroperoxidene and malondialdehyde acid - and decreased levels of ascorbate peroxidase, superoxide dismutase and glutathione in plants exposed to O<sub>3</sub>x2.0. Although the TGR estimated for height, stem diameter and leaf number was similar in both O<sub>3</sub> treatments, the root biomass decreased and the leaf biomass and shoot/root ratio increased significantly in the plants exposed to O<sub>3</sub>x2.0. The changes in these physiological leaf traits seem to indicate that *Eugenia uniflora* is sensitive to high O<sub>3</sub> levels. This assumption will be checked more appropriately in a longer experiment.

**Keywords:** antioxidant defense, Brazilian native tree species, lipid peroxidation, reactive oxygen species, relative growth rates

## **Ozone and biogenic volatile organic compound (BVOC) interactions on leaf surfaces of stressed urban trees: A project plan**

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Stomatal absorption has long been thought to dominate O<sub>3</sub> absorption. However, lately the role of BVOCs in O<sub>3</sub> absorption at a stand level has been highlighted. Close to the leaves and in the intercellular space, BVOCs can be present in relative high concentrations and thus should have the capacity to absorb O<sub>3</sub>. In addition surface assisted ozone reactions are thought to play a role for ozone dry deposition. The absorption of O<sub>3</sub> by BVOCs may be important when the trees' stomata are closed, such as under salt or drought stress. Some BVOCs have been found to increase under stressed conditions, especially when high temperatures and radiation prevail which normally also occur during conditions which induce drought. In this study we want to measure the effect of BVOC emission on the O<sub>3</sub> absorption in the vicinity of the leaf surface under drought and salt stress, both of which lead to a decrease in stomata conductivity. We will compare high BVOC emitting tree species with low emitting tree species and compare the physiological stress level indicated by, for example, peroxidase and polyphenol oxidase activities under different O<sub>3</sub> concentrations. The O<sub>3</sub> absorption and BVOC emissions of 5 common urban tree species in Vienna will be studied under different stress levels using Teflon coated chambers enclosing branches with a constant airflow. Leaf stress will be linked to O<sub>3</sub> absorption and the amount and type of emitted BVOCs. In the second phase of the project, we will attempt to model future ozone concentration in the Viennese air, taking into account the responses of stress on O<sub>3</sub> absorption and BVOC emission combining micro climate- soil vegetation atmosphere transfer-, air chemistry- and tree stress models

**Keywords:** Urban trees, BVOC, chamber measurements, drought stress, salt stress

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## PEPc contribution to CO<sub>2</sub> assimilation in ozone-treated poplars

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Ground-level ozone (O<sub>3</sub>) is a major secondary pollutant that negatively affects plant growth and productivity. O<sub>3</sub> is well known to induce oxidative stress and ROS accumulation that are deleterious for most cell components and their functioning. In C<sub>3</sub> plants, O<sub>3</sub> decrease carbon fixation in lowering Rubisco content and activity. However, a higher PEPc content was also widely reported in O<sub>3</sub>-treated plants. PEPc could contribute to leaf CO<sub>2</sub> fixation and partly compensate for the loss of Rubisco capacity in supplying carbon resources to cell metabolism. Due to technical difficulties to separate CO<sub>2</sub> influx through PEPc or Rubisco, PEPc contribution to CO<sub>2</sub> uptake was never confirmed *in vivo* and quantified under O<sub>3</sub>. Here, we quantified changes in *in vivo* PEPc activity in O<sub>3</sub>-treated poplars using method based on photosynthetic discrimination against <sup>13</sup>C ( $\Delta^{13}\text{CO}_2$ ) measurements and modeling. A lower  $\Delta^{13}\text{CO}_2$  was observed in O<sub>3</sub>-treated poplars compared to control. This decrease was accompanied by a lower mesophyll conductance. However, taking into account changes in mesophyll conductance in the model did not explain the changes in  $\Delta^{13}\text{CO}_2$  under elevated O<sub>3</sub>. Only an enhanced *in vivo* PEPc activity was able to match modeled and measured  $\Delta^{13}\text{CO}_2$  values. Presently, focus is set on the fate of carbon fixed by PEPc across primary metabolism to reveal PEPc-specific metabolic pathways that may contribute to the overall plant-detoxifying arsenal.

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**Keywords:** oxidative stress; carbon uptake; photosynthetic discrimination; PEPc; poplar

## Effects of long-term ambient ozone exposure on xylem morphology of O<sub>3</sub> sensitive poplar treated with ethylenediurea (EDU)

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Wood quality is influenced by many environmental and biotic stressors. Although O<sub>3</sub> is considered one of the most important environmental stressor affecting plant growth, the knowledge on the effects of long-term ambient ozone exposure on wood quality is limited. The chemical ethylenediurea, N-[2-(2-oxo-1 imidazolidinyl)ethyl]-N0- phenylurea (abbreviated as EDU) is used to prevent foliar O<sub>3</sub> injury and to study O<sub>3</sub> effects on woody plants. While the protective effect of EDU against O<sub>3</sub> injury was widely demonstrated in short-term experiments (usually < 2 years), the aim of this study is to study the effects of long-term (6 years) ambient O<sub>3</sub> exposure and EDU treatment on xylem morphology, the main trait for wood quality. Transverse sections of 8–12 µm thickness were dissected out from the last two formed woody rings of the O<sub>3</sub> sensitive poplar clone 'Oxford' (*Populus maximoviczii* Henry x *berolinensis* Dippel) growing at the experimental site of Antella (Central Italy) and subjected for six years (2007-2014) to different treatments: a) WAT (not-protected trees) as ambient O<sub>3</sub> exposure; b) EDU (protected trees) as O<sub>3</sub> protection by weekly soil drenches of EDU solution at concentration of 450 ppm. Xylem morphology traits were analysed by a Nikon Eclipse 800E light microscope connected to a Nikon DS-Fi2 microscope camera. Vessels and fibres density, length and lumen cross-section were measured and frequency distribution analysed. Theoretical Hydraulic Conductivity (K<sub>st</sub>) and hydraulic diameter (d<sub>H</sub>) were calculated as proxy of hydraulic efficiency of the xylem in WAT and EDU plants. The results highlight the importance to investigate the effects of O<sub>3</sub> on xylem morphology and forecast wood properties of trees and hydraulic vulnerability of the xylem under future climate and O<sub>3</sub> pollution.

**Keywords:** vessel density, fibre length, xylem hydraulic efficiency, *Populus maximoviczii*, woody ring

## **Evidence of impacts of ozone on ecosystem services of grasslands**

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Ozone pollution can directly or indirectly cause impacts on many ecological processes that underpin ecosystem services. Over recent years we have performed many experimental studies on the impacts of ozone on grassland vegetation in solardomes. Here we collate datasets from these to provide evidence for ozone impacts on ecosystem services provided by grasslands. The ecosystem services included in this overview are 'supporting', 'provisioning', 'regulating' and 'cultural'. Presented evidence includes: *Impacts of ozone on flower numbers and timing (cultural and regulating services)*. Our results show that several iconic species show a strong decline in flower number with increasing ozone concentration and flux, including harebell (*Campanula rotundifolia*). These results have recently been used to establish critical levels for ozone in Europe. Timing of maximum flowering was significantly advanced by ozone in *Lotus corniculatus*. *Above- and below-ground biomass (supporting and regulating services)*. We have shown that ozone can reduce whole plant photosynthesis, and in addition, following early leaf senescence carbon allocation can be altered to maintain above-ground biomass at the expense of roots. *Litter quantity and quality (supporting services)*. Ozone has been shown to alter the chemical composition of plant material, including the timing, quantity and quality of litter. This can subsequently affect nutrient cycling and other soil processes. *Water cycling (supporting services)*. In addition to alterations in stomatal responses to environmental stimuli following ozone exposure we have shown changes in the water-holding capacity of soils, linked to changes in root biomass. In addition, impacts on provisioning services are considered using effects of ozone on pasture quality and quantity. A case study of how this could affect lamb production in the UK is presented. This showed that modest increases in ozone concentration could have large impacts on lamb production in regions where lamb numbers are high, due to the negative impact on pasture quality.

**Keywords:** grasslands, flowering, ecosystem services, root biomass, nutrient cycling

## Soil water availability affects ozone risk assessment in three European oaks

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In semi-dry ecosystems such as in Mediterranean climate, two main co-occurring climate change factors emerge, i.e. drought and ozone (O<sub>3</sub>) pollution. Limited water availability usually occurs concurrently with climatic conditions that favor O<sub>3</sub> photochemical production, i.e. high sunlight and high temperature. To derive ozone (O<sub>3</sub>) dose-response relationships for three European oak species (*Quercus ilex*, *Q. pubescens* and *Q. robur*) under a range of soil water availability, an experiment was carried out in an ozone FACE (Free-Air Controlled Exposure) facility. The ozone FACE facility is located at Sesto Fiorentino, near Florence, central Italy (43° 48' 59" N, 11° 12' 01" E, 55 m a.s.l.). Two-year-old seedlings of potted *Q. ilex*, *Q. pubescens* and *Q. robur* were grown under the combination of three levels of O<sub>3</sub> (1.0, 1.2 and 1.4 times the ambient concentration) and three levels of water irrigation (1.2, 0.6, 0.12 L day<sup>-1</sup>) from June to October 2015. Total biomass losses were estimated relative to a hypothetical clean air at the pre-industrial age, i.e. at 10 ppb as daily average (M24). A stomatal conductance model was parameterized with inputs from the three species for calculating the stomatal O<sub>3</sub> flux. Exposure-based (M24, W126 and AOT40) and flux-based (POD<sub>0.3</sub>) dose-response relationships were estimated and critical levels (CL) were calculated for a 5% decline of total biomass. Results show that water availability can significantly affect O<sub>3</sub> risk assessment. In fact, flux-based approach explained better the dose-response relationships than exposure-indices when combining results in different water regimes. In a simplified approach where species were aggregated on the basis of their O<sub>3</sub> sensitivity, the best metric was POD<sub>0.5</sub>, with a CL of 6.8 mmol m<sup>-2</sup> for the less O<sub>3</sub> sensitive species *Q. ilex* and *Q. pubescens*, and of 3.5 mmol m<sup>-2</sup> for the more O<sub>3</sub> sensitive species *Q. robur*.

**Keywords:** ozone, drought, water stress, risk assessment, oak, biomass

## Plant-insect interaction of elm seedlings treated with $(\text{NH}_4)_2\text{SO}_4$ spray grown under free-air $\text{O}_3$ fumigation

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Dutch elm disease was found in mature elm trees in Sapporo, northern Japan at around 2005. Not large trees but intermediate size trees had been dead in Hokkaido University campus. Dead trees were slightly suppressed and grown near walkway. We considered the reason why declining symptom occurred. Ground level ozone concentration ( $\text{O}_3$ ) has been increasing in northeastern part of Asia. Elevated  $\text{O}_3$  reduces photosynthetic activities and consequently plant defense capacities would be suppressed. Moreover nitrogen deposition (N ; now ammonium sulfate is dominant in PM2.5) is increasing and changes growth of many kinds of trees. We fumigated  $\text{O}_3$  (70 nmol/mol) and sprayed N as a form of fog (50kgN/ha·yr). We monitored seasonal trend of the grazing damages caused by insect herbivore in the site. We found major insects on elm leaves were follows: leaf beetle of elm (*Pyrrhalta maculicollis*) and alder (*Agelastica coerulea*), and Japanese Argidae (*Arge captiva*). Elm leaf beetle started to graze June to the end of September. Alder leaf beetle was found latter part of July to mid September. Argidae grazed from mid July to the end of September. This insect made oviposition for two times: mid-July and late September. We compared visiting frequency of these insects (elm leaf beetle and Argidae) to host individuals. At the peak season of insect grazing, grazing of elm and alder leaf beetle was tended to be increased by N but was significantly reduced by  $\text{O}_3$ . This tendency is the same for alder leaf beetle on Japanese white birch. According to analysis of the plant defense chemicals, concentration of total phenolics of leaves was higher at elevated  $\text{O}_3$ . N concentration was higher in leaves with N sprayed. These leaves are suitable food for insects. However, Argidae grazed significantly leaves at elevated  $\text{O}_3$  but not N sprayed. Sprayed ammonium sulfate was accumulated at leaf edge and induced to be dead. This dead part of a leaf may inhibit grazing leaves due to the behavior of grazing by Argidae: This insect grazes from leaf edge. Efficiency of photosynthetic oxygen evolution (PS II) was reduced by increased N. Moreover, Chl (a +b) increased by N sprayed. Based on our findings, we may conclude vigor and health of elm trees would be reduced by elevated  $\text{O}_3$  and increased N.

**Keywords:** Leaf phenology, elm, elevated  $\text{O}_3$ , increased nitrogen deposition, leaf beetles, Japanese Argidae

## **Reversible photoinhibition in purple- and green-leafed sweet basil (*Ocimum basilicum*) exposed to ozone**

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A research was conducted to investigate the physiological response of two cultivars of sweet basil (*Ocimum basilicum*) characterized by the different colour of leaves (Purple Ruffle, PR, red, and Green Ruffle, GR, green) to realistic ozone ( $O_3$ ) dosages (60, 80 and 100 nL L<sup>-1</sup> for 3 or 5 h). The aim was to evaluate the influence of anthocyanins on the oxidative stress induced by the pollutant in relation to their photoprotective and antioxidant capacity. At the end of the exposure and during the recovery in filtered air, chlorophyll *a* fluorescence and gas exchange were analysed on mature leaves. Overall, PR showed a lower sensitivity to  $O_3$  as compared to GR as testimony by the appearance of minute roundish browning necrosis only on both the surfaces of the fully-expanded leaves of GR. Chlorophyll *a* fluorescence analysis revealed differences in the responses of genotypes to  $O_3$ . The potential PSII photochemical efficiency ( $F_v/F_m$  ratio) significantly changed in PR only at the highest  $O_3$  dose (0.77 and 0.76 in comparison to ~0.80 in controls, respectively at 3 and 5 h). However, this ratio recovered 2 h after the end of the fumigation reaching the values of controls. Differently, GR showed a decrease in  $F_v/F_m$  at all the dosages (excluded the lowest 60 nL L<sup>-1</sup> for 3 h) and the values did not recover until 3 h after the end of the fumigation (100 nL L<sup>-1</sup>, independently of the duration). Analysis of gas exchanges revealed differences in the response of the two cultivars to  $O_3$  (80 nL L<sup>-1</sup> for 5 h), which negatively affected  $CO_2$  photoassimilation more in GR (-54%) than in PR (-28%), but no differences in stomatal conductance were observed (-46 and -52%, in PR and GR, respectively). A detailed analysis of the mechanisms involved in the reversible photoinhibition is discussed.

**Keywords:**  $O_3$ , photosynthesis, PSII, chlorophyll *a* fluorescence, gas exchange, visible injury, photochemical efficiency

## **Radial growth response to ozone exposure and uptake of sessile oak (*Quercus petraea*) in Mihaesti Level II Forest Monitoring plot, Romania**

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In order to determine specific effects of ozone on sessile oak mature trees, especially in the presence of co-varying influence of other environmental factors, continuous measurements of ozone concentrations, meteorological parameters and tree growth were performed during the 2012-2016 growing seasons in Mihaesti level II plot (405 m a.s.l.). The main species are sessile oak (*Quercus petraea*) – 80% and beech (*Fagus sylvatica*) – 20%, the mean temperature having values of approximately 10°C in all years and the precipitation quantity ranging between 562 mm (2013) and 628 mm (2016). Fluxes of ozone were calculated as described in UNECE Manual (2010), using CCE parameters for the “generic” deciduous tree species, except for which real observations were used. Hourly growth data from four trees were processed as described in Deslauriers et al. (2011). Daily and cycardian cycle basal area increments for each tree were calculated. Growth data was partially correlated (Kendall test) with AOT40 at daily/cycle level, with meteorological parameters as controlling variables. AOT40 calculated for the growing season had decreasing values, from 12.7 ppm in 2012, 9.9 ppm in 2014 to 5.1 ppm in 2016. Although not statistically significant, negative correlations between daily/cycle growth and AOT40 appeared in 2012, 2013 and in 2014. Multiple regression analysis showed a reduction in 2012 daily growth up to 2% due to ozone for 3 of the trees. Further analysis will be performed and presented.

**Keywords:** radial growth, ozone concentrations, AOT40, level II forest monitoring, growing season

## **Physiological and ecological consequences of changes in volatile plant emissions induced by elevated atmospheric ozone and CO<sub>2</sub> concentrations**

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Volatile organic compounds (VOC) emitted from terrestrial vegetation constitute complex olfactory environments (odorscapes) and play major roles in the interactions between organisms. Insects for instance, extract essential cues for their reproduction from their olfactory environment. Plant VOC production and emission are affected by environmental parameters, such heat and drought. As part of ODORSCAPE, a research program funded by French ANR, the effects of combined elevated atmospheric ozone and CO<sub>2</sub> concentrations on plant VOC production are studied, by assessing the stress-induced variations in the VOC emissions and in the corresponding biosynthesis pathways. Two crops representative of temperate agro-ecosystems, maize and poplar, are considered in this study. For CO<sub>2</sub>, 400 ppm or 800 ppm are applied to plants, starting at the germination step for maize and at the acclimatization step for poplar, while 30 ppb or 110 ppb of ozone are applied on fully developed plants, during 6h of the photoperiod, for 2 weeks. At the end of the four treatment combinations, photosynthetic and gas exchange parameters, and VOC emissions are measured and will be related to changes in the potential activity of key enzymes in the major VOC biosynthetic pathways (such as the lipoxygenase, mevalonate and methylerythritol phosphate pathways) and carbon fixing metabolisms (RubisCO and PEP carboxylase). This will allow us to evaluate the extent of photosynthetic carbon allocation toward VOC biosynthesis pathways in response to both elevated ozone and CO<sub>2</sub>. In addition, the stress-induced odorscapes will be tested for their effects on olfaction of herbivorous insects at gene, neural coding and behavior levels. Together, the results of this multidisciplinary project should reveal new aspects of the ecological consequences of climate change.

**Keywords:** elevated CO<sub>2</sub>, tropospheric ozone, maize, poplar, plant volatile organic compounds (VOC), VOC biosynthesis pathways, insect behaviour



## **Estimating the contribution of urban woody plants to the removal of atmospheric pollution: a species-specific case study in Italy**

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A consolidated consensus is now available about the important role of plants in removing air pollutants in the cities. Selecting the right species for urban green infrastructure, however, is still a critical step for a proper planning, due to fragmented knowledge about the species-specific ability of ameliorating air quality. In this work, we collected all knowledge available in the literature and filled the modelling gaps in order to develop an approach suitable to be applied to any city in the world. As a case study, we selected the main cities of the Tuscany Region in Italy. Average data on local pollution and meteorology were used as input into the models. We hypothesized that the plants were isolated, not pruned and healthy. For each woody plant species (trees and shrubs) used in these cities, we estimated the size at maturity (50 years), the balance of ozone (by summing up potential ozone formation based on the emission of volatile organic compounds, and the surface and stomatal deposition of ozone), the total deposition of nitrogen dioxide (stomatal and non-stomatal), the deposition of particles (PM10 and PM2.5) and the uptake and storage of carbon dioxide. All these variables were finally merged into a species air-quality index (SAQI), to summarise the overall ability of the individual woody species in ameliorating urban air pollution. Out of the 278 available species, data in the literature were missing for around 100 species, suggesting further experimental studies are needed. Results suggested that the best species are those with large crowns at maturity and high stomatal conductance e.g. *Fagus sylvatica* and *Acer pseudoplatanus*, while the worst species show little crowns and high emission of volatile organic compounds, e.g. *Prunus* and *Cornus* sp. For instance, an adult plant of *Quercus petraea* shows one of the highest ozone uptake (33.81 g/tree/day) but an even higher potential ozone formation (38.55 g/tree/day). This kind of knowledge will help urban planners and managers of cities with priority issues of air quality in selecting the best species and avoiding the species that are not well suited to these issues.

**Keywords:** Urban forests, urban trees, urban pollution

## **Variable ozone episodes influence on yield and physiology in old and new wheat accessions under a climate change regime with elevated temperature and CO<sub>2</sub>**

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Air pollution effects on vegetation have always been modified by the environment, but in the future crops will be exposed to a, so far, unseen increase in atmospheric carbon dioxide concentration. In addition, other environmental parameters will change e.g. air temperature. These variables will unlikely influence the plant responses to ozone episodes, but the outcome is hard to predict from single factor experiments, since the interaction between parameters is, among other things, depending of timing of ozone episodes and plant development stage. This study, part of the EU Climate Café project, will present the results from a phytotron experiment with three varieties of spring wheat (*Triticum aestivum* L.) varieties of European origins (Lennox, currently used in southern France, KWS Bittern, currently in use in Germany and Denmark and Lantvete, a Swedish landrace) separated into 8 treatments that were grown in 6 different atmospheres. The following environmental conditions were set: Temperature levels (12 /19 °C and 17/ 24 °C night and day resp.), Light level was app. 400  $\mu\text{mol m}^{-2} \text{s}^{-1}$ , day length 16 h, two concentrations of CO<sub>2</sub> (400 and 700 ppm) and three ozone settings: no ozone, episodic ozone and full time ozone. The ozone concentration target in the chambers was 80-100 ppb. We fitted eight climate treatments in the six chambers. We are looking at the impact of ozone and global change under current conditions and that of 2050, as well as the factors of this warming tested separately. Our results show a negative impact of ozone on growth and yields, and the current climate (control) is showing the highest yield. The wheat varieties showed a general response pattern, but with different impact on yield. Warmer temperatures decreases crop yield, and increased CO<sub>2</sub> concentration only partly counterbalance the influence of ozone. Under our experimental conditions, a reduction of about 30% in yields is envisaged between today (2017) and the year 2050.

**Keywords:** Climate change, climate interaction, CO<sub>2</sub> concentration, Ozone, warming, wheat varieties, yield

## Effects of water deficiency and elevated ozone on root traits of three oak species

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In many areas of the world, plants are increasingly subjected to water deficiency in combination with elevated levels of tropospheric ozone. Tree roots are largely neglected in studies of combined effects of both stressors, although their role in water and nutrient absorption is crucial for tree growth and survival. Seedlings of three oak species with different strategies for water use (*Quercus ilex*, *Q. pubescens* and *Q. robur*) were exposed to a combination of water deficiency and ozone stress at a free air ozone exposure facility in Italy. After one growth season, they were destructively sampled. Ectomycorrhizal communities were analysed by morpho-anatomical and molecular tools. Fine roots (< 2 mm) were scanned and analysed with WinRhizo to obtain root morphological parameters. Cross-sections were prepared from 2 mm roots, observed under the light microscope and anatomical features analysed. Fine and coarse roots were dried and weighted to obtain dry weight. Observed effects were root trait specific. Ectomycorrhizal species richness and community composition were significantly affected by combination of ozone and water deficiency in all three species. Due to water deficiency, smaller mean fine root diameter and increased proportion of the thinnest roots (0.0-0.1 mm) were detected in *Q. robur*, while elevated ozone reduced the proportion of the thinnest roots in *Q. pubescens*. Elevated ozone levels resulted in reduced vessel tangential diameter and vessel area in *Q. ilex* and in increased vessel density in all three oak species. Combined effects of ozone and water were detected in bark to secondary xylem ratio. Coarse root biomass was smaller in water deficient seedlings of *Q. pubescens* and *Q. robur*. Observed effects have a potential to affect water, nutrient and carbon cycling in oak forests subjected to combined stressors.

**Keywords:** trees, stress combinations, drought, fine roots, ectomycorrhiza

## Complex responses of volatile organic compound emission to realistic levels of ozone and drought stress in three oak species

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Plants emit biogenic volatile organic compounds (BVOCs) that are important precursors of secondary pollutants such as ozone (O<sub>3</sub>) and organic aerosol. The role of stressors in affecting BVOC emission from plant leaves is still unclear, as both stimulation and inhibition have been recorded. In addition, most of experiments have investigated individual stressors under artificial conditions. Here, we assessed the interactive seasonal effects of O<sub>3</sub> and drought exposure on the BVOC emissions from three oak species showing different O<sub>3</sub> sensitivity, water requirements and BVOC emission patterns. Stomatal regulation types during drought go from isohydric plants – that avoid low leaf water potential by closing stomata - to anisohydric plants – that keep their stomata open and endure lower water potentials. The isohydric isoprene-emitting *Quercus robur* L. is a main component of deciduous broadleaved forests of Europe, and is more O<sub>3</sub>-tolerant than the anisohydric monoterpene-emitting *Q. ilex* L., an evergreen Mediterranean oak. The intermediate isoprene-emitting *Q. pubescens* Willd. is a deciduous oak distributed in Southern Europe. We worked in an ozone FACE (free air controlled exposure) facility under ambient conditions where only O<sub>3</sub> and water availability were manipulated. We successfully tested the following hypotheses: (1) the combined effects of O<sub>3</sub> and drought on BVOCs emission from oaks is additive and not synergistic; (2) these effects are more severe in the most O<sub>3</sub>- and drought-sensitive species; and (3) direction and magnitude of the effects are affected by the time of the growing season.

**Keywords:** BVOC sampling, drought, elevated O<sub>3</sub> monoterpenes emission, O<sub>3</sub> FACE facility, free air controlled exposure, oak species

## Isoprene response to increasing ozone concentrations in Oxford 'O<sub>3</sub>-sensitive' poplar clone grown in enhanced nitrogen and phosphorus soil content

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Tropospheric ozone (O<sub>3</sub>) has been recognized as a significant toxic air pollutant that causes deleterious effects on plants. Such effects may be mitigated by the soil nutritional status. In recent years, nitrogen (N) deposition to forest ecosystems has been rapidly increased. Together with excess of N, phosphorus (P) is considered to be a key limiting factor for tree growth. Isoprene is a major volatile organic compound emitted at high rates by adult and fully expanded poplar leaves and may play an antioxidant role in protecting plants against O<sub>3</sub> stress. However, the interactive effects of O<sub>3</sub>, and enhanced N and P soil content on isoprene emission are still not investigated. Here, for the first time, we have examined both the response of plant performances and isoprene emission in O<sub>3</sub>-sensitive poplar clone (Oxford, *Populus maximoviczii* Henry × *berolinensis* Dippel) grown under various concentration of O<sub>3</sub>, and different amounts of N and P in soil. The experiments were carried out in a last-generation O<sub>3</sub> FACE (Free-Air Controlled Exposure) facility located in Sesto Fiorentino (Florence, Italy). Isoprene emission varied across the growing season. In early summer (July), isoprene emission increased either under exposure to elevated O<sub>3</sub> concentrations (+14% in 1.5 x ambient concentration (1.5AA), +32% in 2.0AA) or by N fertilization (+12%). However, later in the growing season (September-October) the stimulation of isoprene emission due to enhanced N in soil was impaired by the level of O<sub>3</sub> concentration. In fall, isoprene emission declined under increasing O<sub>3</sub> (-60% in 1.5AA, -69% in 2.0AA). Although significant effects of P on the emission rate was not found, P fertilization tended to sustain isoprene emission under 2.0AA. Isoprene emissions and photosynthetic CO<sub>2</sub> assimilation rates declined in parallel in fall. At the same time, O<sub>3</sub> declined the percentage of photosynthetically assimilated carbon that was re-emitted as isoprene. Overall, enhanced N and P amount in soil did not mitigate the inhibition on isoprene emission due to increasing O<sub>3</sub> concentration while increased isoprene emission in early summer and decreased isoprene emission in fall in response to O<sub>3</sub> suggests that the latter induces early senescence.

**Keywords:** poplars, isoprene, ozone, nitrogen, phosphorus

## **Siberian pine and fir decline in the southern Siberian mountains: hypotheses and investigations**

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The mountains of South Siberia are located in the center of the Asian continent and stretches sublatitudinally for three thousand kilometers. Their elevations reach more than 3000 meters. The mountains are the barriers for intercepting moisture-bearing air masses that create favorable conditions for growing of water-loving tree species Siberian pine (*Pinus sibirica* Du Tour) and Siberian fir (*Abies sibirica* Ledeb.). These tree species are shade tolerant and named by darkleaf conifers in Russian geobotanic classifications. From the 1970s scientists observed damage and decline of these darkleaf coniferous forests at middle-to-high elevations. The damage was expressed by necroses of branches with generative organs, by the linear and radial increment decrease, by chloroses, necroses and premature needle falls, the occurrence of stem rot. There exist several hypotheses to explain this phenomenon: - decline of water-loving darkleaf conifers is associated with climate change (aridization). We found from the temperature and rainfall trends for the last 50 years did not change enough across the highlands to cause die-back of Siberian pine and fir; - anthropogenic environmental pollution (various gas emissions, acid rainfall). Specific investigations did not detect increased concentrations of technogenic elements in needles, soils and snow over the mountains; - trees are affected by phytopathogens (*Armillaria mellea*, *Heterobasidion annosum*, etc.); - trees are affected by invasive insect pests. Foresters and ecologists are skeptical about diseases and insect attacks to be the primary causes and consider them as consequences of already weakened trees; - recently, the hypothesis emerged that dark coniferous forest die-backs over the southern Siberian mountains may be associated with the ozone layer depletion caused by tropical volcano eruptions. The increased shortwave ultraviolet radiation (UV-B) that follows the depleted concentration of stratospheric ozone in turn may damage the plant photosynthetic and reproductive apparatus. We hypothesise that the increased ozone concentration in the near-surface air layer may additionally amplify the damage and die-back of the middle-to-high mountain forests.

**Keywords:** forest forming conifer tree species, climate change, ozone concentration, UV-B

## **Potential roles of WRKY transcription factors in regulating oxidative protection and signalling in *Salvia officinalis* plants exposed to ozone**

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Tropospheric ozone (O<sub>3</sub>) is a global air pollutant that causes negative effects on plant metabolism, physiology and growth. Because of its strong oxidative potential, O<sub>3</sub> has a large spectrum of biocidal activities by inducing plant signal molecules that can mediate the stimulation of secondary answers at genetic and metabolic level. The objective of this work is to elucidate the potential roles of WRKY transcription factors in regulating oxidative protection and signalling in *Salvia officinalis* (sage, one of the most well-known aromatic herbs) exposed to a single pulse of O<sub>3</sub> (200 ppb, 5 h day<sup>-1</sup>) in controlled environmental conditions. At the end of the treatment, leaves appeared symptomless. Minute chlorotic spots were recognizable 48 h from the beginning of the exposure and especially on the adaxial leaf surface. To identify WRKY transcriptional factors genes involved in the oxidative burst, BLASTX analysis against the current assembly of the *S. miltiorrhiza* sequence genome was performed using *Arabidopsis* WRKY protein sequences as queries. A total of 14 gene sequences were predicted for sage WRKYs. The relative expression assessment of WRKY genes were analysed by quantitative real-time reverse transcription-PCR and carried out in asymptomatic leaves. Four WRKY were highly induced by O<sub>3</sub> at different time-points. WRKY11 and WRKY46 were significantly up-regulated after 2 h from the beginning of exposure. WRKY4 and WRKY5 have altered expression levels at the end of treatment (about 2-fold higher that controls). Only WRKY23 was significantly down-regulated by O<sub>3</sub>. No significant expression level changes were observed during the recovery period. This result suggests that WRKYs can be important components in the complex signalling processes during O<sub>3</sub> responses. In particular, they could act as redox-responsive sequences and, consequently, as promoter elements specific for O<sub>3</sub> perception/signal transduction. Furthermore, transcriptional and metabolic relationships will be elucidated.

**Keywords:** Oxidative stress, sage, signalling molecules, lipoxygenase activity, reactive oxygen species, phytohormones, antioxidant response

## Photosynthetic performance, oxidative injury and growth of pomegranate plants under salt and ozone stress

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Plants are exposed to a broad range of environmental stresses under natural conditions including ozone (O<sub>3</sub>) and salinity. The combinations of more stressors are common and may have strong impact. The progressive salinization of irrigated lands can limit crop production in the Mediterranean basin. One-year-old plants of *Punica granatum* L. cv. Dente di cavallo were exposed to two levels of O<sub>3</sub> (1.0 and 2.0 times the ambient O<sub>3</sub> concentration: AA and 2.0 × AA) and two levels of salinity (0 and 50 mM NaCl) for three consecutive months in an O<sub>3</sub> free air controlled exposure facility. The aim of the work was to evaluate the cross-talking mechanisms between the two stressors. At the end of the experiment, plants showed leaf yellowing (i.e. typical salt-induced symptoms) and developed visible stipples of browning tissue localized in the interveinal adaxial leaf area (i.e. typical O<sub>3</sub>-induced symptoms). Leaf and root dry weight decreased in plants exposed to 2.0 × AA (-15 and -23% in comparison to AA, respectively) and subjected to salt stress (-2 and -10%, respectively). The effects induced by O<sub>3</sub> were twinned with the alteration of the potential PSII photochemical efficiency (-12%), the production of reactive oxygen species (ROS) [hydrogen peroxide concentrations (H<sub>2</sub>O<sub>2</sub>) were 2-fold higher than AA] and the accumulation of proline (Pro, +46%). Similarly, salinity induced a reduction of the photochemical efficiency of PSII in light conditions (-10%), an oxidative burst (as confirmed by the increase of H<sub>2</sub>O<sub>2</sub>, +5%) and a rise of Pro (+41%). However, the combination of both stressors determined an opposite effect including minor propagation of ROS, lesser modulation of Pro and slight impairment of PSII (-12, -7 and -3%, respectively). The possible “antagonistic” interactions between O<sub>3</sub> and salt will be elucidated.

**Keywords:** O<sub>3</sub>, salinity, *Punica granatum*, reactive oxygen species, photosynthesis, proline, symptoms

**P30**



## What we can't see in the urban forest ecosystem: greenhouse gases or its precursors

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Increasing urban trees and green areas is becoming a greater priority for all boroughs within cities and towns throughout Chile. Our team is working with a group of native and exotic trees to provide scientific insight to support management decisions, particularly into greenhouse gases (CO<sub>2</sub>, H<sub>2</sub>O, CH<sub>4</sub>, O<sub>3</sub>, N<sub>2</sub>O), gas exchange in plants (CO<sub>2</sub> and H<sub>2</sub>O), volatile organic compounds (VOCs) as precursors of O<sub>3</sub>, and particulate matter retention by plants. First results show that the use of perennial native trees as opposed to deciduous exotic fast growing trees in cities of Central Chile may be highly beneficial. First of all, as water use efficiency, or the moles of H<sub>2</sub>O released to the atmosphere compared to the moles of CO<sub>2</sub> uptake by the photosynthesis, is relatively stable for C<sub>3</sub> plants at about 300-500 mol H<sub>2</sub>O per mol<sup>-1</sup> CO<sub>2</sub>, fast growing exotic plants would consume large amounts of water compared to the more conservative native trees, in an environment of climate change. Second, deciduous exotic trees would lose all their foliage through autumn and winter when particulate matter (PM), is at its peak, so that they will be incapable of intercepting any of the PM while native evergreen trees will do the opposite. Third, we have shown that various exotic deciduous trees would emit larger quantities of VOCs producing greater amounts of tropospheric O<sub>3</sub> with known effects on human and plant health. Native and exotic studied trees generally emit small amounts of CH<sub>4</sub> in spring and summer, the day around double the night, while urban soils would sequester CH<sub>4</sub>, although the CH<sub>4</sub> balance in these ecosystems is still under study. A better knowing of the pros and cons of each tree species within the city, provided by scientific research, will better contribute decision makers to carry out better and sounder urban planning.

**Keywords:** Greenhouse gases, gas exchange in plants, particulate matter, volatile organic compounds (VOCs), urban trees and green areas, Chile

## **Biomonitoring of anthropogenic volatile organic compounds in a urban arboreal species. Santiago de Chile**

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Volatile organic compounds (VOCs) are chemical species that actively participate in the formation of photochemical oxidants like as tropospheric ozone and secondary aerosols (SOA). In the Metropolitan Region, Chile, anthropogenic VOCs, represent the third largest contribution in gaseous emissions; it is reported that their toxic or mutagenic characteristics cause a serious damage to human health and decrease the growth and development of plant species. Urban green areas play an important role in the quality of life of the population and within the urban ecology they deliver varied benefits. On the other hand, there is little international information related to the possibility of capturing atmospheric pollutants through the leaves of trees exposed to defined sources of pollution, specifically for anthropogenic VOCs; in Chile, there is no information. The purpose of this work is to study the impact of anthropogenic VOCs emitted by the distribution of fuels. A petrol distribution service station located in the commune of Vitacura was selected, the area where the official monitoring stations of the Region report the highest concentrations of ambient tropospheric ozone; samples of *Platanus orientalis* were collected at different distances from the gas distribution center during austral spring (November) and summer (March). Samples were pulverized with a cryogenic mill; certain amount was weighed for the analysis (triplicate) to take it to a vial of headspace; the remaining material was stored as a counter sample. Determination and quantification of each chemical species were done through the use of analytical equipment GC / MSD. Preliminarily, it was possible to identify chemical species belonging to Organic Range of Gasoline (GRO: range 6-10C), specifically benzene, toluene, xylenes, ethylbenzene and other chemical species that need to be confirmed, in leaves of individual.

## Ozone and climate change impacts on Southern European forests: MITIMPACT project concept

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Climate change and air pollution are two significant stressors affecting forest health and vitality of European forests. Mediterranean area has been identified as one of the most prominent "Hot-Spots" in future climate change projections and is seriously affected by air pollution, in particular ozone (O<sub>3</sub>). The MITIMPACT ALCOTRA project aims to quantify ozone impacts on 42 forest test sites distributed in South East France and Northwest Italy, by evaluating ecosystem health (e.g. crown defoliation and visible foliar injury) and ecosystem services, as well as their vulnerability at medium (year 2035) and long term (year 2055, 2085). The project area can be considered as a case study for the assessment of global change impacts in Mediterranean forests. Based on dose-response functions, new appropriate thresholds to protect Mediterranean forest against the negative effect of O<sub>3</sub> will be recommended. In particular, the influence of soil water availability on the effective dose of O<sub>3</sub> entering into the stomata, the so called Phytotoxic Ozone Dose with a threshold Y (POD<sub>y</sub>) will be estimated. Moreover, O<sub>3</sub>-related forest damages will be economically assessed and mitigation strategies will be identified on the basis of cost-benefit analysis. This innovative aspect of the project can be particularly useful to provide cost-effective measures for forest management in preparation to future climate conditions.

**Keywords:** Ozone, Mediterranean forest ecosystem, soil water limitation, ecosystem health indicators, mitigation strategies, POD<sub>y</sub>, climate scenarios

## **The importance of soil water availability on stomatal conductance regulation: implications for tropospheric ozone**

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Soil moisture and water stress play a pivotal role in regulating stomatal behaviour of plants; however, in the last decade, the role of water availability was often neglected in atmospheric chemistry modelling studies as well as in integrated risk assessments, despite through stomata plants remove a large amount of atmospheric compounds from the lower troposphere. The study aims to evaluate the effects of soil water limitation on stomatal conductance and to assess the resulting changes in atmospheric chemistry testing various hypotheses of water uptake by plants in the rooting zone. We use a multi-model system to reproduce the meteorological conditions and the concentration of gases in the troposphere, specifically the WRF (Weather Research and Forecast Model) regional meteorological model and the CHIMERE chemistry-transport model. The inclusion of soil water stress leads to a reduction of stomatal conductance and thus, the amount of ozone removed by dry deposition is lower. As an example, the amount of ozone removed by dry deposition in one year without considering any soil water limitation to stomatal conductance is about 8.5 TgO<sub>3</sub>, while considering a dynamic layer that ensures plants to maximize the water uptake from soil, we found a reduction of about 10% (7.7 TgO<sub>3</sub>), affecting, the concentration of gases remaining into the lower atmosphere. Our results highlight the importance of improving the parameterizations of processes occurring at plant level (i.e. from the soil to the canopy) as they have significant implications on concentration of gases in the lower troposphere and consequently on risk assessment studies.

**Keywords:** Ozone, soil water limitation, dry deposition, CTM

## Isoprene contribution to ozone production in a context of climate change in a *Quercus pubescens* forest

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Tropospheric ozone is a strong pollutant, which can affect human health, agricultural yields as well as ecosystems functioning. This compound can come from the reaction between isoprene, the most emitted Biogenic Volatile Organic Compound (BVOC), and nitrogen oxides (NO<sub>x</sub>) coming from anthropogenic emissions. For instance, it has been estimated that between 16 and 20% of ozone comes from isoprene emissions in Mediterranean region. This proportion could change in the future with climate change since it has been demonstrated that isoprene emissions can increase, decrease or remains unchanged according to drought. Moreover, there is still a lack of knowledge about the effect of a recurrent drought (applied during several years) on isoprene emissions. In this study, we wanted to evaluate the impact of climate change, expected in Mediterranean region, on isoprene emissions as well as on ozone concentration. Isoprene emissions factors of *Quercus pubescens* were measured, at the branch level, during the first and the third year of a simulated amplified drought expected with climate change (exclusion of 30% of natural rain). An increase of isoprene emissions was observed after the first year whereas a decrease was highlighted after 3 years of drought. Then, CHIMERE model was used to forecast ozone concentration according to three scenarios: a REF scenario (based on summer 2003 known to be extreme in terms of temperature), a Short Drought scenario (after 1 year of drought, +83% isoprene emissions) and a Recurrent Drought scenario (after 3 years of drought, -26% isoprene emissions). Our results showed that short term involved an increase of ozone concentration up to 28.8 µg.m<sup>-3</sup> compared to REF scenario whereas, with recurrent drought scenario, a decrease up to 10.1 µg.m<sup>-3</sup> was observed. Our results indicate that more than drought itself, the duration is an important factor to take into account for ozone modelling.

**Keywords:** recurrent drought, climate change, isoprene emissions, ozone

## Ozone exposure- and flux-based response relationships with photosynthesis, leaf morphology and biomass in two poplar clones

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Poplar clones 546 (*P. deltoides* cv. '55/56' × *P. deltoides* cv. 'Imperial') and 107 (*P. euramericana* cv. '74/76') were exposed to five ozone concentrations in 15 open-top chambers (OTCs). Both ozone exposure (AOT40, accumulation over a threshold hourly ozone concentration of 40 ppb) and flux-based (POD7, Phytotoxic Ozone Dose above an hourly flux threshold of 7 nmol O<sub>3</sub> m<sup>-2</sup> PLA s<sup>-1</sup>) response relationships were established with photosynthesis, leaf morphology and biomass variables. Increases in both metrics showed significant negative relationships with light-saturated photosynthesis rate, chlorophyll content, leaf mass per area, actual photochemical efficiency of PSII in the light and root biomass but not with stomatal conductance (*g<sub>s</sub>*), leaf and stem biomass. Ozone had a greater impact on belowground than on aboveground biomass. The ranking of these indicators from higher to lower sensitivity to ozone was: photosynthetic parameters, morphological index, and biomass. Clone 546 had a higher sensitivity to ozone than clone 107. The coefficients of determination (R<sup>2</sup>) were similar between exposure- and flux-based dose-response relationships for each variable. The critical levels (CLs) for a 5% reduction in total biomass for the two poplar clones were 14.8 ppm h for AOT40 and 9.8 mmol O<sub>3</sub> m<sup>-2</sup> PLA (projected leaf area) for POD7. In comparison, equivalent reduction occurred at much values in photosynthetic parameters (4 ppm h for AOT40 and 3 mmol O<sub>3</sub> m<sup>-2</sup> PLA for POD7) and LMA (5.8 ppm h for AOT40 and 4 mmol O<sub>3</sub> m<sup>-2</sup> PLA for POD7). While in recent decades different CLs have been proposed for several plant receptors especially in Europe, studies focusing on both flux-based dose-response relationships and CLs are still scarce in Asia. This study is therefore valuable for regional O<sub>3</sub> risk assessment in Asia.

**Keywords:** Ozone, Dose-response relationships, Sensitivity, Critical level, Poplar

## **ICP Vegetation smart-phone App for recording incidences of ozone injury on vegetation**

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Exposure of vegetation to ozone pollution can cause visible injury on the leaf surface of ozone-sensitive species. In 2007, the ICP Vegetation published a report documenting over 500 incidences of visible ozone injury on crops, grassland species and shrubs growing in the field under ambient air conditions in 17 countries of Europe (Hayes *et al.*, 2007). Field-based evidence is needed to verify predictions from experiments and to validate risk maps. It also demonstrates the negative impact of ambient ozone to policy makers. To build upon the evidence gathered in the 2007 report, we have developed an ozone smart-phone App, with the aim of creating a global database of injury records. The App, suitable for use on iPhones, Android or Windows phones, allows participants to upload photographs of ozone injury and the coordinates of the location where the injury was detected. There is also an online recording form, for those without a smart-phone. Users are asked a series of questions designed to assist with quality assurance, including details on their previous experience of identifying ozone damage and recent weather conditions. For guidance, the App also contains an 'Ozone Information' section, which provides details of the key visible injury symptoms and example photographs for a variety of different species. Since its development in 2014, we have received injury records from Europe, the USA and Asia for 20 different species. We are keen for many more people to take part to increase the number of records we receive from around the world. One way to collect evidence and report it via the App is to expose sensitive ozone species (SOS) to ambient air, and upload the injury photos. Information on how to download the App and a method for exposing sensitive ozone species are available on the ICP Vegetation website: <http://icpvegetation.ceh.ac.uk/record/index>.

**Keywords:** Smart-phone App; visible ozone injury; ICP Vegetation; field evidence; sensitive ozone species (SOS)

### **Reference**

Hayes, F., Mills, G., Harmens, H., Norris, D. (2007) Evidence of widespread ozone damage to vegetation in Europe (1990 – 2006). Programme Coordination Centre of the ICP Vegetation, Centre for Ecology and Hydrology, Bangor, UK. ISBN 978-0-9557672-1-0.

## **Modelling BVOC potential emission from energy crops – a case study in Brandenburg, Germany**

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Biogenic volatile organic compounds (BVOCs) emitted from vegetation have significant effects on the atmosphere, playing a primary role in formation of tropospheric ozone. As BVOC emissions vary with species, the relative abundance of different crops in the landscape is a major factor determining the contribution of agriculture to ozone pollution. The global mandate of developing renewable energy is promoting widespread adoption of bioenergy crops. For some of them, recent experimental work made available data for calibration of BVOC emission models at the field scale. On these bases, we upscale here –by means of process-based simulation– potential BVOC emissions from maize and rapeseed in the German state of Brandenburg, where such emissions are dominated by agriculture. The crop model MONICA was extended to simulate monoterpene and isoprene emissions from the canopy, according to alternate approaches differing in structure and mechanisms. The model was calibrated using data from field experiments carried out in Dedelow, NE Germany, where BVOC fluxes from maize and rapeseed were monitored during the growing seasons 2015-2016. The model is then applied over the entire state of Brandenburg in the period 1995-2012. Each crop is simulated as monoculture over a 1 km<sup>2</sup> resolution grid covering the study area. The ultimate mechanistic description of the processes underlying the emission of BVOCs from vegetation is yet to come, as their physiological controls remain partially unresolved. However, this does not prevent emission models to achieve acceptable performance. The coupling with a full-blown crop model allows for the dynamic quantification of factors that influence patterns in BVOC emissions, such as the amount of photosynthetically active radiation reaching the leaf surface, phenological development and possible stressors the crop is exposed to. At the scale of the study area, this approach lays the foundations for BVOC emissions budgets from established energy crops.

**Keywords:** BVOC, energy crops, simulation models, MONICA

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## Effects of ozone on two larch species treated with ammonium sulfate or salt loading

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The ground-level ozone (O<sub>3</sub>) concentration has been increasing especially in Northeast Asian region. Elevated O<sub>3</sub> reduces growth and suppresses many physiological processes. In addition, the amount of nitrogen (N) deposition has been increased in Asian region. Effects of N deposition, especially N loading on soil, can promote tree growth and physiological activities in short-term but the excess deposition could also cause nutrient imbalances and acidification in soil i.e. N saturation. Much worse, soil salinization area has been increased due to global warming, especially low precipitation region in the northeastern China. At the wide area of soil salinization in Northeast China, they may employ larch species for making forest plantation. Therefore, we studied on effects of elevated O<sub>3</sub> on larch species grown under high N loading and salinity soil. Here, we investigated on two larch species (Japanese : larch *L. kaempferi*, hybrid larch F<sub>1</sub> : *L. gmelinii* var. *japonica* x *L. kaempferi*). Japanese larch (*L. kaempferi*) has been major afforestation species in Northeast Asian because its high growth rate and adaptability to harsh conditions. However, it has been suffering from biotic stresses, e.g. grazing by voles. To overcome difficulties, we developed hybrid larch F<sub>1</sub> (*L. gmelinii* var. *japonica* x *L. kaempferi*). These seedlings, which were exposed to elevated O<sub>3</sub> [60 nmol O<sub>3</sub> mol<sup>-1</sup>] in open-top chambers (OTCs), were treated with ammonium sulfate [50 kgN ha<sup>-1</sup> yr<sup>-1</sup>] or salt [NaHCO<sub>3</sub> and NaCl] loading. We mainly discuss on the responses the growth of them for the future afforestation.

**Keywords:** Combination effect, Ground level O<sub>3</sub>, Nitrogen deposition, Salinity, Species difference, Larch

## **Effects of elevated ozone concentration on CH<sub>4</sub> and N<sub>2</sub>O emission from paddy soil with two Chinese rice cultivars under fully open-air field conditions**

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We investigated the effects of elevated ozone concentration (E-O<sub>3</sub>) on CH<sub>4</sub> and N<sub>2</sub>O emission from paddies with two rice cultivars (inbred Yangdao 6 (YD6) and hybrid II-you 084 (IY084)) under fully open-air field conditions in China. A mean 26.7% enhancement above the ambient ozone concentration (A-O<sub>3</sub>, 33.7 ppb) significantly reduced the whole-plant biomass by 13.2%, root biomass by 34.7%, dissolved organic carbon in soil solution by 12.3% and CH<sub>4</sub> emission by 29.6%, on average of the two cultivars. Significant varietal difference in CH<sub>4</sub> emission response to E-O<sub>3</sub> was detected in tiller stage because E-O<sub>3</sub> significantly reduced the maximum tiller number of IY084 (-16.5%, P = 0.0311) but that effect was not significant with YD6 (-4.1%, P = 0.2231). Additionally, we found E-O<sub>3</sub> reduced seasonal mean NO<sub>x</sub> flux by 5.7% and 11.8% with IY084 and YD6, respectively, but the effects were not significant in statistical. Our field study has thus supported the findings in controlled-environment chambers that increasing ozone concentration might mitigate the global warming potential of CH<sub>4</sub>, and indicated that the response of tiller number to E-O<sub>3</sub> should be considered in selecting rice cultivars for mitigating CH<sub>4</sub> emissions from paddy soils.

**Keywords:** CH<sub>4</sub> emission, N<sub>2</sub>O emission, elevated ozone, rice cultivar, biomass, paddy soil, FACE

## Diurnal and phenological variations of O<sub>3</sub> and CO<sub>2</sub> fluxes of winter wheat canopy under short-term O<sub>3</sub> exposure

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A dynamic chamber system was designed to measure simultaneously the diurnal and phenological canopy ozone (O<sub>3</sub>) and carbon dioxide (CO<sub>2</sub>) fluxes in the winter wheat field under different O<sub>3</sub> concentrations (0, 40, 80 and 120 nmol mol<sup>-1</sup>). On the diurnal timescale, canopy fluxes usually peaked around noon in early growing stages, while a generally decreasing trend from morning to afternoon was observed in the later stages. O<sub>3</sub> and CO<sub>2</sub> fluxes were positively and negatively correlated with O<sub>3</sub> concentration, respectively. Significant differences were observed in O<sub>3</sub> fluxes but CO<sub>2</sub> fluxes among O<sub>3</sub> treatments. On the phenological timescale, both O<sub>3</sub> and CO<sub>2</sub> fluxes followed the variation of leaf area index (LAI) with the maximum occurring simultaneously at the booting stage. The daytime mean fluxes varied from 10.6 to 17.2 nmol m<sup>-2</sup> s<sup>-1</sup> for O<sub>3</sub> and from 5.9 to 19.6 μmol m<sup>-2</sup> s<sup>-1</sup> for CO<sub>2</sub>. Quantitatively important O<sub>3</sub> deposition (3.1~11.6 nmol m<sup>-2</sup> s<sup>-1</sup>) was also observed at night with the ratios being about 40~70 % relative to the daytime O<sub>3</sub> fluxes for most measuring days, which indicates a significant contribution from non-stomatal components to canopy O<sub>3</sub> removal.

**Keywords:** chamber system; ozone; carbon dioxide; flux; phenology; *Triticum aestivum*

## Effect of ozone and/or EDU on Pinto bean plants' metabolism

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Ambient ozone (O<sub>3</sub>) is considered one of the most widespread phytotoxic air pollutants across the globe. Its levels in the atmosphere are continuously increasing, threatening natural and agricultural ecosystems. It is well documented that ozone enters through stomata, affects plants' metabolism, causes oxidative stress and foliar injury and finally yield reduction. Ethylenediurea (EDU) prevents acute and chronic injury due to ozone stress in various plant species; however, its exact mechanism of action has not been elucidated yet. Metabolomics is a new approach on systems biology, which studies the changes of the intermediates and the final products of metabolism. Here, effects of O<sub>3</sub>, EDU and O<sub>3</sub> x EDU interaction on plants' metabolism were investigated. To this purpose, three week-old plants, of the sensitive to ozone bean (*Phaseolus vulgaris* L.) cultivar Pinto were treated with EDU or water and, after 24 hours, were exposed either to ozone (70 ppb) or to charcoal filtered air for 4 hours. Samples of leaf tissues were taken from the first trifoliolate (fully expanded) leaf of the plants on the end of the exposure. The experiment was conducted in controlled environment chambers under laboratory conditions. Gas chromatography-mass spectrometry (GC/EI/MS) metabolomic analysis was performed. The data were compared to databases (KEGG, NIST) for compounds identification. The findings were further submitted to multifactorial analysis (OPLS-DA). Metabolomic analysis detected 179 metabolic characters, of which 149 were identified compounds (this, in terms of concentrations, corresponds to 95% of the total metabolic amount). Major plant biosynthetic pathways were either up or down-regulated in response to O<sub>3</sub>, EDU and O<sub>3</sub> x EDU. The concentration of many important bioactive metabolites, such as compounds with antioxidant role (GABA, 4-coumarate, etc.) or signal transduction molecules (ethylene, etc.) were affected. The major important results will be extensively presented.

**Keywords:** Phaseolus, GABA, 4-Coumarate, ethylene

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## **Penconazole: A potential ozone protectant of plants? A metabolomics approach**

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Several agrochemicals have been tested as antiozonants and some of them have been found to offer, as a side effect, a kind of protection to plants against ozone phytotoxicity. The near ground level ozone, is gradually increasing over the decades, and is known to cause adverse effects in plants as a result of metabolic disturbances. Metabolomics is among the innovative methods used, nowadays, in studies dealing with the influence of xenobiotics in plants. In this study, we conducted a metabolomics analysis, in order to investigate the potential protective effects of Penconazole against O<sub>3</sub> in plants. To this purpose, seeds, of the sensitive to ozone *Phaseolus vulgaris* L. cv Pinto, were planted in pots and placed in two walk-in chambers, fed with charcoal filtered air, under controlled environment conditions (T: 27 °C, RH: 65-70%, Photoperiod: 14L/10D). When they were three weeks' old, the plants of each chamber, were sprayed either with Penconazole or with water. After 24h, the plants of the one chamber were fumigated by ozone (35 µg/m<sup>3</sup> x 4 hours), while the plants of the other chamber served as control. At the mid (2h) and at the end (4h) of the exposure, samples of leaf tissues were taken from the first trifoliate (fully expanded) leaf of the plants, from both chambers. Samples were submitted to gas chromatography-mass spectrometry (GC/EI/MS) for metabolomic analysis. Bioninformatic tools were used to identify the given chromatographs. Multivariate analysis of metabolic profiles resulted completely distinct groups, differentiating the O<sub>3</sub> and Penconazole treated plants from their control counterparts. The analysis revealed significant main and interaction effects of both (ozone and Penconazole) factors. Some biosynthetic pathways were either up or down-regulated while other remained undisturbed. Complex and contrasting effects were also observed in the concentrations of bioactive metabolites (like linoleate, GABA, L-ornithine, trehalose, ethylene, etc.).

**Keywords:** Phaseolus, GABA, trehalose, ethylene, linoleate

## Effects of elevated tropospheric ozone and N fertilisation on greenhouse gas emission and net ecosystem carbon exchange in British grasslands

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Rising ozone (O<sub>3</sub>) concentration and nitrogen (N) fertilisation can affect plant growth differently, whereas their effects on soil greenhouse gas emission and ecosystem carbon dynamics remain uncertain. Here, we investigated effects of elevated tropospheric O<sub>3</sub> (e[O<sub>3</sub>]) and N fertilisation on CH<sub>4</sub> and N<sub>2</sub>O emission from the lowland and upland grasslands under fully open-air field conditions in North Wales, UK. For each grassland site, intact soil cores were collected and placed inside the rings of O<sub>3</sub> exposure. A two-level factorial experiment with three replicate was established. From May 2017 to this April, we measured the fluxes of soil CH<sub>4</sub> and N<sub>2</sub>O emission. Using the static-chamber method, we measured net ecosystem carbon exchange (NEE) and ecosystem respiration ( $R_{\text{eco}}$ ) across the growing season. Soil moisture, soil temperature, and solar radiation were monitored simultaneously. Our preliminary results showed that CH<sub>4</sub> uptake rates were significantly higher in the upland grassland ( $-10.11$  to  $-3.21 \mu\text{g C m}^{-2} \text{h}^{-1}$ ) than in the lowland grassland ( $-20.82$  to  $-16.78 \mu\text{g C m}^{-2} \text{h}^{-1}$ ;  $P < 0.001$ ), whereas it was not affected by either e[O<sub>3</sub>] or N addition. Similar to N addition ( $P = 0.024$ ), e[O<sub>3</sub>] significantly stimulated soil N<sub>2</sub>O emission ( $P = 0.0013$ ), especially for the lowland grassland with higher flux rates ( $1.23$  to  $8.89 \mu\text{g N m}^{-2} \text{h}^{-1}$ ) than that from the upland grassland ( $0.81$  to  $5.48 \mu\text{g N m}^{-2} \text{h}^{-1}$ ).  $R_{\text{eco}}$  was significantly increased by an average of 19% under N addition, and there was a marginal interaction between e[O<sub>3</sub>] and N addition ( $P = 0.089$ ), especially for the lowland grassland. Across all treatments, N addition significantly increased the aboveground biomass of both grasslands by c. 40%, despite the absence of effects of e[O<sub>3</sub>] and grassland type. Taken together, our current results that rising tropospheric [O<sub>3</sub>] may have negative impacts on soil N<sub>2</sub>O emission and ecosystem carbon balance.

**Keywords:** semi-natural grassland; elevated ground-level ozone; greenhouse gases; fertiliser management; soil respiration; GEP

## Relationships of CO<sub>2</sub> assimilation rates with exposure- and flux-based O<sub>3</sub> metrics in three urban tree species

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The relationships of CO<sub>2</sub> assimilation under saturated-light conditions ( $A_{\text{sat}}$ ) with exposure- (AOTX, Accumulated Ozone exposure over a hourly Threshold of X ppb) and flux-based (POD<sub>Y</sub>, Phytotoxic Ozone Dose over a hourly threshold Y nmol. m<sup>-2</sup>. s<sup>-1</sup>) O<sub>3</sub> metrics was studied on three common urban trees, *Fraxinus chinensis* (FC), *Platanus orientalis* (PO) and *Robinia pseudoacacia* (RP). Parameterizations for a stomatal multiplicative model were proposed for the three species. RP was the species showing lower species-specific maximum stomatal conductance ( $g_{\text{max}}$ ) and experiencing lower cumulative O<sub>3</sub> uptake along the experiment, but in contrast it was the most sensitive to O<sub>3</sub>. POD<sub>Y</sub> was slightly better than AOTX metric at estimating R- $A_{\text{sat}}$  changes for PO and RB but not for FC. The best fittings obtained for the regressions between relative  $A_{\text{sat}}$  (R- $A_{\text{sat}}$ ) and AOTX for FC, PO and RP were 0.904, 0.868, and 0.876, when the thresholds of X were 60 ppb, 55 ppb and 30 ppb, respectively. However, AOT40 performed also well for all of them, with R<sup>2</sup> always >0.83. For POD<sub>Y</sub>, the highest R<sup>2</sup> values for FC, PO and RB were 0.863, 0.897 and 0.911 at thresholds Y=7, 5 and 1, respectively. Given the potentially higher O<sub>3</sub> removal capacity of FC and PO by stomatal uptake and their lower sensitivity to this pollutant with regard to RP, the former two species would be appropriate for urban gardens and areas where O<sub>3</sub> levels are high. Parameterization and modeling of stomatal conductance for the main urban tree species may provide reliable estimations of the stomatal uptake of O<sub>3</sub> and other gaseous pollutants by vegetation, which may support decision making on the most suitable species for green urban planning in polluted areas.

**Keywords:** Ozone, Photosynthesis, Flux-response relationships, China, urban trees

## Ozone pollution impacts on crops and forests in China

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Global mean tropospheric ozone concentration has been predicted to double over the rest of this century, predominantly due to emissions associated with fossil fuel and biomass burning. Tropospheric ozone is known to have detrimental effects on carbon uptake by land ecosystems through damaging photosynthesis, thereby reducing plant growth and biomass accumulation, limiting crop yields, and affecting stomatal control over plant transportation of water vapour between the leaf surface and atmosphere. The current and future food production in China is highly vulnerable to high levels of ozone concentration; however, it remains unclear how the different plant functional types (PFTs) and crops respond to increased concentrations of ozone for different regions only based on a single ozone flux (e.g. stomatal conductance). The UK Met Office land surface JULES-crop model represents a set of 9 PFTs characterised by leaf traits, including Leaf Mass Area (LMA), and a marginal cost of water-use parameter. Linking ozone sensitivity to LMA provides a powerful unifying explanation for variation in plant ozone sensitivity, and make the modelling of ozone plant damage at the large-scale become possible. In this work, results will be presented based on JULES-crop for China using a resolution up to 0.5 degrees and calibrated and validated with multiple measurement datasets (FACE etc.). Results will be presented illustrating the effect of predicted increased ozone levels (e.g. LMA-ozone) on net primary productivity (NPP) and main crop (Rice, Maize, Wheat, and Soybean) yield for China. An estimation of ozone effect on PFTs and crop yield under future scenarios and present day will also be shown.

**Keywords:** JULES-crop, PFTs, NPP, crop yield, China



## Effects of nutrient availability on O<sub>3</sub>-caused damage of poplar Oxford clone

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Ozone pollution is often associated with nitrogen (N) deposition because nitrogen oxides (NO<sub>x</sub>) are the main O<sub>3</sub> precursor. In addition, N deposition may limit the availability of phosphorus (P) through soil acidification. However, to our knowledge, there are only few reports about the impacts of N and P availability individually or in combination on the O<sub>3</sub>-caused damage of forest species. In the present study, the potential impacts of N and/or P enrichment on poplar Oxford clone under different O<sub>3</sub> concentrations in a free air controlled exposure (FACE) system were investigated. Compared to AA (ambient O<sub>3</sub> concentration), O<sub>3</sub> decreased total annual biomass by 18% in 1.5xAA and 23% in 2.0xAA. Nitrogen enrichment changed the response of total annual biomass to O<sub>3</sub>, but P addition did not. The O<sub>3</sub>×N×P interaction on biomass was significant indicated that the combination of N and P enrichment affected the O<sub>3</sub>-caused biomass loss. Ozone decreased the leaf amount and increased the number of leaf trace. When P levels were low and medium, N addition mitigated the O<sub>3</sub>-caused leaf loss, but it was aggravated by N enrichment under high P. In August, plant injury index (PII) was much higher in 1.5xAA and 2.0xAA than in AA. Under 2.0xAA, the addition of N decreased PII. During the same period, net photosynthetic rate was decreased by elevated O<sub>3</sub> and the relative loss in 1.5 xAA could be alleviated by N addition. At the end of the growth season, Fv/Fm and performance index (PI) were reduced by 2.0xAA, while the addition of N or P singly mitigated the relative loss. In summary, nutrient availability and O<sub>3</sub> are interrelated to change the visible injury, phenology, photosynthesis and biomass. The imbalance of N and P should be emphasized for forest protection under O<sub>3</sub> pollution.

**Keywords:** biomass, nitrogen, ozone, phenology, phosphorus, photosynthesis, visible injury

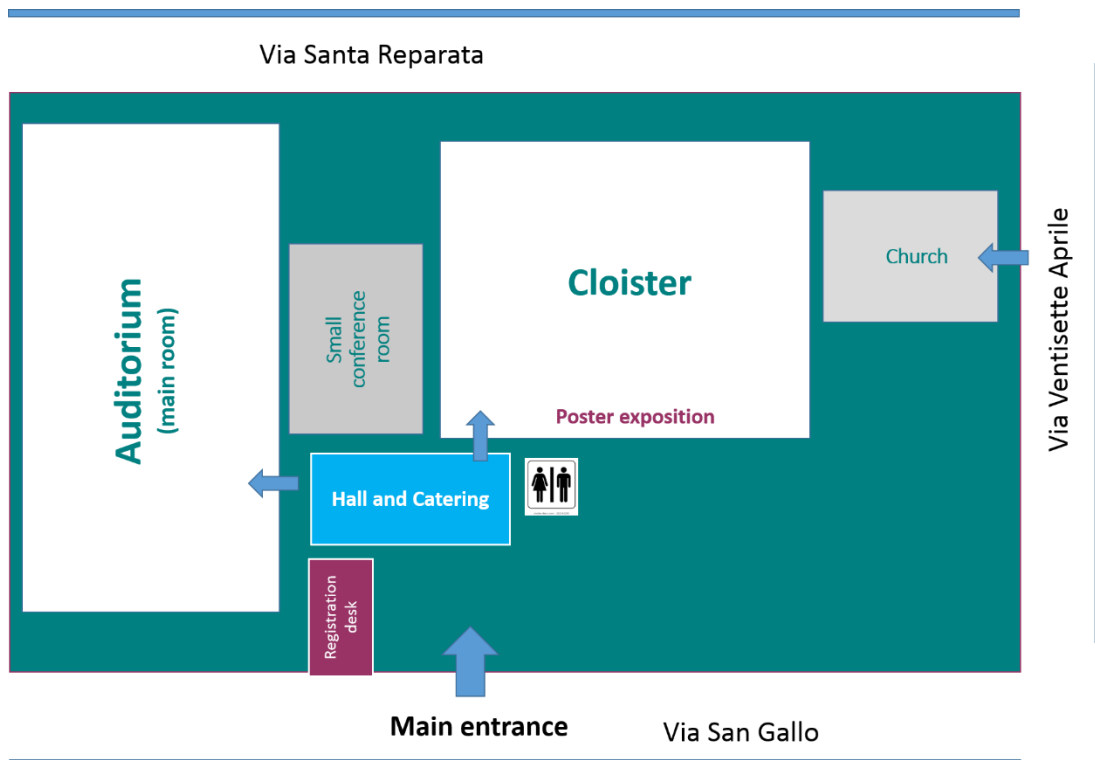


## 6. Maps and useful tips

### Auditorium of Sant'Apollonia – The Conference Venue

The Auditorium of Sant'Apollonia is located in Via San Gallo 25, Firenze. It is about 15 minutes walking distance from the main train station of Firenze S.M.N. (Santa Maria Novella).

The Auditorium is a portion of the monastic complex of the Church of Sant'Apollonia. Sant'Apollonia was a former Benedictine convent, founded in 1339. The structures of the convent, suppressed since the 19<sup>th</sup> century, are now put to different uses, e.g. event venue with two rooms with 200 (Auditorium) and 40 (small conference room) seats.



The small church building is still present on the corner of Via Ventisette Aprile and San Gallo. The best known component is the former refectory or dining hall of the convent, the Cenacolo of Sant'Apollonia now part of the Museums of the Comune of Florence, with entrance through a nondescript door near the corner of Via Ventisette Aprile and Reparata. The refectory (1445) harbors the well-conserved fresco, The Last Supper by the Italian Renaissance artist Andrea del Castagno. Castagno created the rich marble panels that checkerboard the trompe-l'oeil walls and broke up the long white tablecloth with the dark figure of Judas the Betrayer, whose face is painted to resemble a satyr, an ancient symbol of evil.



*The Last Supper by Andrea del Castagno*

### Palazzo Budini-Gattai – Venue of the Social Dinner

Budini Gattai Palace, formerly Grifoni Palace, was built by Baccio d'Agnolo and Bartolomeo Ammannati, in 1563, 16th century.

Ugolino di Iacopo Grifoni, secretary to Duke Cosimo de' Medici, purchased a number of houses in the area close to Piazza Santissima Annunziata and via de' Servi. His intention was to demolish them and construct a *palazzo* (palace) that would bring honor to his family. Baccio d'Agnolo, a student of Michelangelo, started the project, but it was completed by Bartolomeo Ammannati, who was likely the architect of the garden as well. The Italian garden was created around 1573, as a monumental fountain with statues of Giasone, Venus and sea monsters attributed to sculptor Giovanni Bandini. The garden was enlarged in the 18<sup>th</sup> century and the fountain moved and substituted with a walled fountain where the statue of Venus was housed. The Grifoni family died out in the 18<sup>th</sup> century and the property passed into the Riccardi family before going to the Budini Gattai family, to whom it still belongs today. The current garden, further modified in the late 1800s boasts curved flowerbeds and beautiful collections of camellias and azaleas. Other decorative elements of the garden include a glass and iron greenhouse dating back to 1892; a banana grove; and the "Monument to the lost tree" completed in 1908 in memory of an ancient *Cinnamomum camphora*

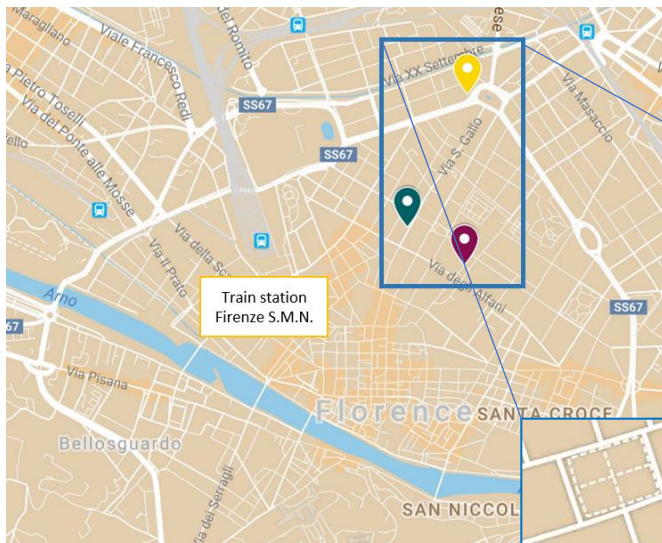
#### *A story of lovers and ghosts*

*The far right window on the second floor of the Palazzo has been open for centuries. One of the legends says that at the end of 1500, a son of the Grifoni family was called off to war and had to leave his young bride. The wife had been waiting for the return of his beloved, seating at open window for decades, but her loved one never came back. Only when she died the window was shut. Two versions are told of this story: according to the first, the neighborhood was so touched that the window was opened again in memory of this story of love and devotion. The second argues that when the family attempted to close the window, the furniture started shaking, books flew off the shelves, paintings fell of the walls. The palace was back in order only when finally the shutters were reopened: the window has always been left ajar ever since. Another legend tells that the room with the open window belonged to a woman who was the secret lover of Ferdinando I de' Medici, Grand Duke of Tuscany: the statue depicting him, placed exactly in front of the Grifoni Palace, in fact keeps his eyes focused on that window...*



*The facade of Palazzo Budini Gattai with the always-open window*

## Maps of Conference places



Walking distance:

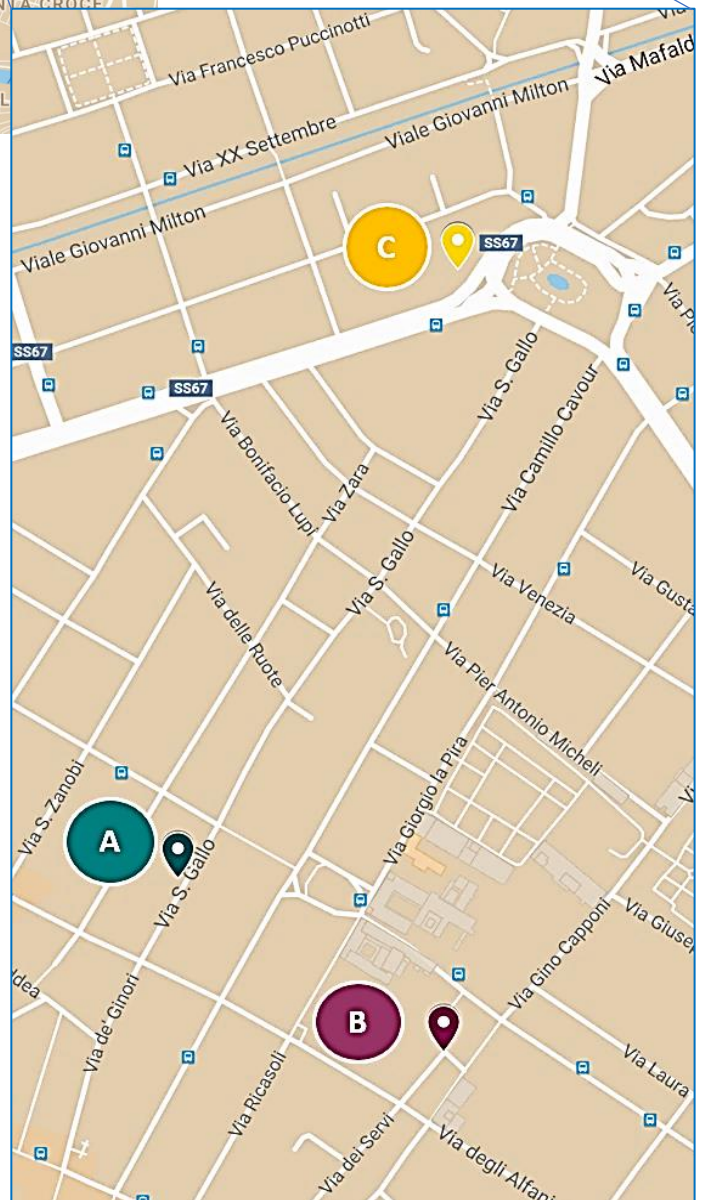
A → B 6 minutes

A → C 11 minutes

**A** Auditorium di Sant'Apollonia-  
Conference Venue,  
Via San Gallo 25

**B** Palazzo Budini-Gattai,  
Venue of the Social  
Dinner,  
Via De Servi 51

**C** Meeting point for  
Excursion,  
Piazza della Libertà 3



In case you need, please contact **Elisa +39 338 1580798; Elena +39 329 8061717**



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**Notes:**

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