Modelling ozone stomatal flux of wheat under mediterranean conditions

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HIGHLIGHTS

- ► Models to estimate ozone deposition flux need to be tested in the field.
- ► A new ozone flux model parameterization for Mediterranean wheat is proposed.
- ► Modelled ozone flux for Mediterranean wheat was higher than previously estimated.
- ► Regional model parameterizations are more suitable for ozone risk assessment.
- ► Field validation of ozone effects on wheat yield in Southern Europe is still scarce.

ABSTRACT

Correct estimation of leaf-level stomatal conductance (g_{sto}) is central for current ozone (O_3) risk assessment of wheat yield loss based on the absorbed O_3 phytotoxic dose (POD). The g_{sto} model parameterizations developed in Europe must be checked in the different climatic regions where they are going to be applied in order to reduce the uncertainties associated with the POD approach.

This work proposes a new g_{sto} model parameterization for estimating POD of *Triticum aestivum* and *Triticum durum* under Mediterranean conditions, based on phenological observations over 25 years and g_{sto} field measurements during 5 growing seasons. Results show that POD in the Mediterranean area might be higher than previously estimated. However, caution must be paid when assessing the risk of yield loss for wheat in this area since field validation of O_3 impacts is still limited.

Keywords:
Triticum aestivum
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Phenology
Stomatal conductance
Ozone risk assessment
Phytotoxic ozone dose

1. Introduction

Tropospheric ozone (O_3) is considered one of the most phytotoxic air pollutants, able to induce negative effects on the growth, productivity and yield quality of many crops (Ashmore, 2005; Booker et al., 2009) as well as native plant species (Davison and Barnes, 1998; Skärby et al., 1998). Ozone negative effects have been identified in many parts of the world, including Europe, Australia, North and South America and Asia (Ashmore, 2005; Booker et al., 2009; Feng and Kobayashi, 2009).

In Europe, peak O_3 concentrations are gradually declining but background ozone concentrations are still rising and predicted to continue this upward trend in the next decades if current emission levels persist (Dentener et al., 2006; Jonson et al., 2006). In the Mediterranean area, high precursor emission rates of both anthropogenic and natural origin add up to pollution transported from central Europe to produce O_3 concentrations above the air quality standards (Cristofanelli and Bonasoni, 2009; EEA, 2011).

Indeed, current ambient O_3 levels have been shown to induce negative impacts on the production and quality of over 20 agricultural and horticultural crop species of economical importance across the Mediterranean basin (Fumagalli et al., 2001).

Ozone critical levels (CLe) have been proposed for the protection of vegetation under the framework of the Convention on Long-Range Transboundary Air Pollution (CLRTAP/UNECE), being the base of the European Directive on Air Quality (2008/50/EC) objectives for plant protection. Current O_3 CLe and target values for the protection of vegetation in the European legislation are based on the AOT40, the cumulative exposure to O_3 atmospheric concentrations over 40 nl l^{-1}. Recently, an improved approach has been introduced where effects can be assessed in relation to stomatal ozone uptake or flux and new flux-based CLe have been derived for certain plant species (Mills et al., 2011b). The flux approach for O_3 CLe derivation relies on the ability to adequately estimate stomatal conductance (g_{sto}) and the availability of species-specific dose–response relationships. The stomatal uptake of ozone at the leaf level over the sensitive phase of plant development or Phytotoxic O_3 Dose (POD) is modelled using the Deposition of Ozone and Stomatal Exchange deposition model (DO$_3$SE) (UNECE, 2010). DO$_3$SE is currently included within the European Monitoring and Evaluation Programme (EMEP) photo-oxidant chemical transport model, which is used by the CLRTAP to inform European air pollution abatement strategies (Simpson et al., 2007).

Much attention has focused on assessing the risk posed to crop yield by O_3 pollution under present and future climate conditions (Ashmore, 2005; Avnery et al., 2011; Feng and Kobayashi, 2009). AOT40 and POD maps across Europe are being used for this task (Simpson et al., 2007). However, uncertainty levels in the estimation of POD are dissimilar in different regions and are especially high in Southern Europe (Mills et al., 2011a, b). Climatic conditions in Southern Europe differ from Northern Europe due to higher temperature and vapour pressure deficit conditions, less rain and severe summer droughts. The response of g_{sto} under such conditions has not been tested for many crops, studies about the cultivar variability on maximum g_{sto} (g_{max}) in Southern Europe are still lacking and phenological models have not been validated in the Mediterranean area. For these reasons, current g_{sto} model parameterizations for the estimation of O_3 risk in Europe based on the POD approach, developed using measurements from Central and Northern Europe except for g_{max} estimation which considers data from Spain (Grünhage et al., 2012; Pleijel et al., 2007; UNECE, 2010), must be validated in different regions before being applied at pan-European level.

Wheat is an important O_3 sensitive crop (Feng et al., 2008; Mills et al., 2007; Pleijel et al., 2007), the largest food crop grown on a European scale and also in Northern Mediterranean countries (Spain, Italy, Portugal and Greece), where wheat (durum + bread wheat) covers 5.1 million ha (EU, 2011) mostly under rainfed growing conditions. Eight times more surface of wheat is grown under rainfed than under irrigated conditions in Spain (MARM, 2010). Approximately half of the surface covered by wheat in the Mediterranean countries of Europe is durum wheat (*Triticum durum* Desf.), but in Italy and Greece durum wheat area is two times greater than bread wheat (*Triticum aestivum* L.). The opposite is the case in Spain and Portugal, where bread wheat is more important. However, the relative importance of bread and durum wheat can vary within a country. In Spain, the area of bread wheat decreases from North to South, from 94% of the surface covered by wheat in the Northern third of the country through to 58% and 35% in the Central and Southern thirds respectively (MARM, 2010) in parallel with increasingly warmer and drier climates.

Considering its importance for the European agriculture and its sensitivity to O_3, wheat was designated a representative species for O_3 risk assessment of agricultural crops across Europe (Mills et al., 2011b). Dose–response relationships have been developed experimentally across a range of climatic conditions in Europe (Grünhage et al., 2012; Pleijel et al., 2007), but g_{sto} model parameterizations have not been compared with g_{sto} field measurements in a sufficient number of areas to be representative of all European bioclimatological regions. The objectives of the present study were to define a relevant POD accumulation period for wheat based on phenological observations in Spain, to develop a g_{sto} multiplicative model parameterization for estimating POD under Mediterranean conditions, to test current g_{sto} parameterization for wheat under Mediterranean conditions and evaluate the uncertainty associated with the use of the POD approach to estimate the risk of O_3 damage to agricultural production under rainfed Southern European climatic conditions. The work was based on a combination of field data and published values. Long-term phenology datasets from 2011 back to 1982 from different regions in Spain were used to define a relevant accumulation period for POD. Furthermore, field gas exchange data collected on durum and bread wheat cultivars for 5 growing seasons combined with a review of the peer-review literature reporting on gas exchange data measured under Mediterranean conditions were used to parameterize a g_{sto} multiplicative model for durum and bread wheat growing in Southern Europe.

2. Materials and methods

2.1. Phenology

Phenology observations were obtained from field performance tests developed by the agricultural research institutes ITGA (Navarra, Northern Spain), IRTA (Cataluña, North-eastern Spain), IMIDRA (Madrid, central Spain) and published reports of the GENVCE network (Spanish Group for the Evaluation of New Varieties of Extensive Crops, Ministry of Agriculture, Food and Environment) (GENVCE, 2012). The data from Navarra, Cataluña and Madrid was analysed with the objective of defining the POD accumulation period for wheat under Mediterranean conditions. The data at national scale from GENVCE was used to validate the estimations obtained with the other three regions.

Table 1 presents some detailed information of the phenology dataset. Three regions, Navarra, Cataluña and Madrid, which were considered as representative of Mediterranean with Atlantic climate influence, coastal Mediterranean and continental Mediterranean climates respectively were used to estimate the mean dates of selected phenological events. They constitute a wide range of the most common climates where wheat is grown in Spain. Each region contains data from two to seven sites grouped by agro-climatic zones, areas with homogeneous climatic characteristics for agricultural production, and a number of years per site varying between two and 17 (Table 1). Phenological observations in Navarra, Cataluña and Madrid included sowing and inflorescence emergence (IE) dates of a total of four durum and nine bread wheat commercial cultivars from 14 sites over a period of 25 years. Navarra and Madrid datasets also included booting and maturity (M) dates while the period 1999–2002 in one of the sites in Madrid included weekly phenological records as part of the field measurements described in Section 2.3.

In the Mapping Manual methodology for POD calculation (UNECE, 2010), is stated that the POD accumulation period coincides with the lifespan of the flag leaf, from flag leaf stage (FL) to M (FL – M), and phenological events are expressed relative to anthesis (A) date. Dates of FL, A and M in the phenology dataset were

Table 1

Summary of phenology observations from the GENVCE network. Datasets from Navarra, Cataluña and Madrid were used to define the most relevant dates for the flag leaf – maturity growing stage period in Spain. The dataset covering Spain was used to test the anthesis temperature sum at country level. The years in parenthesis show the time range covered by each dataset.

Region	Climate	Agroclimate	No. sites	Years	Wheat cultivars
Navarra (ITGA)	Mediterranean with Atlantic climate influence	Baja Montaña	4	8	*T. aestivum* (Marius, Soissons)
		Intermedia	1	8	
		Media	2	7 (1992–2001)	
Cataluña (IRTA)	Coastal Mediterranean	Secans Semifrescals	2	5	*T. aestivum* (Etecho, Marius, Soissons, Tremie)
		Secans Frescals	2	5	
		Girona Interior	1	6 (1995–2000)	
Madrid (IMIDRA)	Continental Mediterranean	Zona II	1	23	*T. aestivum* (Aragón03, Arganda, Exotic, Pane247, Pirón)
		Zona III	1	6 (1982–1986, 1989, 1991–1996, 1999–2006, 2008–2010)	*T. durum* (Camacho, Don Pedro, Roqueño, Vitrón)
Spain (GENVCE)				5 (2007–2011)	*T. aestivum* (CCB Ingenio, Marius, Paledor, Soissons) *T. durum* (Amilcar, Avispa, Don Pedro, Gallareta, Simeto, Vitrón)

estimated from IE observations using the average FL – IE, IE – A and IE – M number of days of phenology records from Navarra and Madrid.

Phenological events were then expressed in terms of effective temperature sum accumulation (°C.day), where daily averaged temperatures above zero are accumulated over a base temperature of 0 °C. Temperature sum to A was accumulated from the 1st January or from plant emergence if later (UNECE, 2010). Temperature sum to FL and M were calculated over a base temperature of 0 °C at A (UNECE, 2010). Daily maximum and minimum temperatures to calculate temperature sums (McMaster and Wilhelm, 2003) were obtained from the closest meteorological stations managed by the Meteorology and Climatology Network of Navarra, Agrometeorology Network of Cataluña, the Madrid Air Quality Network and the Spanish Agency of Meteorology (AEMET) as appropriate. The meteorological stations were located within a radius of 10 km except in three cases, where the closest stations were located at less than 22 km.

Temperature sums to A were compared within and across agroclimatic zones using data from those trials that were performed simultaneously in the different sites in order to minimize the variability of the data.

The GENVCE data (Table 1), including five year IE dates of bread and durum wheat cultivars, was used to check the validity at national scale of estimated temperature sum to A. A date was estimated using the average IE – A number of days obtained analyzing data from Navarra and Madrid. Mean A date at national scale was calculated for each year and it was assumed that A date in Navarra, Cataluña and Madrid coincided on average with the national mean date during the 2007–2011 period. Temperature sum to A was accumulated from January 1st until the annual mean A date using temperature data from one site per region analysed (Navarra, Cataluña and Madrid) during the same 5-year period. Sites were selected according with the availability of suitable meteorological data and abundance of phenological records. Finally, temperature sums obtained using national scale data (2007–2011) were compared with the average (1982–2011) obtained in Navarra, Cataluña and Madrid.

2.2. Literature review of g_{max} under Mediterranean conditions

A review of the literature reporting g_{sto} measurements for wheat raised under Mediterranean conditions was performed to identify the range of g_{max} to O_3 values measured under these climatic conditions. All g_{sto} values were normalised on a projected leaf area basis multiplying adaxial g_{sto} by 1.43, the adaxial to abaxial g_{sto} ratio from measurements reported in the literature (Amundson et al., 1987; Araus et al., 1989). Stomatal conductance to CO_2 was transformed to H_2O and then to O_3 using the ratio of molecular diffusivity between CO_2 and H_2O and between O_3 and H_2O in air, $D_{CO_2/H_2O} = 0.64$, $D_{O_3/H_2O} = 0.663$ respectively (Larcher, 2003; Massman, 1998).

2.3. Gas exchange field measurements

Wheat g_{sto} was measured at El Encín Experimental Station – IMIDRA (Alcalá de Henares, Madrid, 40°31′N; 3°17′W), in central Spain. This site has a Mediterranean climate, with mild, humid winters followed by dry, hot summers. Average annual rainfall in the area is 378 mm and the annual mean temperature is 14.1 °C (1971–2000) (AEMET, 2012). Wheat was grown following common agricultural practices in the region, mostly under rainfed conditions but some measurements in watered fields were also included. Over 500 measurements of gas exchange were performed during five growing seasons (1998–2002 and 2009–2010) in winter sown bread wheat, cultivars Arganda (5 seasons), Exotic (1 season) and Pirón (1 season), and durum wheat, cultivars Camacho (5 seasons) and Peñafiel (1 season). Measurements were taken using a portable infrared gas exchange analyzer (LiCor models 6200 and 6400, LiCor Inc., Lincoln, NE, USA) on fully developed flag leaves, from the flag leaf stage until flag leaf senescence.

Meteorological values, photosynthetic active radiation, air temperature and humidity and wind speed at 3 m high were collected from the closest air quality monitoring station operated by the Air Quality Network of Madrid (Comunidad de Madrid) located in Alcalá de Henares (40°28′N; 3°22′W), at 6 km from the experimental site. Meteorological measurements were not corrected for vertical profiles down to canopy height. Soil humidity was determined weekly in the field using a tensiometer (Delta T Devices, HH2 Moisture Meter, PR1 profile probe) between 1998 and 2002. Continuous soil moisture measurements were collected in 2010 using ECH$_2$O SH20 probe (Decagon Devices, Inc. Pullman, WA, USA) buried at 10 cm depth.

2.4. Stomatal conductance modelling and POD calculation

Measurements of g_{sto} were used to parameterise a multiplicative model (Jarvis, 1976) where g_{sto} is estimated from functions describing the response of stomata to key species-specific and environmental variables. Equation (1) shows the g_{sto} model used in this study (UNECE, 2010):

$$g_{sto} = g_{max} \cdot f_{phen} \cdot f_{light} \cdot \max\left\{f_{min}; \left(f_{temp} \cdot f_{VPD} \cdot f_{SWC}\right)\right\} \quad (1)$$

where, g_{sto} represents the stomatal conductance to O_3 per unit projected leaf area (mmol O_3 m^{-2} s^{-1}); g_{max} represents maximum g_{sto} while f_{phen}, f_{light}, f_{temp}, f_{VPD} and f_{SWC} represents the influence of phenology, photosynthetically active radiation, air temperature, vapour pressure deficit and soil water content on g_{max}, respectively; f_{min} represents minimum stomatal conductance. g_{max} was calculated based on the average g_{sto} above the 90th percentile measured under optimum meteorological conditions for stomatal opening (González-Fernández et al., 2010). Optimum conditions were defined using the relationships obtained between g_{sto} and meteorological values from the measurements. The relationship between g_{sto} and the climatic variables was analysed by applying a boundary line approach to the data cloud (González-Fernández et al., 2010; Pleijel et al., 2007).

Two functions were tested in this model in order to describe the stomatal closure of wheat due to reductions of the plant water potential during the course of the day that prevents stomatal reopening in the afternoon: the f_{time} boundary line (Danielsson et al., 2003) and a $\sum$VPD routine (UNECE, 2010).

Calculations of O_3 uptake per unit leaf projected area or POD, were based on the guidelines provided in the CLRTAP Mapping Manual (UNECE, 2010). Hourly mean O_3 concentrations, obtained from the closest air quality monitoring station (Air Quality Network, Comunidad de Madrid) at Alcalá de Henares, were corrected to canopy height (1 m) concentration using the tabulated gradient method (UNECE, 2010). POD was accumulated on an hourly basis over the life-span of the flag leaf. The time window was selected accordingly with the model parameterizations.

POD was calculated for a field site in El Encín, central Spain. Four g_{sto} model parameterizations were used: two Mediterranean specific parameterizations for bread and durum wheat developed in this study; and two bread wheat parameterizations, full and generic, of the Mapping Manual (UNECE, 2010). The full parameterization considers all the modifying functions (Equation (1)) for g_{sto} modelling and is intended for quantifying the effect of O_3 on wheat yield using a specific dose–response relationship (UNECE, 2010). The generic parameterization is a simplified version which only takes into account the effect of temperature, light and VPD. The generic parameterization is designed for indicating the degree of risk of O_3 induced damage to crops (UNECE, 2010).

The comparison of the results is based on the POD over thresholds of 0, 3 and 6 nmol m^{-2} s^{-1}. Thresholds 3 and 6 nmol m^{-2} s^{-1} are used for pan-European O_3 risk assessment of wheat yield loss (using the generic parameterization) and flux-based O_3 CLe exceedance (using the full parameterization) respectively (UNECE, 2010).

2.5. Statistical analysis

Differences between means were analysed using ANOVA after normality and variance homogeneity checks. When these assumptions were not met, non-parametric Kruskal–Wallis test was used instead.

Simple regression analyses were used to quantify the relationship between measured and modelled g_{sto}. The goodness of fit for each model parameterization was tested with the R^2 coefficient, the root mean squared error (RMSE) and the difference with the $x = y$ line using 95% confidence intervals of the slope and the intercept of the regression between measured and modelled values.

3. Results

3.1. Phenology

Table 2 shows mean bread and durum wheat sowing and inflorescence emergence dates during the assays and the mean number of days for the periods sowing – plant emergence, FL-IE, IE-A and IE-M. Despite bread wheat sowing dates varied from region to region, IE dates were very stable across regions each year. Durum wheat showed a slightly shorter cycle, it was sown latter but IE and M occurred sooner than for bread wheat in Madrid.

Temperature sum to A was again fairly stable within and across regions (Table 3). No differences were found within Navarra agro-climatic zones, but statistically significant differences were found between the most Southern and Northern agro-climates in Cataluña (Kruskal–Wallis $P = 0.02$). No differences were found comparing temperature sum to A in Navarra and Cataluña nor Navarra and Madrid sites. Significant differences ($P = 0.05$; $df = 18$) were found comparing temperature sum to A of bread and durum wheat in Madrid.

The comparison between temperature sum to A (Navarra, Cataluña and Madrid) and the value obtained from GENVCE at national scale, showed that temperature sum to A was on average 66 ± 165 °C.day (4 ± 10 days) greater in GENVCE data for bread wheat and 138 ± 145 °C.day (9 ± 10 days) smaller for durum wheat. Significant differences were only found comparing temperature sum to A for durum wheat ($P < 0.001$, $df = 36$).

Average temperature sum to A, FL and M of bread wheat (Table 3) showed differences from values in the Mapping Manual (UNECE, 2010). Under Mediterranean conditions, A occurs 181 °C.day (12 days) later compared with current parameterizations in Europe (Grünhage et al., 2012; UNECE, 2010). FL-A temperature sum under Mediterranean conditions was 97 °C.day longer and A-M was 90 °C.day shorter than in the Mapping Manual (UNECE, 2010) and in Grünhage et al. (2012). The net effect is that the whole POD accumulation period for bread wheat under Mediterranean conditions is delayed, starting and finishing on average 8 and 5 days latter respectively compared with the Mapping Manual (Grünhage et al., 2012; UNECE, 2010) while durum wheat is delayed 5 and 3 days at the start and end of the accumulation period compared with the Mapping Manual.

Table 2
Mean dates of wheat phenological phases and mean number of days for the periods sowing – plant emergence, FL-IE, IE-A, IE-M. Flag leaf stage (FL), inflorescence emergence (IE), anthesis (A), maturity (M).

Wheat	Region	Sowing	Sowing - plant emergente (days)	FL – IE (days)	IE	IE – A (days)	IE – M (days)
Bread	Navarra	9/11			05/05		39 ± 6
	Cataluña	14/11			07/05		
	Madrid	24/11	28 ± 9	14 ± 4	07/05	8 ± 2	47 ± 8
Mean of bread wheat		13/11	28 ± 9	14 ± 4	07/05	8 ± 2	41 ± 7
Durum	Madrid	29/11	27 ± 8	14 ± 4	04/05	8 ± 2	42 ± 6

Table 3
Effective temperature sum of relevant phenological events for 3 regions in Spain and comparison with Mapping Manual (UNECE, 2010) values. Temperature sums to anthesis accumulated from 1st January, or plant emergence if latter, over a base temperature of 0 °C.day. Values to flag leaf stage and maturity relate to anthesis temperature sums. Mean ± SD. The number of assays used in each mean value is represented between brackets.

Wheat	Region	Species	Anthesis (°C.day)	Flag leaf (°C.day before anthesis)	Maturity (°C.day after anthesis)
Bread	Navarra	*T. aestivum*	1234 ± 113 (59)	−288 ± 28 (59)	565 ± 88 (59)
	Cataluña	*T. aestivum*	1268 ± 130 (99)	−299 ± 43 (99)	619 ± 54 (99)
	Madrid	*T. aestivum*	1256 ± 137 (16)	−319 ± 37 (16)	734 ± 143 (16)
Durum	Madrid	*T. durum*	1192 ± 98 (35)	−294 ± 59 (23)	673 + 136 (23)
Bread	Mean	*T. aestivum*	1256 ± 126 (174)	−297 ± 39 (174)	610 ± 89 (174)
	UNECE, 2010	*T. aestivum*	1075 °C.day	−200 °C.day	700 °C.day

3.2. *Maximum stomatal conductance*

Following the quality criteria outlined in Pleijel et al. (2007), papers reporting g_{sto} measurements performed under optimum environmental conditions providing full details of measuring conditions, in field-grown plants under Mediterranean climate conditions were used to estimate the range of g_{max} values measured in the field. Values reported in the peer-review literature were analysed in combination with those obtained from g_{sto} measurements performed in El Encín (Table 4).

No differences were found comparing bread and durum wheat g_{max} values. However, g_{max} from Mediterranean studies (bread + durum wheat) was significantly lower ($P = 0.007$, $df = 20$) than values reported in the Mapping Manual (UNECE, 2010).

3.3. *Stomatal conductance modelling*

Table 5 and Fig. 1 shows boundary line parameterizations derived for bread and durum wheat growing under Mediterranean conditions and, for comparison, the parameterization currently used for pan-European O_3 damage estimation (Grünhage et al., 2012; UNECE, 2010).

The Mediterranean g_{sto} parameterizations explained 75% and 43% ($P < 0.001$) of the variation in bread wheat (vars. Arganda, Pirón, Exotic) and durum wheat (vars. Camacho, Peñafiel) g_{sto} measurements under field conditions respectively. The regression of modelled and observed g_{sto} values (Fig. 2) showed statistically significant differences from the $x = y$ line intercept in bread wheat, 95% CI: (0.02, 0.07), and in durum wheat with the slope, 95% CI: (0.69, 0.94), and intercept, 95% CI: (0.03, 0.09). Including an f_{time} function improved model results in bread ($R^2 = 0.79$, RMSE = 0.15 mol m^{-2} s^{-1}, n.s different from $x = y$ line) and durum wheat ($R^2 = 0.51$, RMSE = 0.13 mol m^{-2} s^{-1}, slope 95% CI: (0.72, 0.96)). However, the analysis of field measurements revealed that g_{sto} remained high until daily $\sum$VPD values close to 16 kPa (data not shown) and its inclusion in the g_{sto} algorithm did not improve modelling results.

The Mapping Manual g_{sto} model full parameterization explained 45% ($P < 0.001$) of the variation in bread wheat g_{sto} measurements, with significant differences from the $x = y$ slope 95% CI: (0.31, 0.39) and RMSE = 0.29.

3.4. *Ozone stomatal fluxes under Mediterranean conditions*

The POD was modelled for central Spain field conditions in 2010. The results obtained using 0, 3 and 6 nmol m^{-2} s^{-1} POD thresholds can be compared in Table 6.

Considering full parameterizations, modelled POD0 using the Mediterranean parameterization yielded the highest value for bread wheat, increasing the value estimated using the Mapping Manual full parameterization by 6%. Fig. 3 presents the values of the f functions of the g_{sto} model using the Mediterranean parameterization for bread wheat during the 2010 POD accumulation period. POD using this parameterization was mainly limited by soil humidity, plant phenology and temperature. The inclusion of an f_{time} function that reduced g_{sto} in the afternoon hours decreased POD0 by 67% compared with the Mediterranean parameterization (data not shown). The generic wheat parameterization of the Mapping Manual resulted in much higher POD values (genPOD) compared with the rest. genPOD3 values were 2.1 and 2.8 times those estimated for bread wheat using the Mediterranean or full Mapping Manual parameterizations respectively.

The Mediterranean durum wheat g_{sto} parameterization yielded 26% higher POD0 values than its equivalent parameterizations for

Table 4
Maximum stomatal conductance (g_{max}) values reported in field studies performed under Mediterranean conditions. Values were measured on flag leaves under optimum environmental conditions for maximum stomatal opening. Mean ± SD.

Reference	g_{max} (mmol O_3 m^{-2} PLA s^{-1})	Wheat species and type	Cultivar	Country	Device
Araus et al., 1989	479	*T. aestivum*	Kolibri	Spain	LICOR 1600
Araus et al., 1989	372	*T. aestivum*	Astral	Spain	LICOR 1600
Araus et al., 1989	363	*T. aestivum*	Boulmiche	Spain	LICOR 1600
Araus et al., 1989	459	*T. durum*	Senatore Capeli	Spain	LICOR 1600
Araus and Tapia, 1987	361	*T. aestivum*	Kolibri	Spain	LICOR 1600
del Pozo et al., 2005.	338	*T. aestivum*	Alcalá	Spain	CIRAS-2
Sato et al., 2006	450	*T. aestivum*	Cham4, Cham6, Bloyka1, Qafza8 and Qimma5	Syria	LICOR 1600
Field study	631	*T. aestivum*	Arganda	Spain	LICOR 6400
Field study	362	*T. durum*	Camacho	Spain	LICOR 6400
Mean values					
T. aestivum	Mean = 428 ± 103		Range: 338–631		
	Median = 372		$n = 7$		
T. durum	Mean = 410 ± 68		Range: 362–459		
	Median = 410		$n = 2$		
UNECE, 2010	Mean = 497		Range: 366–660		
	Median = 492		$n = 17$		

Table 5
Parameterization of the multiplicative model to estimate g_{sto} for bread and durum wheat growing under Mediterranean conditions and that used in the Mapping Manual (UNECE, 2010) for bread wheat, where f_{min} is the fraction of g_{max} at minimum g_{sto}; A_{start} and A_{end} represent the start and end of the accumulation period in degree-days relative to anthesis (defined using phenological information from Table 1); a and b are the difference between the maximum g_{max} fraction (1) and the f_{phen} value at the start of flag-leaf senescence and A_{end} respectively; fraction of g_{max}; e, f, g, h, i, are the temperature sums at A_{start}, anthesis, end of maximum g_{sto}, start of flag-leaf senescence and temperature sum at which f_{min} is reached; α is the rate of saturation of g_{sto} in response to photosynthetic active radiation; *Morning, max* and *afternoon* are the time of day when f_{min}, g_{max} and f_{min} are recorded; T_{min} and T_{max} denote the temperatures below and above g_{sto} is limited to f_{min}; T_{opt} is non-limiting temperature for g_{sto}; VPD_{max} and VPD_{min} define the level when vapour pressure deficit starts to limit g_{sto} and f_{min} are reached respectively; t is the minimum non-limiting percentage of plant available soil water; SWC_{max} and SWC_{min} are the minimum non-limiting soil water content and the value bellow which g_{sto} is limited to f_{min} respectively.

Function	Parameter	Units	Bread wheat *(T. aestivum)*	Durum wheat *(T. durum)*	Mapping Manual 2010
f_{min}		Fraction	0.01	0.01	0.01
f_{phen}	A_{start}	°C.day	−300	−300	−200
	A_{end}	°C.day	600	675	700
	a	Fraction	0	0	0.3
	b	Fraction	0	0	0.7
	e	°C.day	−300	−300	200
	f	°C.day	0	0	0
	g	°C.day	70	100	100
	h	°C.day	0	0	525
	i	°C.day	550	675	700
f_{light}	α	Constant	0.0105	0.0105	0.0105
f_{time}	*Morning*	Hour	–	7	–
	Max	Hour	12	13	–
	Afternoon	Hour	17	19	–
f_{temp}	T_{min}	°C	12	11	12
	T_{opt}	°C	28	28	26
	T_{max}	°C	39	45	40
f_{VPD}	VPD_{max}	kPa	3.2	3.1	1.2
	VPD_{min}	kPa	4.6	4.9	3.2
f_{PAW}	t	%	–	–	50
f_{SWC}	SWC_{max}	%	18.6	18	–
	SWC_{min}	%	4.7	4.1	–

Mediterranean bread wheat and 34% higher compared with the Mapping Manual full parameterization (Table 6). Again, as a result of the inclusion of an f_{time} function that reduced g_{sto} during the morning and afternoon hours, estimated POD0 was decreased by 26% (data not shown). The Mediterranean durum wheat parameterization showed the highest POD3 estimated under central Spain climatic conditions for wheat species but, still, estimated genPOD3 was 63% higher.

The effect of important sources of uncertainty on POD0 estimation in the Mediterranean area is presented in Fig. 4. The maximum uncertainty ranges, 38% and 32% for bread and durum wheat respectively, were calculated using the standard deviation of the start and end of the POD accumulation period and g_{max} values observed in the phenology and g_{max} datasets (Tables 3 and 4).

4. Discussion

Wheat POD modelling in Southern Europe shows higher uncertainties than in other parts of the continent due to the fact that current parameterizations in the Mapping Manual (UNECE, 2010) have been developed mostly using measurements from Central and Northern Europe. The main sources of uncertainty for POD modelling under Mediterranean conditions identified in this study are the definition of the accumulation period, the g_{max} value and the g_{sto} response to VPD and plant phenology.

One of the key issues for O_3 risk assessment is the accumulation period. A temporal matching between the highest O_3 concentrations and sensitive plant phenological phases and/or maximum physiological activity is not always found under Mediterranean rainfed growing conditions. The most O_3 sensitive phase for wheat yield is the reproductive one (Pleijel et al., 1998), which coincides approximately with the life span of the flag leaf (FL-M) and with maximum O_3 deposition velocities in the Mediterranean area over cereal crops like barley (Gerosa et al., 2004). Timing of this sensitive phase can be described with the help of a temperature sum (McMaster and Wilhelm, 2003; Pleijel et al., 2007). The dataset from Cataluña was used to evaluate the effect of using maximum and minimum daily temperature to calculate temperature sums to A. Temperature sums to A were increased by 5% compared with the value obtained using daily mean temperatures. Our results show that this method is appropriate to estimate A date of bread wheat growing under present climatic conditions in Spain, as indicated by the lack of major differences across Spanish regions on average temperature sum to A based in a dataset over 25 years. The validation of the mean temperature sum with phenological observations at national level for five years confirms that the value obtained is suitable to be used at national scale for bread wheat. Differences of temperature sum to A in durum wheat between the experimental site in central Spain and the value at national scale, though relatively small, indicate the need to extend the database, especially from Southern Spain and other Mediterranean countries where *T. durum* is the most abundant wheat species.

The comparison between FL-M temperature sum for bread wheat in Spain with the Mapping Manual (UNECE, 2010), show that the observed start and end of the POD accumulation period happened on average 6.5 days later under Mediterranean conditions, despite A date happened on average 12 days later in that region. Differences varied across years, reaching a maximum shift of 15 days at the start and 6 days at the end of the growing season. The small differences in the accumulation period despite the relatively large difference in A date were a result of the longer FL-A period and shorter A-M period shown by the field data. This could be reflecting the importance of the booting-A phase for yield which has been favoured in wheat breeding under Mediterranean conditions (Isidro et al., 2011). The shorter A-M period is probably due to faster development of plant senescence under increasingly stressful conditions at the end of the growing cycle, a usual situation in Mediterranean regions (Hafsi et al., 2007; Wardlaw, 2002).

The estimation of g_{sto} in response to environmental variables and plant phenology is also a central issue for O_3 effect

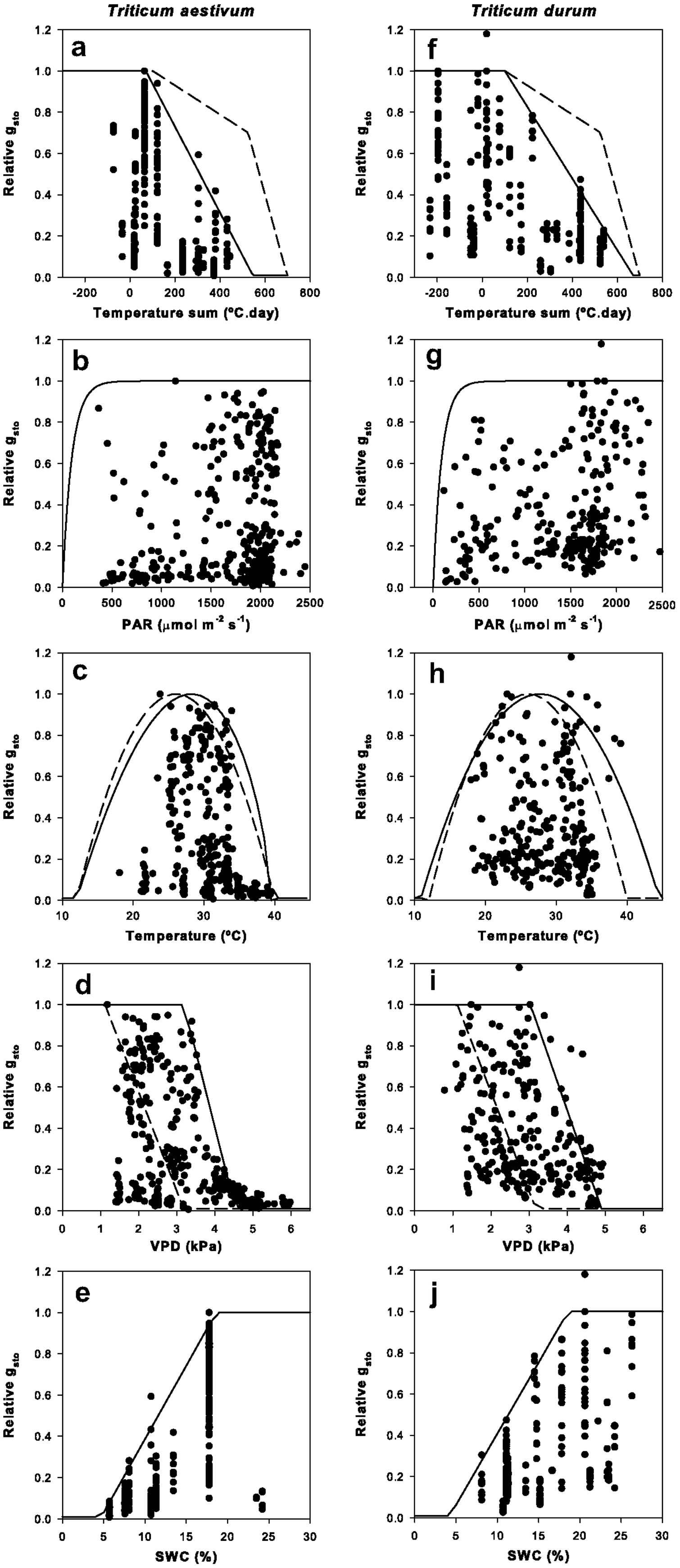

Fig. 1. Stomatal conductance (g_{sto}) measurements of bread wheat (*T. aestivum*) (a, b, c, d, e) and durum wheat (*T. durum*) (f, g, h, i, j) and boundary lines used in g_{sto} multiplicative models. The figure includes the boundary lines used for the phenology function (a, f), the photosynthetic active radiation (PAR) response function (b, g), the temperature function (c, h), the vapour pressure deficit (VPD) function (d, i) and the soil water content (SWC) function (e, j). The solid lines represent the Mediterranean parameterizations for each species. The dashed line is the wheat full parameterization of the Mapping Manual (UNECE, 2010) shown for comparison.

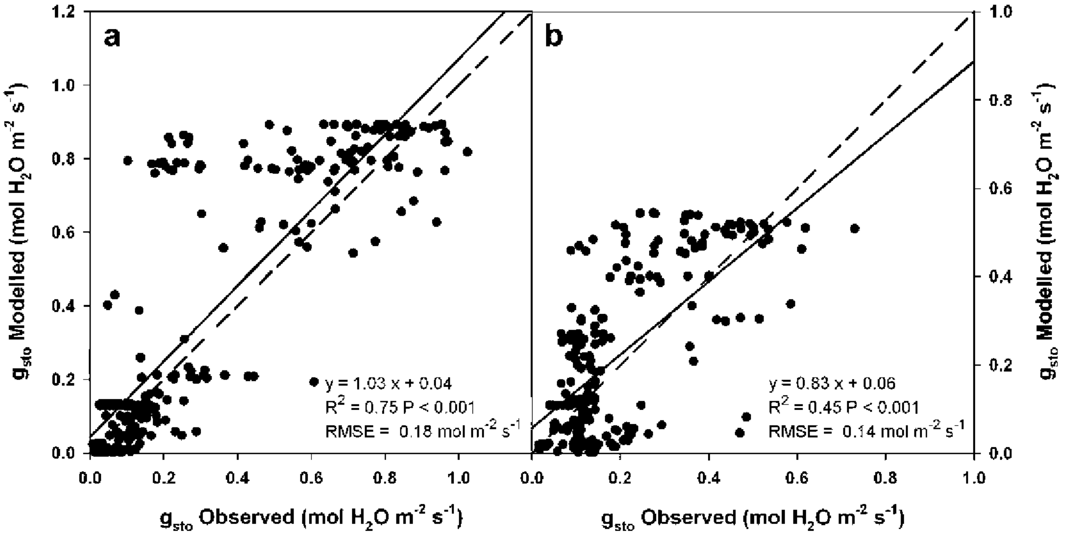

Fig. 2. Modelled vs. measured leaf level stomatal conductance in *Triticum aestivum* cultivars Arganda, Pirón and Exotic (a) and *Triticum durum* cultivars Camacho and Peñafiel (b).

quantification and risk analysis. g_{max} constitutes one of the most important parameters due to its influence on final estimates of POD (González-Fernández et al., 2010). Different approaches were used to calculate g_{max} in the studies listed in Table 4. The g_{max} calculated using values above the 90th percentile for cultivars Arganda and Camacho are based on the mean of 15 and 12 values, compared with 4–10 measurements in other studies. The coefficient of variation (CV) of g_{max} of Arganda and Camacho, 1.6 and 4% respectively, show that the variability of the g_{sto} values used in the calculation was small and using a different approach would result in a similar g_{max} value. Thus the methodologies were judged comparable in this case. The comparison between studies revealed that g_{max} of wheat growing under Mediterranean conditions was lower compared with that used in the Mapping Manual (Grünhage et al., 2012; UNECE, 2010). However, no indication was found in the literature of constitutively lower g_{sto} of wheat growing under Mediterranean conditions due to regional adaptations of wheat to Mediterranean growing conditions, though environmental conditions like early-season drought may cause persistent reductions of g_{sto} in certain cultivars (El Hafid et al., 1998). Significant g_{max} variability was observed among the different Mediterranean studies and the extent of the variation (22%) is consistent with that of studies reporting data for multiple cultivars, ranging from 8 to 40% (González-Fernández et al., 2010; Grünhage et al., 2012; Jiang et al., 2000; Xue et al., 2002). The variability observed in g_{max} values across cultivars was more important than phenology in its contribution to the uncertainty range found in the final POD0 estimation (Fig. 4). This uncertainty is greater than that associated with the measurement of environmental variables, the estimation of leaf-level ozone concentration and the calculation of g_{sto} to O_3 – excepting the g_{max} choice – which was estimated to be around 1% of the final POD0 value (Gerosa et al., 2012a).

Table 6
Modelled phytotoxic O_3 dose (POD, mmol m^{-2}) under field conditions in 2010 in central Spain over thresholds of 0, 3 and 6 nmol m^{-2} s^{-1} using different g_{sto} model parameterizations for bread and durum wheat.

Wheat species	Parameterization	POD0	POD3	POD6
Bread wheat (*T. aestivum*)	Mediterranean	5.0	1.5	0.2
Durum wheat (*T. durum*)	Mediterranean	6.3	1.9	0.2
Bread wheat (*T. aestivum*)	Mapping Manual - full	4.7	1.1	0.0
Bread wheat (*T. aestivum*)	Mapping Manual - generic	10.7	3.1	0.1

The g_{sto} response to environmental variables differed between Mediterranean cultivars and cultivars from Central and Northern Europe (Table 5, Fig. 1). Mediterranean bread and durum wheat cultivars showed greater g_{sto} than Northern and Central European cultivars under warm and dry conditions. The response of g_{sto} under high VPD observed in the field measurements (Fig. 1) has been previously reported in other studies showing that wheat can keep its stomata open under VPD values over 3.0 kPa, this response being subject to considerable intra-specific variability (Feng et al., 2012; Xue et al., 2004). In agreement with this result, our field measurements show that g_{sto} of Mediterranean cultivars was less sensitive to the accumulated hourly VPD ($\sum$VPD) than Central and Northern European cultivars. $\sum$VPD is used in the multiplicative model to describe a reduction of the plant water potential during the course of the day that prevents stomata reopening in the afternoon when $\sum$VPD is over 8 kPa (Pleijel et al., 2007). This process was also observed in this study but field measurements showed high g_{sto} until $\sum$VPD two times the threshold established in the Mapping Manual for stomatal closure (UNECE, 2010). The stomatal closure in the afternoon can also be incorporated by means of an f_{time} function that has been already used in previous g_{sto} modelling algorithms (Danielsson et al., 2003; González-Fernández et al., 2010). In this study, the use of f_{time} improved modelling results for durum and bread wheat. However, the use of the hour of the day as an input variable by f_{time} is probably a local-specific variable and is not recommended for POD estimation at regional scales.

High g_{sto} under high T and VPD may also suggest that adaptations of Mediterranean cultivars to unfavourable conditions have occurred. Several studies indicate that drought tolerance and productivity of wheat and barley in dry environments of the Mediterranean basin is associated with relatively high values of g_{sto} and lower canopy temperature (El Hafid et al., 1998; Fischer et al., 1998; Giunta et al., 2008; González et al., 1999). One of the mechanisms explaining the g_{sto} tolerance to heat and drought of barley and wheat growing under Mediterranean conditions is their high capacity of osmotic adjustment, allowing optimum leaf turgor under drought stress (Blum and Pnuel, 1990; González et al., 1999). Also historical perspectives have shown that the spread of agriculture has triggered regional and local adaptations of wheat to a wide array of environmental conditions (Araus et al., 2007) and that these local adaptations in the Mediterranean basin could result

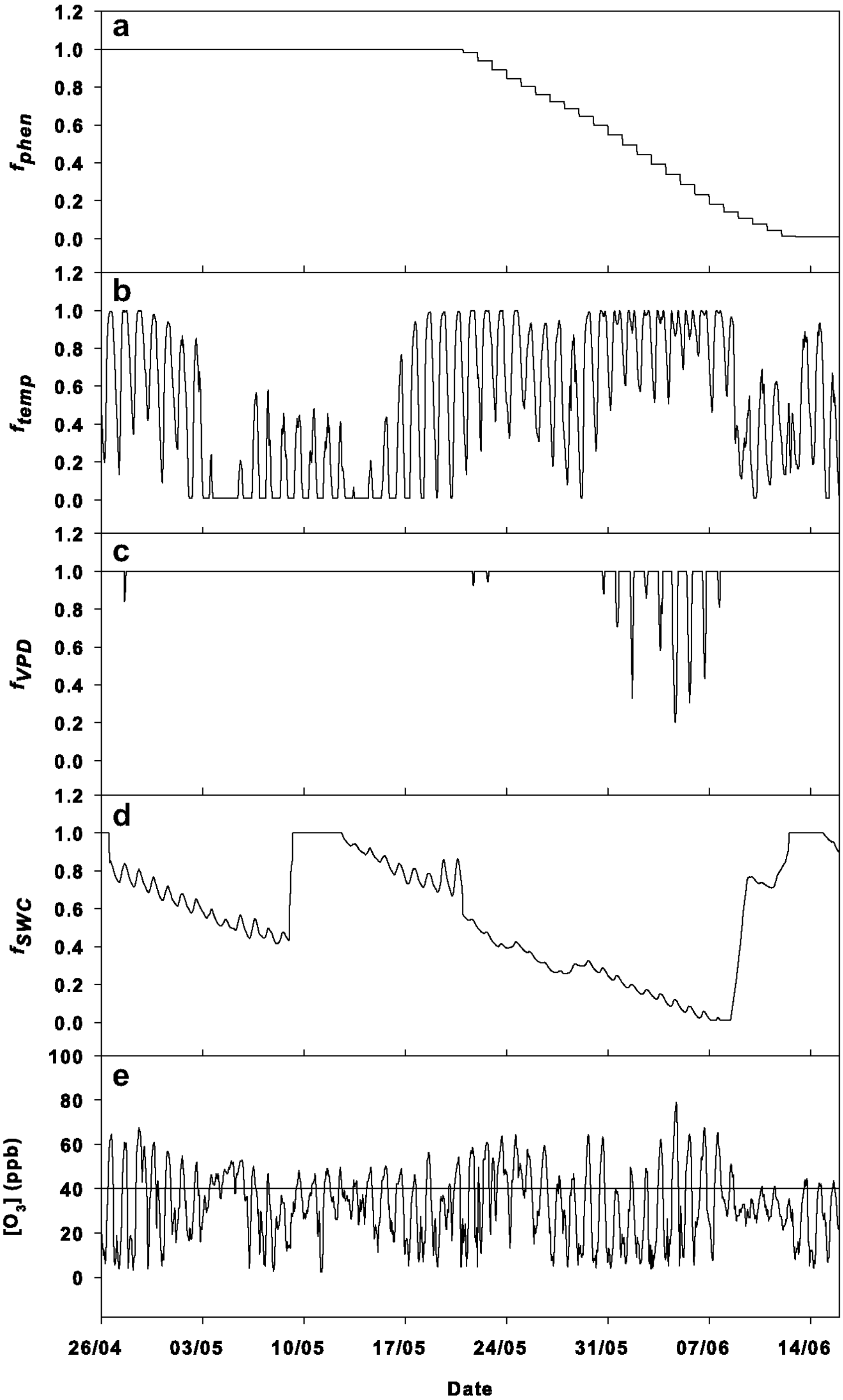

Fig. 3. Values taken by the modifying functions of the g_{sto} multiplicative model using the Mediterranean bread wheat parameterization during the POD accumulation period in 2010: f_{phen} (a), f_{temp} (b), f_{VPD} (c), f_{SWC} (d); and ozone concentrations (e) during the same period.

in tolerance differences to drought and heat stress among cultivars (Moragues et al., 2006).

Reparameterizing g_{sto} model to fit the response of Mediterranean wheat cultivars to environmental variables greatly improved the estimation of g_{sto} measured under field conditions. The new Mediterranean g_{sto} parameterizations explained 30% more variability of field g_{sto} data and reduced RMSE by 38% compared with the Mapping Manual parameterization (UNECE, 2010). This parameterization should be the choice for wheat g_{sto} modelling in Spain in flux based O_3 risk assessment exercises.

Despite lower g_{max}, the Mediterranean g_{sto} parameterizations yielded higher POD values than the Mapping Manual 2010 parameterization as a result of higher gas exchange rates under dry conditions, meaning that O_3 risks for wheat could be higher than previously estimated. A similar result was obtained modelling POD values across Spain only by setting up VPD_{max} and VPD_{min} with the same values of the present parameterization (de Andrés et al., 2012). However, the differences observed in the accumulation period and stomatal behaviour resulted in unexpectedly low changes in POD0 between model parameterizations in 2010.

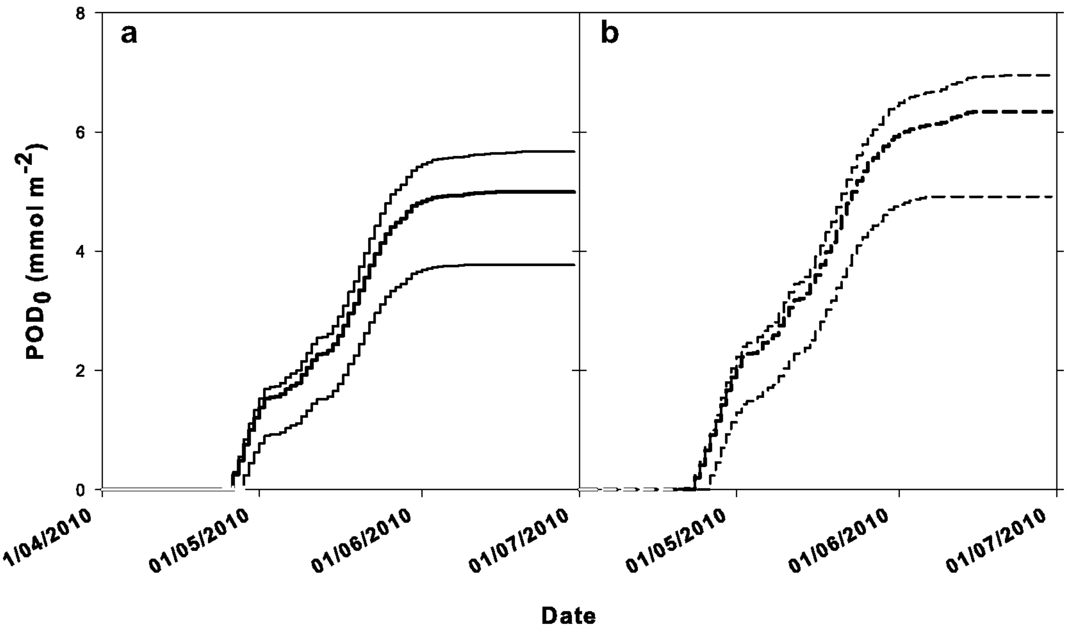

Fig. 4. Phytotoxic O_3 Dose without threshold (POD0) calculated in 2010 for a site in central Spain using Mediterranean-specific bread (a) and durum (b) wheat parameterizations for g_{sto} modelling. Dark grey lines in both graphs represent the uncertainty range of POD0 for bread and durum wheat respectively due to the variability in the accumulation period and g_{max} found in the databases.

This result was due to a compensation effect between g_{max}, f_{VPD} and f_{phen} under the specific environmental conditions of the growing season in 2010 in central Spain and is susceptible of changing depending on prevailing meteorological conditions during the growing season. These comparisons should be performed in different sites with appropriate data availability to derive firmer conclusions about the net effect of the Mediterranean parameterization.

The comparison of POD6 values derived from the different parameterization deserves particular attention since this index is currently used to estimate O_3 CLe exceedance of damage to bread wheat yield (UNECE, 2010). None of the parameterizations tested exceeded the POD6 CLe of 1 mmol m^{-2} established for wheat (Mills et al., 2011b) despite the AOT40 (1st April–30th June) exceeded (3.7 μl l^{-1} h) the concentration based CLe of 3 μl l^{-1} h (UNECE, 2010). Low fluxes were mainly caused by soil water content limitations to g_{sto} when O_3 concentrations were above 40 nl l^{-1} (Fig. 3). This result exemplifies the overestimation of O_3 risk to Mediterranean rainfed agriculture using exposure indices like the AOT40 that do not consider the modulation exerted by environmental conditions on plant physiological activity.

The new and more adjusted g_{sto} parameterizations for Mediterranean wheat proposed in this paper can help to reduce the uncertainty related with the use of flux-based CLe for wheat in Southern Europe. However, further information is required to check the representativeness of this parameterization in more sites of the Mediterranean area and to define the range of O_3 sensitivity of Mediterranean bread and durum wheat cultivars. Field validations are needed before quantifications of O_3 induced wheat yield losses can be performed in the Mediterranean area, since available results show that both sensitive and resistant cultivars are grown in this region (Badiani et al., 1996; Gerosa et al., 2012b; De Marco et al., 2010).

The genPOD3, currently used for pan-European risk assessment of crop loss (UNECE, 2010), resulted in non negligible discrepancies with POD3 estimated with the Mediterranean parameterizations, as illustrated in Table 6. Soil humidity and phenology are not considered by the generic wheat g_{sto} parameterization, which represents a significant simplification in areas where soil humidity is a key driver of crop physiology and O_3 absorption (Fig. 3) and interannual temperature variability can shift the phenological development of plants and change the timing of maximum O_3 sensitivity. A similar result was presented in a study from Germany, where the assumption of no water limitation in the g_{sto} model resulted in a strong increase in estimated POD6 and the degree of risk of O_3 damage (Grünhage et al., 2012). Although the generic approach might represent a worst case scenario, risk maps at European scale based on genPOD3 should be interpreted with caution as major departures from the estimated risk using regional parameterizations can occur.

The results of the present study stress the delicate equilibrium that have to exist between accuracy and model generalization. While the Mapping Manual full parameterization accumulation period for POD, f_{light} and f_{temp} function showed an acceptable agreement with the g_{sto} measurements performed in Spain, some other functions, like f_{VPD} or f_{phen} showed large discrepancies. The net effect of these changes on POD0 was small for one season in central Spain but it can vary depending on environmental conditions. Regional-specific g_{sto} model parameterizations have been already suggested as a way to improve flux-based risk assessment results in Europe, reducing the uncertainties associated with the estimation of g_{sto} which is central in POD modelling (Feng et al., 2012; González-Fernández et al., 2010; Paoletti and Manning, 2007). Where more general parameterizations are needed, models should cover the whole range of environmental conditions (temperature, air humidity, light, etc) where the model will be applied. In particular, the main drivers of g_{sto} in each area, such as soil water content in the Mediterranean area, need to be considered in larger scale modelling so that the accuracy of the results is sufficient in the different regions. Furthermore, estimations of POD should be accompanied by their uncertainty ranges derived from the variability of the parameters used by the models and other sources of error, which in the present study was 35% on average for POD0.

5. Conclusions

A new g_{sto} model parameterization for Mediterranean bread and durum wheat is proposed for O_3 risk assessment, following the methodology adopted within the LRTAP Convention. Results show that POD values in the Mediterranean area might be higher than previously estimated using current wheat full parameterizations.

The generic approach for POD estimation applied under Mediterranean conditions resulted in non negligible discrepancies with the regional parameterization since soil water availability and phenology, important limiting factors for g_{sto} in this region, are not considered. This study proved that Mediterranean-specific g_{sto} parameterizations can reduce the uncertainties associated with POD modelling for wheat in this area through direct consideration of cultivar characteristics and environmental conditions common in this area. Different wheat parameterizations could be used in the different European climatic regions for increasing the confidence of O_3 risk assessment studies. Still, the uncertainty range of regional-specific POD calculation was 35%.

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