How does soil water availability control phytotoxic O₃ dose to montane pines? Modelling and experimental study from two contrasting climatic regions in Europe

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Abstract. Montane forests are exposed to high ambient ozone (O₃) concentrations that may adversely affect physiological processes in internal cells when O₃ molecules enter the plants through the stomata. This study addresses the model results of Phytotoxic Ozone Dose metric (POD) based on estimation of stomatal O₃ flux to dwarf mountain pine (*Pinus mugo*) and Swiss stone pine *(Pinus cembra)*. We focused on two different bioclimatic regions: (1) the temperate mountain forests in the High Tatra Mts (SK–HT) of the Western Carpathians, and (2) the Mediterranean forests of the Alpes–Mercantour (FR–Alp) in the Alpes–Maritimes. Field measurement of O₃ concentration and meteorological data incorporated into deposition model DO₃SE showed lower O₃ flux in FR–Alp than in SK–HT plots for the 2016 growing season. Model outputs showed that soil humidity play a key role in stomatal O₃ uptake by montane pines at the alpine timberline. We found that temperate climatic conditions in SK–HT with sufficient precipitation did not limit stomatal conductivity and O₃ uptake of *P. mugo* and *P. cembra*. On the other hand, the Mediterranean mountain climate characterised by warm and dry summer reduced stomatal conductance of pines in FR–Alp. POD without threshold limitation i.e. POD₀ as a recently developed biologically sounded O₃ metric varied near around and below critical level (CLef) depending upon different conditions of sunshine exposure in SK–HT plots. Field observation at these plots showed relatively weak visible O₃ injury on *P. cembra* (2 % and 7 %) when compared with *P. mugo* (8 % and 18 %) for one year (C+1) and two year (C+2) old needles, respectively. Despite of low POD₀ values, clearly below CLef, the highest level of visible O₃ damage on average from 10 % (C+1) to 25 % (C+2) was observed on *P. cembra* needles in Mediterranean (FR–Alp) area. Further research is needed to clarify the effect of real soil moisture regime on stomatal closure in dry areas (FR–Alp) and resistance of pine species against visible O₃ injury in wet subalpine zones (SK–HT). More attention should be paid to O₃ fluxes covering a year-round growing season as well as intra-daily dynamics, especially the night hours, since these time spans appear to play significant role in O₃ uptake by mountain conifers.

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1 Introduction

Surface ozone (O_3) is one of the most common air pollutants according to on a recent review of the accumulated scientific evidence (WHO, 2006; EPA, 2014; EEA, 2016; UNECE, 2016). During recent decades, trends in mean O_3 concentrations have varied by regions (Cooper et al., 2014) nevertheless did not appear to be well associated with some exposure metrics applicable for assessing human health or vegetation effects (Lefohn et al., 2017). A variety of O_3 metrics are used in the risk assessment for forest trees. Initially developed (or original) exposure standards (AOT40) based only on measured O_3 concentration does not take into account environmental factors affecting responses of vegetation. Therefore in late 1990s a discussion on developing new, flux-based critical levels, started. New approaches focus on the principles of O_3 transport from atmosphere to the plant interior through the stomata, and control of O_3 uptake by leaves *via* environmental factors (Fuhrer et al., 1997; Massman et al., 2000; Grünhage et al., 2001; Ashmore et al., 2004; Musselman et al., 2006; Karlsson et al., 2007; Matyssek et al., 2007). A stomatal conductance based model was developed to estimate O_3 uptake for a number of the most widespread tree species (Emberson et al., 2000). The new flux-based critical levels revised by the LRTAP Convention (CLRTAP, 2015; Mills et al., 2011) were named as the Phytotoxic Ozone Dose (POD_Y), i.e., the accumulated stomatal O_3 flux above a threshold (Y) flux. There is strong support among biologists for the use of the threshold O_3 flux that includes the detoxification capacity of the trees (Karlsson et al., 2007). Expert judgement was used to set Y=1 nmol m^{-2} PLA s^{-1} (PLA is the projected leaf area) based on observation of O_3 sensitivity under controlled conditions (Dizengremel et al., 2013). De Marco et al. (2015) recommend applying POD_Y without threshold limitation (Y=0) i.e., POD_0 rather than POD_1. It is based on the fact that any O_3 molecule entering into the leaf may induce a metabolic response (Musselman et al., 2006). Various studies have provided information on how O_3 interacts with the plant at the cellular level (Bussotti et al., 2011; Gottardini et al., 2014; Braun et al., 2014; Mills et al., 2016). In addition, the physiological consequences of the O_3 induced effects may impair resistance of trees to the abiotic (frost, drought) and biotic (nutrient deficiencies, pathogens, bark beetle) stress factors (Vollenweider and Günthardt-Goerg, 2006).

The major challenge in the development of O_3 standards is their validation against biologically based field data (Paoletti and Manning, 2007). Recent epidemiological studies show better correlation between POD_0 and visible foliar O_3 injury than AOT40 (Sicard et al., 2016). The most sensitive conifers are *Pinus* species (Dalstein and Vas, 2005), however different visible O_3 injury response may be expected under natural conditions (Coulston et al., 2003; Nunn et al., 2007; Braun et al., 2014). Based on large literature evidence, mountain forest in the Carpathians (Bytnerowicz et al., 2004; Hůnová et al., 2010; Zapletal et al., 2012; Bičárová et al., 2016), and in the Alps (Smidt and Herman, 2004; Sicard et al., 2011) are exposed to high O_3 concentrations. Ozone damage rates increase with altitude in response to increasing O_3 mixing ratios and O_3 uptake due to favourable microclimatic conditions (Díaz-de-Quijano et al., 2009). However there is still lack of empirical data concerning vulnerable mountain forest tree species.

The objectives of this study were: (i) to map ozone metrics (AOT40, POD_1, POD_0) for the growing season 2016 and assess them with respect to related critical levels including the innovative species specific flux-based critical level (CLef) for forest

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protection against visible O_3 injury; (ii) to appraise the role of physiological parameters and soil water availability in O_3 uptakes

65 under contrasting climate conditions; and (iii) to analyse the relation between model results of POD_0 and field observation of visual O_3 injury for high O_3 sensitive conifers such as Swiss stone pine (*Pinus cembra* L.) and dwarf mountain pine (*Pinus mugo* Turra). To achieve these goals we focused on two different mountain bioclimatic regions of Europe such as (1) the High Tatra Mts (SK–HT) in the Western Carpathians with temperate climate and (2) the Alpes-Mercantour (FR–Alp) in the Alpes-Maritimes where forest vegetation is influenced by Mediterranean climate.

70 **2 Study area**

The study area (Fig. 1) covers montane forest sites situated in the Tatra National park (SK–HT) and in the Mercantour National Park (FR–Alp). SK–HT region is the highest mountain range of the Western Carpathians located in the north Slovakia. The elevation in this region rises from foothills at 800 m a.s.l. to the highest peak at 2,655 m a.s.l.. Climate is mostly cold and humid. According to standard reference climate period (1961–1990), mean annual air temperature ranges from 5.3 °C at foothills

75 to –3.8 °C in zone above 2,600 m a.s.l. Mean annual precipitation ranges from 760 to 2,000 mm. In the growing season (Apr–Sep) precipitation reaches nearly 65 % of annual sum with culmination in June or July. The coniferous forests cover area up to 1,600 m a.s.l. Subalpine zone (up to 1,800 m a.s.l.) is almost completely covered by dwarf mountain pine. Swiss stone pine occurs sparsely at the timberline. In recent decades, the massive windstorms and consecutive bark beetle (*Ips typographus)* outbreaks damaged SK–HT forests weakened by various abiotic factors such as elevated temperature (Mezei et al., 2017) or

80 long-range transport of air pollutants (Bytnerowicz et al., 2004).

The Mercantour National Park with several peaks above 3,000 m, and wide altitudinal range of more than 2,500 metres has a specific climate mixing Mediterranean and Alpine influences. In conjunction with multiform geological terrains ranging from limestone to crystalline, the habitats of the southwestern Alps are extremely diverse and this region belongs to the richest hotspots of biodiversity in Europe (Dole-Olivier et al., 2015). Climate in (FR–Alp) is temperate Mediterranean with moderately

85 cool and dry summer. Minimum monthly precipitation occurs in July, exactly the opposite as in SK–HT region. In this region the Holm oak, the Mediterranean olive tree, rhododendron, fir, spruce, Swiss stone pine and larch are widespread species. Previous studies have shown (Dalstein and Vas, 2005; Sicard et al., 2011) that the rural alpine Mediterranean area of the Mercantour National Park may be affected by considerable quantities of localized O_3 generated by photochemical reactions of O_3 precursors from the regional road traffic in presence of high temperatures and solar radiation in very hot summers of the

90 Mediterranean climate. Visible leaf injury on particularly sensitive species is one of the O_3 air pollution symptoms.

Six study sites were established in different altitudinal zones to map exposure and phytotoxic O_3 doses in contrasting climate conditions: A–foothills, B–submontane zone, C–subalpine zone, D–subnival zone and different aspects: 1 – south, 2 – north (Table 1, Fig. 1).
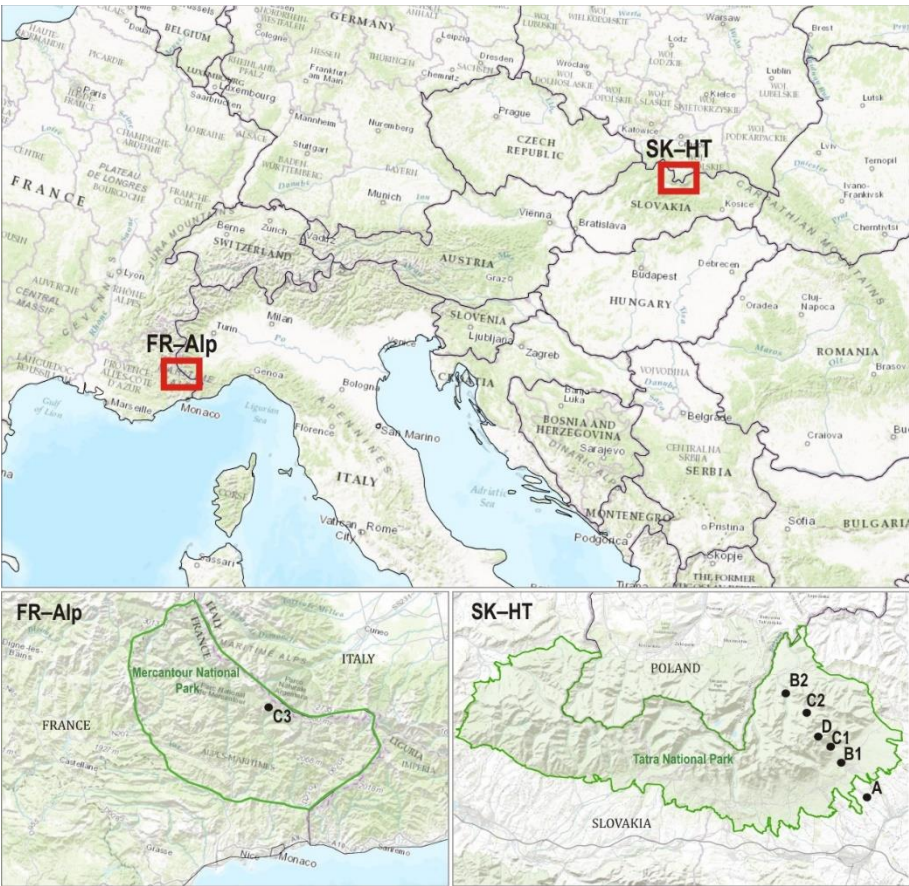
Figure 1. The geographical position of the Tatra National Park (SK–HT) in the Carpathian mountain range and the Mercantour National Park in the Alpes-Maritimes region (FR–Alp) including experimental sites in order of altitudinal zonation; A–foothills: Stará Lesná; B–submontane zone: B1–Štart, B2–Podmuráň; C–subalpine zone: C1–Skalnaté pleso, C2–Kolové pleso, C3–Col de Salèse; D–subnival zone: Lomnický štít.

Table 1. Description of experimental sites (CODE used in relation to Fig. 1)

CODE site	GPS Latitude Longitude	Altitude Zone/Aspect	Climate (Köppen classification) Local specification	Tree species composition	Soil type Soil texture
SK–HT					
A Stará Lesná	49°09'08" N 20°17'19" E	810 m a.s.l. Foothill/S	Temperate continental (Dfb); Moderately warm and humid	*Picea abies, Betula verucosa, Pinus sylvestris, Larix decidua, Alnus glutinosa*	Haplic Cambisols; Silt loam (medium coarse)
B1 Štart	49°10'30"N 20°14'48" E	1,150 m a.s.l. Submontane/S	Temperate continental (Dfb); Moderately cool and humid	*Picea abies, Larix decidua, Pinus sylvestris, Abies alba, Acer pseudoplatanus*	Haplic Podzols; Sandy loam (coarse)
B2 Podmuráň	49°15'00" N 20°09'25" E	1,100 m a.s.l. Submontane/N	Temperate continental (Dfb); Moderately cool and extremely humid	*Picea abies, Abies alba, Sorbus aucuparia, Fagus silvatica, Acer pseudoplatanus*	Haplic Podzols; Loam (medium)
C1 Skalnaté pleso	49°11'21" N 20°14'02" E	1,778 m a.s.l. Subalpine/S	Cool continental (Dfc); Cool mountain and extremely humid	*Pinus mugo*	Folic Leptosols; Sandy loam (coarse)

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C2 Kolové pleso	49°13'22" N 20°11'27" E	1,570 m a.s.l. Subalpine/N	Cool continental (Dfc); Cool mountain and extremely humid	*Pinus mugo, Pinus cembra, Picea abies*	Folic Leptosols; Silt loam (medium coarse)
D Lomnický štít	49°11'43" N 20°12'54" E	2,635 m a.s.l. Subnival summit	Cool continental (Dfc); Cold mountain and extremely humid	:	Lithic Leptosols; Hyperskeletic
FR–Alp					
C3 Col de Salèse	44°08'18" N 07°14'11" E	1,993 m a.s.l. Subalpine/S	Temperate Mediterranean (Csb); Moderately cool and dry summer	*Pinus cembra, Larix decidua*	Lithosols Hyperskeletic

3 Methods

3.1. Ozone metrics

All O_3 metrics (AOT40, POD_1, POD_0) were calculated using the multiplicative model DO_3SE (Deposition of Ozone for Stomatal Exchange). AOT40 (ppb h) is the accumulated amount of ozone over the threshold value of 40 ppb for daylight hours
105   during the relevant growing season (Apr–Sept). Concentration based critical level $CLec$ of AOT40 was set to 5,000 ppb h (Directive 2008/50/EC). An algorithm for model estimation of POD_Y (mmol m^{-2} PLA) incorporates effects of meteorological conditions such as air temperature (f_{temp}), vapour pressure deficit (f_{VPD}), solar radiation or light (f_{light}), furthermore soil water potential (f_{SWP}), plant phenology (f_{phen}) and O_3 concentration (f_{O3}) on the maximum stomatal conductance (G_{max}). Passage rate of O_3 entering through the stomata is expressed as the stomatal O_3 conductance G_{sto} (m s^{-1}):

110    $$G_{sto} = G_{max} * [min(f_{phen}, f_{O_3})] * f_{light} * max\{f_{min}, (f_{temp} * f_{VPD} * f_{SWP})\} \qquad (1)$$

Stomatal O_3 flux F_{st} (nmol m^{-2} PLA s^{-1}) is then given by:

$$F_{st} = G_{sto} * c(z_1) * \left(\frac{r_c}{(r_b + r_c)}\right) \qquad (2)$$

where $c(z_1)$ is concentration of O_3 (nmol m^{-3}) at the top of the canopy measured in the tree height (z_1), r_b and r_c are the quasi laminar resistance and the leaf surface resistance (s m^{-1}), respectively.

115   Phytotoxic ozone dose POD_Y is sum of hourly values of F_{st} (Eq. 2) over threshold Y=1 (POD_1) or without threshold Y=0 (POD_0) aggregated over the growing season. Stomatal flux-based critical levels $CLef_1$ of POD_1 is proposed to be 8 mmol m^{-2} PLA for evergreen coniferous, especially Norway spruce (Mills et al., 2011). An innovative species-specific CLef of POD_0 is proposed to be 19 mmol m^{-2} PLA for forest protection against visible O_3 injury for high O_3 sensitive tree species such as Swiss stone pine (Sicard et al., 2016). More specific description of the algorithm and derivation of the physical relationships for the
120   final calculation of POD_Y is given in the manual for modelling and mapping of the critical level exceedance (CLRTAP, 2015; Mills et al., 2011). The parameterization of DO_3SE model reflects the recommendations in different scientific papers, the generic values are also given in manual ICP Modelling and Mapping (ICP, 2016). In this work, the preset built in version 3.0.5 of DO_3SE model (SEI, 2014) with collection of parameters for coniferous forests (CF) was used (Table S1). Maximum level of stomatal O_3 conductance G_{max} (mmol O_3 m^{-2} PLA s^{-1}) of dwarf mountain pine and Swiss stone pine were obtained according
125   to field experiments in SK–HT. Model requires input files that include measured O_3 concentration and meteorological data, for each experimental site separately.

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3.1.1 Ozone and meteorological data

In SK–HT, O_3 concentration was measured with active monitors (Horiba–APOA360, Thermo Electron Environmental 49C and 2B Tech Ozone Monitor M106-L) based on the well established technique of UV absorption by O_3 at wavelenght 254 nm.

130   Hourly mean data at three experimental forest stands (A, B2, C2) were recorded in continuous regime without major gaps during year 2016. Furthermore, O_3 concentrations measured at experimental site Lomnický štít (D) were considered for illustration of vertical O_3 profile in altitudinal range between 810 and 2,635 m a.s.l. In sites without electricity power (B1, C2) ozone monitors (2B Tech Ozone) were powered by solar panels. In FR–Alp (C3), passive samplers (Svenska Miljöinstitutet) were used for estimation of O_3 concentration. These passive O_3 sensors allow large-scale monitoring in remote areas (Krupa

135   and Legge, 2000) but do not provide real-time O_3 concentrations which are essential for O_3 flux calculations. Method proposed by Loibl (Loibl et al., 1994; Loibl and Schmidt, 1996) was used for recalculation of aggregated data from passive samplers to hourly O_3 concentrations. The method is based on a model (Eq. 3) that describes daily O_3 profile as a function of relative elevation (h_r in meters), day time hours (t from 0 to 23) and coefficients obtained from the fitting (a1, a1, a2, a3, a4 and b1, b2, b3, b4, b5, b6) (Loibl et al., 1994).

140   $$C(h_r,t) = a_1 + a_2 e^{-(t-a_3)^{2a_4}} \ln\left(\frac{h_r}{100} + \frac{b_1 t^2 + b_2 t + b_3}{b_4 t^2 + b_5 t + 10,000} e^{-b_6 t}\right) \qquad (3)$$

In FR–Alp, real-time O_3 concentrations continuously measured at Cians (distance about 15 km from C3) by active monitor (type Environnement SA) were used for recalculation to hourly values at C3. This approach supported small difference in seasonal average values (up to 5%). The meteorological variables (air temperature, relative humidity, wind speed, air pressure, solar radiation and precipitation) were continuously monitored at all experimental sites in SK–HT region using automatic

145   weather stations (Physicus, SK; EMS, CZ). In FR–Alp region, the meteorological data in hourly intervals were obtained from the station Isola 2000 (the Meteoblue weather archive at meteoblue.com) situated in the vicinity of Col de Salèse.

3.1.2 Stomatal conductance of montane pines

Maximum level of stomatal conductance (G_{max}, mmol O_3 m^{-2} PLA s^{-1}) as key parameter (Eq. 1) for calculation of stomatal O_3 flux (Eq. 2) has not been specified neither for dwarf mountain pine nor for Swiss stone pine. Data required for G_{max}

150   parameterization were obtained by measurement on both pine species in SK–HT. For this purpose LI-6400 photosynthesis system (Li-Cor, Inc., Lincoln, NE) equipped with a standard Licor 6400-22 Opaque Conifer Chamber and 6400–18 RGB Light Source was used. To capture wide range of climatic conditions stomatal conductance was measured from June to November at study sites situated in two different elevations: site A (Stará Lesná, 810 m a.s.l.) and site C1 (Skalnaté Pleso, 1,778 m a.s.l.). During the experiment, inside chamber temperature ranged from 5 to 35 °C, VPD from 0.2 to 3.5 kPa, photosynthetic photon

155   flux density (PPFD) from 0 to 2,500 µmol photons m^{-2} s^{-1}, CO_2 concentration was set to 400 ppm. Before each measurement, gas exchange was permitted to stabilize for approximately 6–10 min. Gas exchange measurements were conducted frequently on attached healthy looking sunlit terminal shoots of lateral branches from middle part of the plant. On average 8–9 measurements under different conditions were recorded per shoot. We consider G_{sto} (mmol O_3 m^{-2} PLA s^{-1}) from G_{sto} (mmol

H_2O m^{-2} PLA s^{-1}) using a conversion factor of 0.663 (Massman, 1998) to account for the difference in the molecular diffusivity

160 of water vapour (measured by LI-6400 photosynthesis system) to that of ozone. Similar values of G_{sto} with the top 5 percent in range between 110 and 160 mmol O_3 m^{-2} PLA s^{-1} were noticed for both *Pinus* species. Maximum stomatal conductance G_{max} was derived as the 95 percentile of all measured data of stomatal conductance for O_3 flux rates (about 2,700 measurements of G_{sto}) after removal of outliers. Based on this derivation, maximum level of stomatal conductance for O_3 was determined as G_{max}=110 mmol O_3 m^{-2} PLA s^{-1} for both studied montane pines.

3.1.3 Soil water potential

165 Soil moisture data were obtained by two approaches: field measurement and modelling. Both, real-time and modelled data of soil water potential (SWP) are useful for specification of the f_{SWP} function (Eq. 4).

$$f_{SWP} = min \left\{1, \{f_{min} \left((1 - f_{min}) * (SWP_{min} - SWP)/(SWP_{min} - SWP_{max})\right) + f_{min}\}\right\} \qquad (4)$$

Function of f_{SWP} defines the effect of soil moisture on G_{sto} (Eq. 1). It is expressed in relative terms (i.e., it takes values between

170 0 and 1 as a proportion G_{max}). Additional parameters such as f_{min}, SWP_{min}, SWP_{max} are listed in Table S1. Differences between f_{SWP} based on measured and modelled SWP allow to verify the reliability of the soil moisture module included in DO$_3$SE model. Verification cannot be done at FR–Alp plot due to deficiency of SWP data measured in field conditions. Field measurement of SWP at three soil depths (–0.1, –0.2, –0.4 m) was carried out only in SK–HT region at all experimental sites (except the cliff of Lomnický štít). SWP values were measured with gypsum blocks in the range up to –1.5 MPa (GB2,

175 Delmhorst Instrument, U.S.A.) and stored with integrated data loggers (MicroLog SP3, EMS Brno, CZ). Modelling approach was used in both study areas and incorporated hydraulic resistance (steady state, SS) to water flow through the plant system (Büker et al., 2012). In model options is possible to choose appropriate alternative for simulation of soil water influence on G_{sto} (Eq. 1). We selected an alternative using factor f_{SWP} (Eq. 4) in calculation of G_{sto}. The choice with disabled f_{SWP} should show different results G_{sto}, primarily in dry areas we expect higher values of G_{sto}, F_{st} (Eq. 2) and consequently also POD$_y$.

3.2 Visible ozone injury

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Observation of visible injury symptoms on Swiss stone pine and dwarf mountain pine needles collected in the autumn 2016 was undertaken in accordance with the methods recommended for analysis of the effects of air pollution on forests (ICP, 2016). Swiss stone pine branches sampled from four plots at elevation between 1,000–1,600 m a.s.l. (SK–HT) and one plot at altitude 2,000 m a.s.l. (FR–Alp) were used for visible O_3 injury assessment. Samples of dwarf mountain pine branches from eleven

185 plots situated along altitudinal profile from 800 to 2,000 m a.s.l. only for SK–HT area were evaluated. At each plot we selected 5 sample trees exposed to sun. For each tree, 5 branches with at least 30 needles per each needle age class (current year foliage (C), one year old (C+1) and two year old needles (C+2)) were removed from the upper third of the crown by using telescopic secateurs. For each branch, the percentage of total needle surface affected by visible foliar O_3 injury was scored for C, C+1 and C+2. Finally, a mean percentage of needles surface affected by visible foliar O_3 injury was calculated per every plot.

4 Results

4.1 O$_3$ concentration and environmental conditions

During the growing season (Apr–Sept 2016) mean O$_3$ concentrations ranged from 29.6 (B2) to 53.6 ppb (D). Measured mean hourly O$_3$ concentration (Table 2) confirmed the expected increase of O$_3$ concentration along the altitudinal profile (Fig. 2a). Therefore high altitude areas of the subalpine (C1, C3) and subnival zone (D) are most affected by O$_3$ pollution. Monthly O$_3$ means in the foothills and submontane zones varied between 20 and 40 ppb while in the subalpine and subnival zones were above 40 ppb. In SK–HT, monthly mean O$_3$ concentrations culminated in May (Fig. 2b) and achieved values between 36.3 ppb (B2) and 59 ppb (D). On the contrary, in FR–Alp (C3) the highest monthly O$_3$ average was recorded in July and reached 50 ppb. The real air temperatures (AT, deg C in Table 2) with respect to the optimum and limit range of DO$_3$SE model parameterization (Table S1) were favourable for stomatal conductance (Fig. 3a) and potentially also for photosynthetic capacity of these two coniferous species. In FR–Alp, the average air temperature of 9.2°C at altitude close to 2,000 m a.s.l. was comparable with those of 1,600–1,800 m a.s.l. in SK–HT. Average values of vapour pressure deficit (VPD, kPa) ranged from 0.24 to 0.39 kPa (Fig. 3b) and were sufficient for unlimited stomatal conductance (Table S1). In SK–HT, precipitation totals (P, mm) in the submontane and subalpine zones varied between 669 mm and 1,280 mm and ensured a sufficient supply of soil water for roots. On contrary, low seasonal precipitation 469 mm in the foothill zone in SK–HT (A) and 425 mm in the subalpine zone in FR–Alp (C3) suggested soil drought. The amount of global solar radiation (R, kW m^{-2}) in FR–Alp was considerable higher than in SK–HT.

Table 2. Statistics of hourly O$_3$ data (ppb) and meteorological variables for the Apr–Sept 2016 period

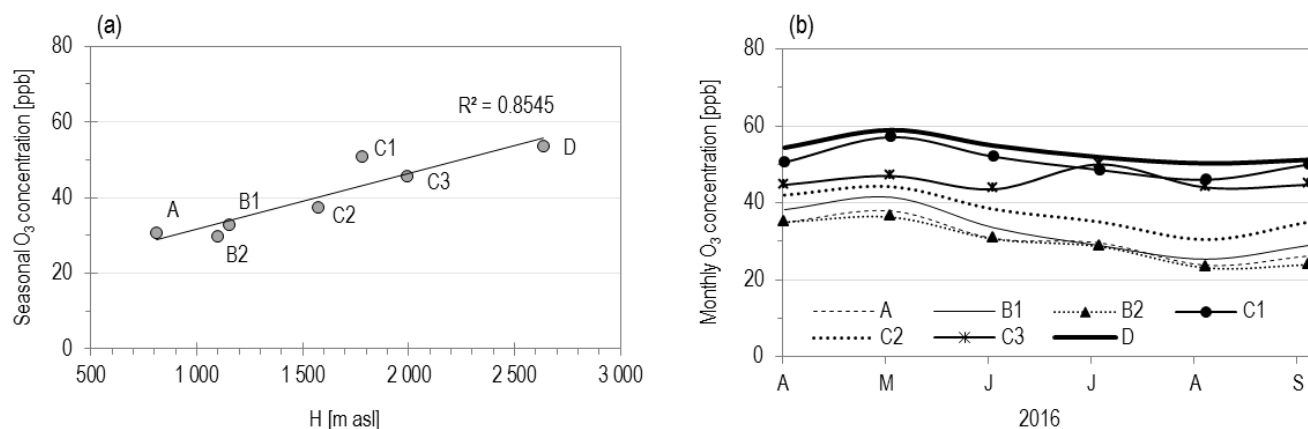| Exp. site	O$_3$ concentration (ppb)				AT (°C)				VPD (kPa)				P (mm)	R (kW m^{-2})
	Min	Max	Mean	STD	Min	Max	Mean	STD	Min	Max	Mean	STD	Sum	Sum
SK–HT														
A	3.7	64.0	30.6	13.0	-4.4	29.2	13.0	6.1	0.05	2.30	0.43	0.39	469	863
B1	1.6	67.7	32.8	13.2	-4.6	26.5	11.6	5.6	0.00	2.42	0.38	0.46	669	587
B2	1.7	68.6	29.6	16.4	-6.9	26.9	10.6	5.9	0.02	1.80	0.24	0.29	1,044	707
C1	17.2	81.2	50.7	8.0	-7.8	22.4	8.3	5.2	0.03	1.56	0.24	0.20	945	750
C2	1.3	87.4	37.5	10.7	-9.5	25.3	8.6	5.6	0.00	2.72	0.39	0.47	1,280	543
D	19.2	86.0	53.6	7.4	-14.3	16.6	2.5	5.2	0.00	1.51	0.17	0.22	890	798
FR–Alp														
C3	17.0	92.1	48.3	11.3	-6.3	21.6	9.2	5.2	0.00	1.81	0.38	0.27	425	931

Figure 2. (a) Change of seasonal O_3 concentration with increasing altitude, and (b) course of monthly O_3 means in different altitudinal zones during the Apr–Sep 2016 period.
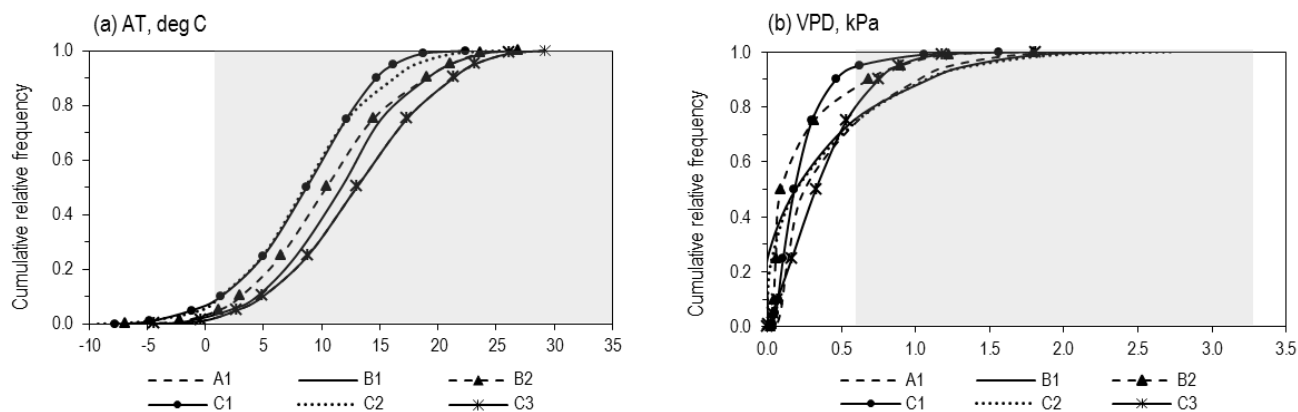

Figure 3. (a) Empirical cumulative distribution of hourly data for air temperature (AT, deg C) and (b) vapour pressure deficit (VPD, kPa) in different altitudinal zones during the Apr–Sep 2016 period; grey area illustrates ranges of AT and VPD for effective stomatal O_3 conductance (Table S1).

4.2 Ozone metrics

The DO$_3$SE model results (Table 3) show values of ozone metrics (AOT40, POD$_1$ and POD$_0$) that indicate different effect of O_3 pollution on mountain pines. Accumulated O_3 concentration exposure index AOT40 (8.3–23.3 ppm h) clearly exceeded CLec (5 ppm h) for protection of European forest (ICP, 2016) at all study sites. Phytotoxic ozone dose metric (POD$_1$) with a threshold Y=1 varied in range from 6.4 to 13.7 mmol m^{-2} PLA and showed an exceedance of CLef$_1$ flux based critical level for POD$_1$ (8 mmol m^{-2} PLA) proposed for coniferous forests (such as Norway spruce) at all sites in SK–HT. Accumulated stomatal O_3 flux without a threshold (Y=0), POD$_0$, ranged from 12.5 to 22.4 mmol m^{-2} PLA for Swiss stone pine and 11.4 to 19.3 mmol m^{-2} PLA for dwarf mountain pine. POD$_0$ exceeded critical level for highly O_3 sensitive conifers (CLef=19 mmol

m^{-2} PLA) such as Swiss stone pine only at sites in SK–HT with favourable sunshine exposure (A, B2, C1). The concentration of O_3 and sufficient amount of soil water in submontane and alpine zones of SK–HT contributed to higher level of POD_0 (16.9–22.4 mmol m^{-2} PLA) for Swiss stone pine compared with dwarf mountain pine (12.2–19.3 mmol m^{-2} PLA). These differences between the two species may be associated with their different canopy height and root depth listed as model

225 parameters in Table S1. POD_1 and POD_0 values were generally higher in SK–HT than in FR–Alp. Lower stomatal O_3 flux and POD_0 value (12.5 and 11.4 mmol m^{-2} PLA, for Swiss stone pine and dwarf mountains pine, respectively) which is clearly below CLef was determined in FR–Alp where the climate is considerably drier than in SK–HT. As we expected, higher POD_0 value (18.9 and 16.3 mmol m^{-2} PLA for Swiss stone pine and dwarf mountains pine, respectively) which were closer to CLef resulted from the simulation that eliminated soil water influence on stomatal conductance (disabled soil moisture module in

230 DO_3SE model).

Table 3. DO_3SE model outputs for AOT40, POD_1 and POD_0 ozone metrics (G_{max}=110 mmol m^{-2} PLA s^{-1})

CODE site	AOT40 (ppm h) CLec = 5ppm h	POD_1 (mmol m^{-2} PLA) with threshold (Y=1) $CLef_1 = 8$ mmol m^{-2} PLA	POD_0 (mmol m^{-2} PLA) without threshold (Y=0) CLef = 19 mmol m^{-2} PLA (highly sensitive species)	
			Swiss stone pine	Dwarf mountain pine
SK–HT				
A	8.3	13.3	21.8	18.4
B1	8.9	10.1	18.1	15.6
B2	13.7	13.7	21.3	18.9
C1	23.3	14.1	22.4	19.3
C2	13.8	9.3	16.9	12.2
FR–Alp				
C3	13.4	6.4	12.5	11.4
C3*	13.4	11.1	18.9	16.3

Note: *for disabled soil moisture module in DO_3SE model

4.3 Soil moisture and stomatal conductance

The effect of soil moisture regime on G_{sto} was analysed with modelled and measured SWP values and f_{SWP} function (Eq. 4).

235 There is an acceptable agreement between SWP values modelled via DO_3SE and SWP measured in SK–HT for submontane (B1, B2) and subalpine zone (C1, C2) with respect to f_{SWP} (Table 4). Values of f_{SWP} mostly at the level of 1 (i.e. SWP > SWP_max = –0.76 MPa) confirm the assumption that soil moisture at higher altitudes in SK–HT is sufficient for unlimited stomatal conductance and O_3 uptake. However, at the foothill site (A) differences between modelled and measured SWP as well as f_{SWP} were larger. It appears that the model insufficiently reflected soil moisture deficit when SWP dropped below –1.2

240 MPa (Fig. 4). Water deficit can reduce stomatal conductance as well as stomatal O_3 flux to forest vegetation. The course of stomatal O_3 flux and POD_Y, taking into account the measured SWP values for foothill site (A) was tested using the algorithm recommended by the working group ICP Modelling and Mapping (ICP, 2016). In this case POD_0 value (20.2 mmol m^{-2} PLA) was slightly lower than DO_3SE model output (21.8 mmol m^{-2} PLA). Validation of SWP in FR–Alp (C3) is not possible due to the absence of field measurement in 2016. In this area, the modelled average SWP (–1.02 MPa) was close to its limit (SWP_min

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245   $= -1.20$ MPa). This fact, together with mean f_{SWP} (0.71) suggest a decrease of G_{sto} as a response to the serious soil moisture

deficit in the summer season. The DO₃SE model using the soil moisture module application resulted in substantially decreased

POD_Y (C3) when the soil water influence on G_{sto} in model options was considered (Table 3). Soil moisture conditions can have

a significant effect on stomatal conductance (Fig. 4) and therefore for modelling of the stomatal O_3 flux it is important to take

into account field measurement of SWP, especially in areas where soil drought events in association with lower precipitation

250   amount (A, C3) occur.

Table 4. Measured and modelled values of SWP and f_{SWP}

Exp. site	Measurement						DO₃SE model						Difference					
	SWP (MPa)			f_{SWP}			SWP (MPa)			f_{SWP}			SWP (MPa)			f_{SWP}		
	Min	Max	Mean	Min	Max	Mean	Min	Max	Mean	Min	Max	Mean	Min	Max	Mean	Min	Max	Mean
SK–HT																		
A	**-1.44**	-0.02	-0.46	0.10	1.00	0.79	-0.08	-0.01	-0.02	1.00	1.00	1.00	-1.36	-0.01	-0.44	-0.90	0.00	-0.21
B1	-0.77	-0.02	-0.03	0.98	1.00	1.00	-0.12	-0.02	-0.03	1.00	1.00	1.00	-0.65	0.00	0.00	-0.02	0.00	0.00
B2	-0.02	-0.01	-0.02	1.00	1.00	1.00	-0.04	-0.02	-0.02	1.00	1.00	1.00	0.02	0.01	0.00	0.00	0.00	0.00
C1	-0.05	-0.03	-0.04	1.00	1.00	1.00	-0.04	-0.02	-0.02	1.00	1.00	1.00	-0.01	-0.01	-0.02	0.00	0.00	0.00
C2	-0.03	-0.02	-0.03	1.00	1.00	1.00	-0.02	-0.01	-0.01	1.00	1.00	1.00	-0.01	-0.01	-0.02	0.00	0.00	0.00
FR–Alp																		
C3		:			:		**-4.00**	-0.02	-1.02	0.10	1.00	0.71		:			:	

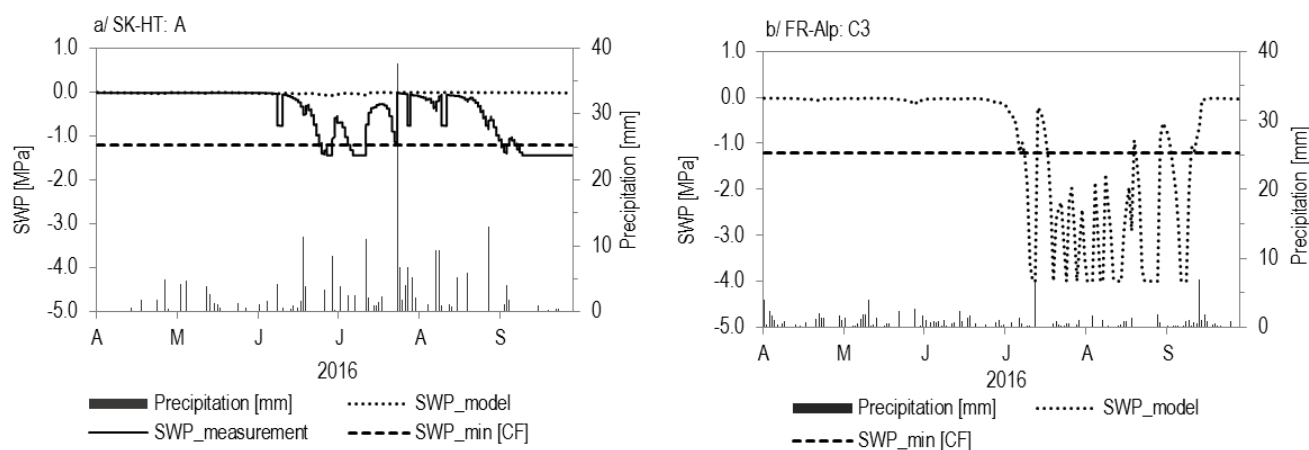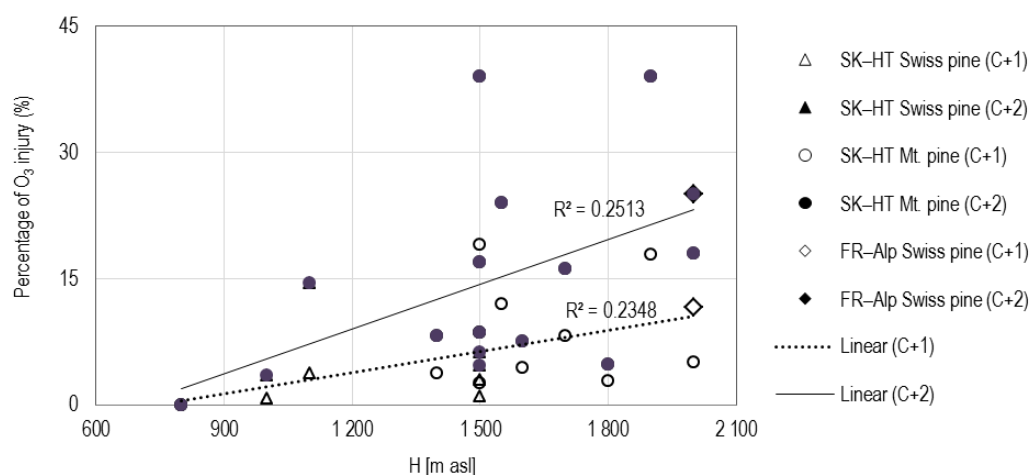Note: Values in bold are SWP < SWPmin = −1.20 MPa for CF (Table S1)

Figure 4. Hourly data of precipitation (mm) and soil water potential (SWP, MPa) for two experimental sites: (a) A in SK–HT, and (b) C3 in FR–Alp as response to dry events which occurred during summer 2016. SWP_min (−1.2 MPa) is a limit value of
255   soil water potential for minimal stomatal conductance for the coniferous forest (CF) (Table S1).

4.4 Visible ozone injury

Swiss stone pine and dwarf mountain pine branches sampled along the vertical profile from 800 to 2,000 m a.s.l. showed an

obvious visible O_3 injury at higher altitudes for both bioclimatic regions (Fig. 5). In SK–HT plots, more pronounced visual

symptoms were observed for dwarf mountain pine (7.7 ± 1.1 % for C+1 needles and 18.2 ± 2.3 % for C+2 needles) than for

Swiss stone pine (2.2 ± 0.4 % for C+1 needles and 7.2 ± 2.0 % for C+2 needles) plots. The oldest needles of both species were more frequently damaged by O_3 injury at all plots. The youngest, current year needles did not show any signs of O_3 injury. No significant differences were found between southern and northern transects. Higher damage of dwarf mountain pine by O_3 could be caused by mild winter of 2015/2016 with unusually low snow cover in SK–HT plots. The dwarf mountain pine which is usually covered by snow in spring was exposed to ambient air O_3 maxima in 2016.

Chlorotic mottle and marbling as characteristic markers of O_3 damages were clearly visible on the Swiss stone pine in FR–Alp plot with the highest percentage of visible O_3 injury from 10.0 ± 0.6 % for C+1 needles and 25.0 ± 0.9 % for C+2 needles, respectively. This observation confirms that the Swiss stone pine could be considered as a sensitive bioindicator of O_3 exposure in the FR–Alp area.

Figure 5. Percentage of O_3 injury visible on needles of different age for pines within the SK–HT and FR–Alp regions.

5 Discussion

Estimation of phytotoxic effect of ozone on coniferous trees at high elevation and vulnerable mountain forests needs a special approach. Model simulation of stomatal O_3 uptake requires continuous field measurement of hourly O_3 concentration and various meteorological parameters. Precise parameterization of the DO_3SE model is also important for calculation of an accumulated stomatal O_3 flux i.e., POD_Y metrics. Such data is widely available for some commonly occurring trees species, such as Norway spruce (ICP, 2016; Mills et al., 2011) with low sensitivity to O_3 exposures (Coulston et al., 2003). However, there is still a lack of empirical data concerning potentially vulnerable mountain forest tree species. Therefore modification of input parameters reflecting real environment of specific coniferous species may be very useful, especially maximal stomatal conductance of mountain pines in different bioclimatic regions.

In this work, we modified maximal stomatal conductance $G_{max}=160$ mmol O_3 m^{-2} PLA s^{-1} generally used in DO_3SE model as a standard for coniferous tree species. Based on real-time measurements of mountain pines in SK–HT we changed this preset value to the adjusted $G_{max}=110$ mmol O_3 m^{-2} PLA s^{-1} (Table S1). This modification resulted in POD_0 values substantially lower

(about 10 mmol m^{-2} PLA) than those predicted by the DO$_3$SE model with preset G$_{max}$ value. Further change of POD$_0$ value can be expected after modification of G$_{max}$ based on real-time measurements of G$_{sto}$ in much warmer FR–Alp conditions.

295   Our modelling of POD$_0$ showed that FR–Alp experienced lower O$_3$ uptake than the sites in SK–HT. Contrary to this, visible O$_3$ injury of Swiss stone pine was more serious in FR–Alp than in SK–HT. This might be influenced partly by different altitudes of Swiss stone pine plots where visible O$_3$ injury observations were carried out: in FR–Alp at elevation of 2,000 m a.s.l., and in SK–HT at elevations between 1,000 and 1,600 m.a.s.l.. In addition, it is necessary to consider some degree of uncertainty of the DO$_3$SE model in animation of stomata functioning driven mostly by soil water potential. Real low soil

300   moisture in FR–Alp should notably reduce stomatal conductance, and consequently yield low values of POD$_0$ leading to avoidance of visual O$_3$ symptoms. Based on a strong visual evidence of injuries we suspect that the used model underestimated stomatal conductance. The importance of correct incorporation of soil moisture into modelling of stomatal O$_3$ uptake was recently reported by De Marco et al. (2016).

Sufficient amount of precipitation and favourable soil moisture eliminated influence of f$_{SWP}$ (Eq. 4) on stomatal conductance

305   (Eq. 1) at most of the study sites in SK–HT. This fact caused that f$_{SWP}$ was nonessential for the POD$_Y$ calculation (Eq. 2). On the contrary, in FR–Alp the modelled SWP values were markedly lower and the POD$_0$ values changed. These findings underline the necessity to include site specific real–time SWP data into the DO$_3$SE model.

Beside these findings the study revealed an importance of the nighttime plant transpiration and stomatal conductance for more precise estimation of POD$_o$ values. The DO$_3$SE model sets the night time values to zero and thus understimate stomatal

310   conductance during night. Our preliminary night time physiological measurement on dwarf mountain pine (unpublished data) indicate the role of night time fluxes similarly as in other tree species (Zeppel et al. 2013).

An earlier onset of real growing season and its extended duration in the FR–Alp region could be another explanation of discrepancy between serious visual injury symptoms and relatively low POD$_0$ value. For the purpose of comparison and limited data availability only the period from April to September 2016 was considered. It is possible that relevant O$_3$ uptake was

315   present in time prior to the considered period. All round year field measurement campaign at higher altitudes of Mediterranean mountains should be realized to verify this assumption.

Based on the outputs of our study we can confirm that visible O$_3$ injury increases with rising altitude (Díaz-de-Quijano et al., 2009; Benham et al., 2010; Kefauver et al., 2014). A fact that O$_3$ concentrations and O$_3$ uptake into the foliar tissues rise with altitude is supported by many previous studies (Chevalier et al., 2004; Bičárová et al., 2013). Our findings concerning visible

320   O$_3$ injury and altitude confirm findings from other studies (e.g. Dalstein et al., 2004; Ulrich et al., 2006).

Our observations of visible symptoms indicate that, the Swiss stone pine in the Carpathians seems to be less sensitive to O$_3$ and consequently show lower visible injury than dwarf mountain pine. Although Mediterranean vegetation is considered to be better adapted to oxidative stressors than mesophilic vegetation (Paoletti, 2006), we found that in FR–Alp the visible symptoms on Swiss stone pine (chlorotic mottles and marbling) were more evident, evolved over time and their number increased with

325   age. The marbling was also found on the oldest needles and a cumulative effect was more pronounced. Different visible O$_3$ injury response may be expected under natural conditions due to differences in O$_3$ sensitivity controlled by a genotype and

micro-site conditions of growth, exposure, and O_3 flux (Coulston et al., 2003; Nunn et al. 2007; Braun et al., 2014). Despite genus mutuality of Swiss stone pine and dwarf mountain pine we found surprisingly notable differences in their visible O_3 injury. It raised questions if this is the result of different species tolerance to O_3 injury and different rate of O_3 uptake or simply

330 an inadequacy of the visual injury assessments. Evaluation of O_3 injury symptoms on conifers is not easy and therefore may produce some questionable results as discussed by several authors (e.g. Wieser et al., 2006). Although visible injury is commonly used as an indicator of phytotoxic O_3 concentrations in ambient air, it is not always a reliable indicator of damage or other injury endpoints (EPA, 2007). Due to high sensitivity of Swiss stone pine proven in FR–Alp it can be considered as local bioindicator of O_3 exposure. In SK–HT, dwarf mountain pine with pronounced visual symptoms seems to be an

335 appropriate local conifer species for future biomonitoring of O_3 injury.

6 Conclusions

Swiss stone pine and dwarf mountain pine are typical tree species of the European timberline. Determinations of selected O_3 phytotoxicity metrics (AOT40, POD_1, POD_0) with respect to exceedance of relevant critical levels (CL_{ec}, CL_{ef_1}, CL_{ef}) suggests different adverse O_3 effect on mountain pines in two contrast bioclimatic regions: moist in the High Tatra Mts in

340 Slovakia (SK–HT) and dry in the Alpine Mercantour Mts in France (FR–Alp). Values of AOT40 were found to be substantially higher than the critical level CL_{ec} for both moist and dry regions. However, modelled results of POD_0 values that reflect influence of climatic and environmental conditions on the opening of the stomata showed lower O_3 uptake to conifer needles in the Mediterranean region of the Alps, where the climate is considerably drier than in the Carpathians. Humid environment leads to a greater capture of O_3 gas in tissues of plants exposed to high O_3 doses. Observations of visible O_3 injury confirmed

345 that older needles were more damaged by O_3 than the younger ones. Despite the favourable humid soil conditions for stomatal O_3 uptake, we observed relatively weak visible O_3 injury on two year needles of Swiss stone pine at SK–HT. Dwarf mountain pine with more pronounced visual symptoms seems to be an appropriate conifer species for further monitoring of O_3 injury at SK–HT. Severe foliar O_3 injury symptoms were identified on Swiss stone pine at the FR–Alp experimental site.

Presented results confirm high phytotoxic potential of O_3 air pollution in different bioclimatic regions of Europe, although the

350 biologically-based field data do not completely correspond with the calculated O_3 metrics. For better explanation of differences in visual O_3 symptoms, further studies concerning O_3 resistance of pine species in changing real soil humidity regime in subalpine zone are needed. In future research, closer attention should be paid to the entire growing season as well as night time O_3 fluxes, since both these time spans appear to play an important role in estimates of POD in mountain forests.

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Supplement Part SI–1: Details in section Methods: DO$_3$SE model parameterization (Table S1)

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