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# Ozone and water use efficiency

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**Abstract.** This study has been carried out in the southern Italy, under conditions favourable to ozone pollution, together with soil and atmospheric drought. Soybean was subjected to well-watered and water stress conditions, and three levels of ozone (filtered, low and high) in open top chambers during three growing seasons. Cumulated AOT40 values were zero, 3400 and 9000 ppb.h for filtered (control), low and high ozone concentration treatments, respectively. Under well-watered conditions, the increase of ozone concentration levels leads to a reduction in daily actual evapotranspiration (AET) and yield. On the contrary, under water stress conditions, the increase of ozone concentration levels does not affect the examined parameters. Compared to the control treatment under well-watered conditions, total AET reduction was 14% and 28% at low and high ozone levels, respectively. In well-watered conditions, at a high level of ozone concentration, a yield reduction of 47% was observed, whereas an increase in ozone had no effect on yield of plants in water-stressed conditions. Cumulated (during the soybean season) data of AET ( $\Sigma$ AET) and grain yield (Y) allow to calculate the water use efficiency (WUE) by the ratio  $WUE = Y/\Sigma AET$ . As a result, during the 3-year study, significant relationships were found between AOT40 and relative (low or high to control ozone treatments) water use efficiency.

**Keywords.** Mediterranean region – Soybean – Drought – Irrigation – Atmospheric pollutants – Yield.

## Ozone et efficence d'utilisation de l'eau

**Résumé.** Cette étude a été réalisée au sud de l'Italie dans des conditions, favorables à la pollution par l'ozone, combinées à la sécheresse du sol et de l'atmosphère. Le soja bien irrigué ou sous stress hydrique, a été soumis dans des enceintes découvertes, à trois niveaux d'ozone (filtré, bas et haut) pour trois saisons. Les valeurs cumulées du AOT40 étaient nulles, de 3400 et 9000 pp b.h, pour le traitement filtré (le contrôle), à basse et à haute concentration, respectivement. Dans le soja bien irrigué, l'augmentation de la concentration de l'ozone porte à une réduction, de l'évapotranspiration actuelle journalière (AET) et du rendement. Au contraire, sous stress hydrique, l'augmentation de la concentration de l'ozone n'affecte pas les paramètres examinés. La AET totale sous des conditions de basse et haute concentration d'ozone, a subi une réduction du 14% et du 28% respectivement, en comparaison avec le contrôle bien irrigué. Le rendement a été réduit du 47% dans le traitement bien irrigué à haut niveau d'ozone, alors qu'une augmentation de l'ozone n'a pas eu d'effet sur le rendement des plantes sous conditions de stress hydrique. Les données cumulées du AET ( $\Sigma$ AET) (pour toute la période de croissance du soja) et le rendement en graines (Y) permettent de calculer l'efficence d'utilisation de l'eau (WUE) par le rapport  $WUE = Y/\Sigma AET$ . En conséquent, pour les trois années d'étude, des rapports significatifs ont été trouvés entre AOT40 et l'efficence relative de l'utilisation de l'eau (par rapport aux niveaux d'ozone bas et haut).

**Mots-clés.** Région Méditerranéenne – Soja – Sécheresse – Irrigation – Polluants atmosphériques - Rendement.

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## I – Introduction

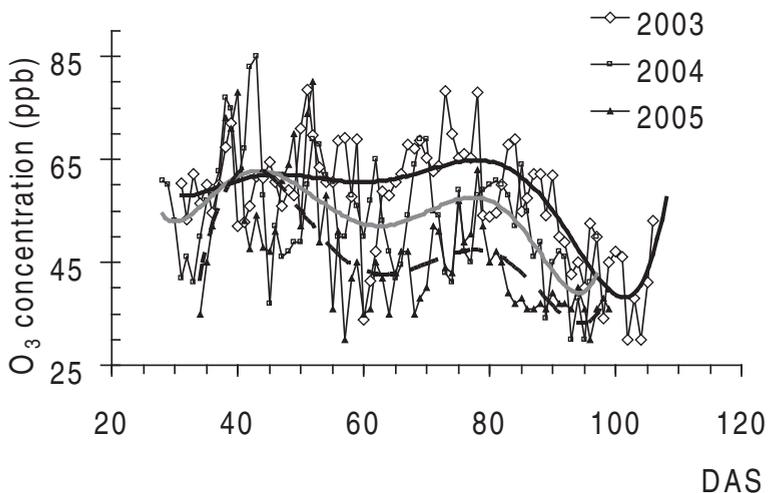
The Mediterranean region is an area prone to the development of photochemical oxidants (Bussotti and Ferretti, 1998; Alonso *et al.*, 2001; Forlani *et al.*, 2005). The typical climatic conditions of this region (high temperatures and solar radiation combined with stable air masses and high emission of air pollutants) favour the formation of secondary pollutants such as ozone (O<sub>3</sub>).

The objective of the study was to analyse, under natural conditions and for the entire growing season, the effect of ozone on the water use efficiency (WUE) of soybean. The effect was studied on soybean plants characterized by different conditions of water status.

## II – Materials and methods

Field trials were conducted during three successive years (2003-2005), at the experimental farm of CRA-SCA (Agricultural Research Council-Research Unit for Cropping Systems in Dry Environments). This site is located in Rutigliano (lat 40°59' N, Long 17°59' E, 147 m a.s.l.). Soybean (cv. Casa) was chosen for the study because it grows during the occurrence of high ozone concentration levels, together with a reduction in soil water content (due to the high evaporative demand). The variety had a late maturity, that insures a relatively longer ozone exposure period. As a reminder, soybean is considered sensitive to ozone according to the classification made by Mills *et al.* (2002).

During soybean crop season, an hourly basis monitoring of ozone concentrations was carried out over the experimental plots at 1m height from soil level, using an ozone analyser. Figure 1 presents the daily maximum ozone values, as well as average values on a weekly basis.

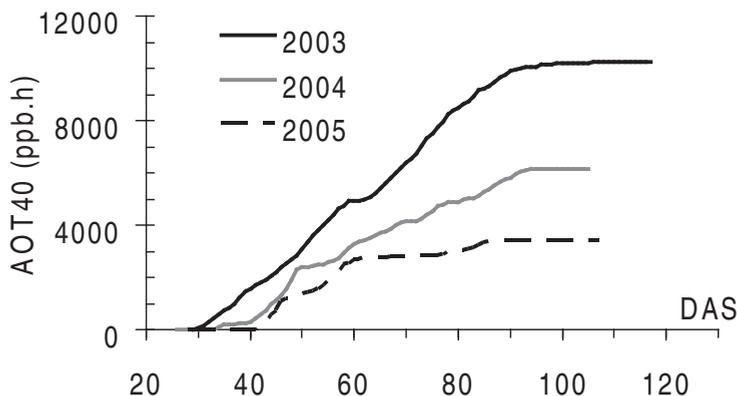


**Figure 1.** Daily maximum and mean values on a weekly basis of ozone concentration measured in the open field during 3 soybean growing seasons (2003, 2004 and 2005). DAS = Days After Sowing.

Maximum ozone values were generally higher during 2003, in comparison with the two following years. Ozone content in the atmosphere was evaluated through indices as AOT40, that is the accumulated hourly ozone values over the threshold of 40 ppb (Fuhrer *et al.*, 1997). When AOT40 cumulated during 90 days is higher than the threshold of 3 ppm.h, according to the EU directive 3/2002), plant functions, mainly gas exchange, leaf growth and yield, are affected (*i.a.* see the review by Bou Jaudé *et al.*, 2008 a and b). Cumulated AOT40 over soybean crop season varied by a ratio of 1 to 3 between 2005 (3392 ppm.h) and 2003 (10237 ppm.h). Even during the season less favourable to ozone rise (2005), the thresholds limits indicate by the EU directive have been exceeded.

Four Open Top Chambers (Heagle *et al.*, 1973) were used in this study. The ozone treatments were: 1) Control (C) involves OTC's supplied with a charcoal filter. This filter can reduce the amount of ozone in ambient air up to 50%. In general, ozone concentrations in control treatments are lower than the 40 ppb threshold. 2) High ozone concentration treatments (H) involve OTC's equipped with an ozone generator OZOMATIC (mod. 4, VKAU147974, Wedeco). Air was enriched with ozone in order to reach a maximum value of 75 ppb whenever the hourly ozone concentrations were lower than 60 ppb. The objective was to reach an AOT40 value of about 10000 ppb.h, which corresponds to the highest value observed in the open field during the study period. 3) Low ozone concentration treatments (L) involve natural ambient air. The objective was to reach an AOT40 value close to 3500 ppb.h, which corresponds to the lowest value observed in the open field during the study period.

Ozone concentration measurements were performed by an ozone analyser (OZ 2000 G), at a 1m height above soil level, in ambient air as well as in the OTC's.



**Figure 2. Daily cumulated AOT40 measured in the open field during 3 soybean growing seasons (2003, 2004 and 2005).**

Ozone effects were studied in combination with the plant water status. The two conditions of plant water status were: 1) Well-watered (W); 2) Water-stressed (S). An original method was adopted for irrigation scheduling. It was based on the experimental relationship found between the predawn leaf water-potential and the stomatal conductance of soybean (Mastrorilli *et al.*, 1993). Irrigation was scheduled whenever the predawn leaf water-potential reached  $-0.4$  MPa for the well-watered treatments (W) and  $-0.8$  MPa for the water-stressed treatments (S).

The symbols CW and CS represent the control OTC groups, well-watered and stressed, respectively. The symbols HW and HS represent the OTC's with high ozone concentration levels, well-watered and stressed, respectively. The symbols LW and LS represent OTC's with low ozone concentration levels, well-watered and stressed, respectively.

Daily actual evapotranspiration was calculated for each OTC through a simplified soil water balance method as follows:  $AET = P \pm \Delta Sw - Dr$ , where AET is the actual evapotranspiration in mm; P is irrigation (or rain) in mm;  $\Delta Sw$  is the daily variation in soil (root zone) water content measured by the time domain reflectometry method (TDR) in mm; Dr is drainage in mm. Drainage is the difference between soil water content (SWC) and field capacity (FC), when daily  $SWC > FC$ . The previous equation supposes that runoff is nil due to the use of a drip irrigation system and to the slopeless topography of the site; moreover capillary rise is nil due to the presence of a calcareous and impervious parent rock. The previous equation supposes that runoff is nil (due to the use of a

drip irrigation system and to the level topography of the site) and the capillary rise is nil (due to the presence of a calcareous and impervious parent rocky layer). These hypotheses were verified by Mastrorilli *et al.* (1998), who showed coherence between the AET measured by the Bowen ratio method and that calculated through a simplified soil water balance method. Cumulated (during the soybean season) data of AET allow to calculate the seasonal evapotranspiration ( $\Sigma$ AET).

At harvest, yield was analyzed for all the plants grown within each OTC. According to the review of Katerji *et al.* (2008), the water use efficiency (WUE) was calculated as the ratio between the yield ( $Y$  = dry matter of the grains) and  $\Sigma$ AET ( $WUE = Y/\Sigma AET$ ).

### III – Results

Some reviews were addressed to evaluate the effect of ozone on crops cultivated under Mediterranean climate of Italy (Ferretti *et al.*, 2007), Australia (Muray, 2003), South Africa (Van Tinhooven and Scholes, 2003), and Egypt (Abdel-Latif, 2003). However these syntheses do not provide any information about the relationship between water use efficiency (WUE) and ozone exposure. Actual evapotranspiration is difficult to measure inside the Open Top Chambers (OTC) which have been conceived to analyze in field the ozone effect on crops.

In the following the measurements of AET realized in the OTC's are presented for each growing season. Successively the data on grain yield are reported, and finally the results on WUE.

#### 1. Daily Evapotranspiration (AET)

Figure 3 shows the evolutions of AET measured in the 3-year experiment, inside the OTC's for the different ozone and irrigation treatments.

In 2004 season soybean was growing under well-watered conditions and each ozone treatment was repeated twice in the open top chambers (OTC1 and OTC2). Significant differences of AET values were observed between CW and HW treatments starting from 39 DAS (days after sowing). Daily evapotranspiration of HW treatments decreased progressively in comparison with CW treatments. Hence, cumulated AET values over soybean growing season were 386 mm and 274 mm for CW and HW treatments, respectively.

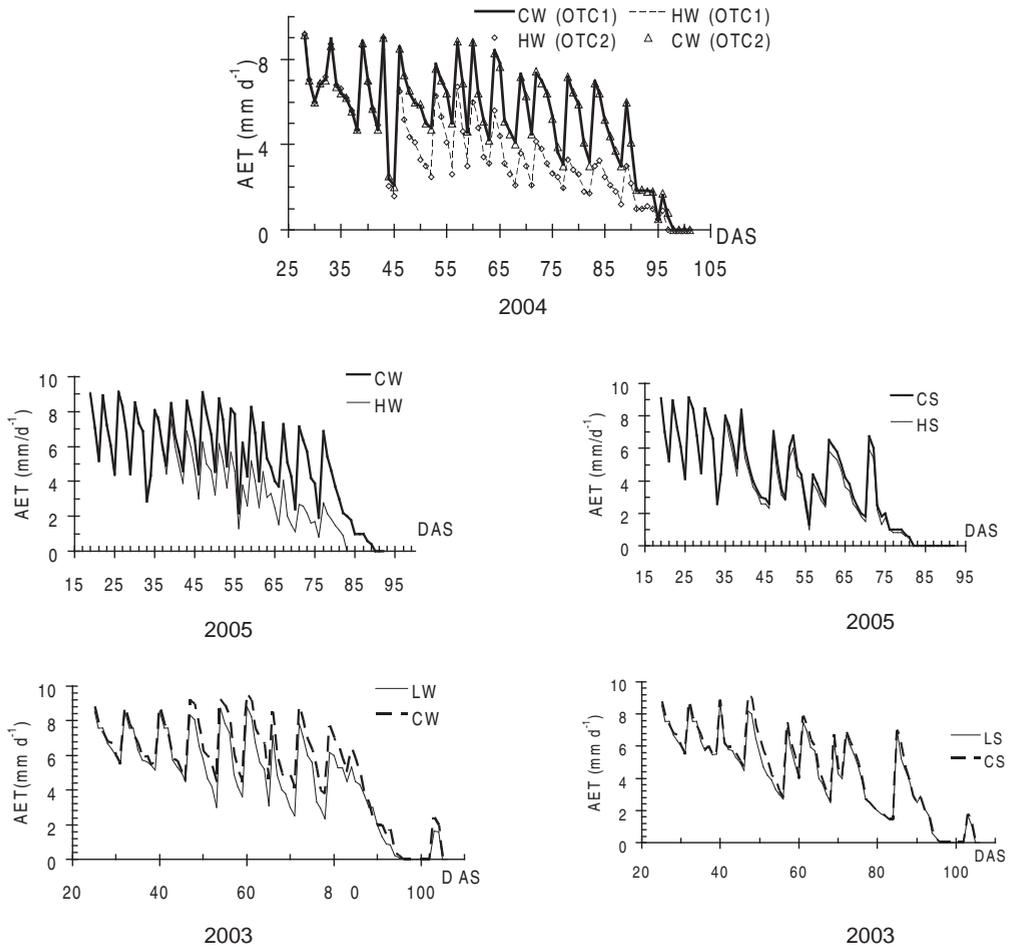
In 2005 soybean was growing under well-watered and stressed conditions. Cumulated AET values over soybean growing season were 389 mm and 285 mm for CW and HW treatments, respectively. These values were similar to the cumulated AET values obtained in 2004. Under water stress conditions, daily AET in 2005 shows a larger range of variation, between two irrigations. However, AET values for CS and HS treatments were similar over all the study period. Water stress minimized the effect of high ozone concentrations on daily AET. Cumulated AET values over the growing season were 291 mm and 272 mm for CS and HS treatments, respectively. These values showed a slight increase for the control treatment. The effect of water stress on plant water consumption is higher for the control treatment (-26%) than for the high ozone concentrations treatment (-5%).

The AET values cumulated over the 2003 soybean growing season were 344 mm and 295 mm for CW and LW treatments, respectively. The AET for LW treatments was reduced by 14% in 2003; whereas, reduction was 27% and 29% for HW treatments in 2004 and 2005, respectively. The daily AET values measured for water stressed treatments (LS, CS) during 2003 were similar to those observed for CS and HS treatments in 2005. Cumulated AET values during 2003 were 278 and 264 mm for CS and LS treatments, respectively. The difference between LS/CS treatments was 9%, a value very similar to HS/CS treatments in 2005.

## 2. Grain yield

In 2003 a low level of ozone concentration did not produce significant reductions in grain yield in either well-watered or water-stressed conditions (tab. 1). Differences between the ozone treatments under well-watered (CW and LW) or stressed (CS and LS) conditions were not statistically significant.

In 2004 (tab. 1), mean yield values obtained from the two ozone treatments (CW and HW) were significantly different ( $P < 0.05$ ). The HW treatment shows a grain yield loss of 47%, which is the result of a reduction in the number of pods per plant and in the 1000 grain weight (Bou Jaudé *et al.*, 2008b).



**Figure 3.** Time (in days after sowing, DAS) changes of daily actual evapotranspiration (AET). Values were measured during soybean growing seasons on well-watered (W) conditions in two open top chambers (OTC, high, H, and control, C, ozone treatments) in 2004, and on well-watered and stressed (S) conditions in 2003 (low, L, and C ozone treatments) and 2005 (H and C ozone treatments).

**Table 1. Actual evapotranspiration (AET), yield, and water use efficiency (WUE) observed for different ozone treatments during the three soybean growing seasons.**

Growing season	2003				2004				2005			
	0		3400		0		8979		0		8564	
[O <sub>3</sub> ] + water-status treatment	CW	CS	LW	LS	CW	HW	CW	CS	HW	HS		
AET m <sup>3</sup> /m <sup>2</sup>	0.34	0.28	0.30	0.26	0.39	0.28	0.39	0.29	0.29	0.27		
Grain yield (kg/m <sup>2</sup> )	0.30	0.32	0.27	0.31	0.28	0.15	0.28	0.19	0.15	0.18		
WUE (kg/ m <sup>3</sup> )	0.86	1.15	0.91	1.16	0.73	0.55	0.73	0.65	0.52	0.66		

In 2005 (tab. 1), under well-watered conditions, the HW treatment significantly reduced grain yield (47%) in comparison with the control treatment (CW), which corresponds to a loss in the number of pods and 1000-grain weight (as found in 2004). In water-stressed conditions, control (CS) and high (HS) ozone treatments showed yield values which were not statistically different.

### 3. Water use efficiency

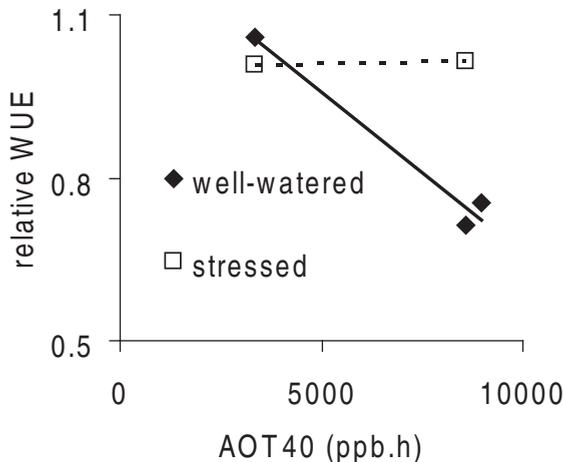
Table 1 summarizes for each treatment the seasonal AET, the grain yield and WUE.

In 2003, a low level of ozone concentration did not produce reductions WUE in either well-watered or water-stressed conditions.

In 2004, the WUE of the HW treatment was 25% lower than the control treatments.

In 2005, under well-watered conditions, the WUE of the HW treatment was 29% lower than the control treatment. In water-stressed conditions, control and high ozone treatments had similar WUE. WUE was characterised by a 11% reduction for the CS/CW treatments, and a 27% increase for the HS/HW treatments.

In synthesis (fig. 4), under well-watered conditions WUE was affected by the ozone. Notably, WUE decreased with an increase of AOT 40. In water-stressed conditions, yield and WUE were not affected by ozone and no correlation was found with AOT 40 values.



**Figure 4. Relationships between AOT40 and relative values of WUE observed for well-watered and stressed soybean crops. Relative WUE was calculated as the ratios of WUE from the ozone treatments (low levels of ozone concentration in 2003, and high in 2004 and in 2005) and the corresponding control treatments (AOT40 = 0).**

## IV – Conclusions

Mainly in the Mediterranean region, AOT40 values observed during the growing seasons of winter and summer crops are rare. This represents an additional cause of the scarce knowledge concerning the effect of ozone on water requirements in the Mediterranean region, although this region is particularly interested by this kind of pollution.

Results here reported show that WUE of well-watered soybean subjected to high level of ozone during the vegetative cycle (cumulated AOT40 = 8500 pbb.h), decreased by 30% in comparison with a control treatment with air filtered (cumulated AOT40 = 0). Conversely, ozone had a not-significant effect on WUE of a soybean which was stressed by water rationing. According to the results reported by Bou Jaudé *et al.* (2008 a and b), the absence of effect on water-stressed plants is due to stomatal closure, which reduces ozone flux towards leaves (Karlsson *et al.*, 2000), and thus its action on plant functions. Drought induced stomatal closure has also been demonstrated to reduce ozone damages. In the presence of ozone, the smaller reduction in stomatal conductance observed in stressed compared to well-watered plants may be consistent with a protective effect of drought (Fagnano and Merola, 2007; Ferretti *et al.*, 2007), as well as of salinity (Maggio *et al.*, 2007), on ozone-induced stomatal closure. Nevertheless, this response should be considered only in relative terms since the water stress by itself reduced the stomatal conductance, independently on ozone stress.

These results agree with those observed on wheat (Khan and Soja, 2003), on cotton (Heagle *et al.*, 1988), and on white clover (Fagnano and Merola, 2007). The ozone level in the atmosphere is often a neglected parameter in studies on irrigation; however, it is susceptible to creating variability in WUE for irrigated crops in the Mediterranean region. The literature shows that few studies have been conducted on this subject, which is why the effect of ozone on plants needs to be analysed deeply in the future.

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