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Effects of Ozone on CO_2 Assimilation and PSII Function in Two Tobacco Cultivars with Different Sensitivities

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Abstract

Two tobacco cultivars (*Nicotiana tabacum* L.), Bel-B and Bel-W3, tolerant and sensitive to ozone, respectively, were grown in a greenhouse supplied with charcoal filtered air and exposed to 200 ppb ozone for 4 hr. Effects on chlorophyll fluorescence, net photosynthesis, and stomatal conductance are described. Quantum yield was calculated from chlorophyll fluorescence and the initial slope of the assimilation-light curve measured by the gas exchange method. Only the sensitive cultivar, Bel-W3, developed visual injury symptoms on up to 50% of the 5th leaf. The maximum net photosynthetic rate of ozone-treated plants was reduced 40% compared to control plants immediately after ozone fumigation in the tolerant cultivar; however, photosynthesis recovered by 24 hr post fumigation and remained at the same level as control plants. On the other hand, ozone exposure reduced maximum net photosynthesis up to 50%, with no recovery, in the sensitive cultivar apparently causing permanent damage to the photosystem. Reductions in apparent quantum efficiency, calculated from the assimilation-light curve, differed between cultivars. Bel-B showed an immediate depression of 14% compared to controls, whereas, Bel-W3 showed a 27% decline. Electron transport rate (ETR), at saturating light intensity, decreased 58% and 80% immediately after ozone treatment in Bel-B and Bel-W3, respectively. Quantum yield decreased 28% and 36% in Bel-B and Bel-W3, respectively. It can be concluded that ozone caused a greater relative decrease in linear electron transport than maximum net photosynthesis, suggesting greater damage to PSII than the carbon reduction cycle.

Key words : Ozone fumigation, Chlorophyll fluorescence, Gas exchange, Bel-W3, Bel-B

1. INTRODUCTION

Ozone (O_3) is formed by photolysis of nitrogen dioxide (NO_2) via a series of complex photochemical reactions with both natural and anthropogenically-derived gases. When atmospheric conditions are right, phytotoxic levels of O_3 occur (Saitanis and Karandinos, 2002; Schraudner *et al.*, 1997). Researches show that the increasing production rate of O_3 is consistent with precursor emissions and it is expected that the tropospheric O_3 concentrations continue to increase in Northern Hemisphre (Chameides *et al.*, 1994) O_3 induces visible injury, reduces growth, decreases the rate of net photosynthesis, and accelerates foliar senescence (Davison and Barnes, 1998; Torsethaugen *et al.*, 1997; Runeckles and Chevone, 1992; Darrall, 1989). O_3 enters the leaf through stomata and can react with membranes and other cell components causing leaf injury and impairing photosynthesis (Runeckels and Chevone, 1992; Kerstiens and Lendzian, 1989). Leaves which

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are expanding, or just fully expanded, are generally most susceptible so that ozone injury is usually found on older foliage (Ribas *et al.*, 1998).

Chlorophyll fluorescence has been widely used to assess photosynthetic activity in plants since the introduction of pulsed-fluorescence monitoring systems (Schrieber *et al.*, 1986). The measurement of chlorophyll fluorescence is a non-invasive technique to access the physiological state of the photosynthetic apparatus, not only indicating changes in overall photosynthetic capacity, but also allowing for localization of sites of damage (Genty *et al.*, 1990). The yield of chlorophyll fluorescence is determined by two distinct quenching processes, photochemical (qP) and non-photochemical (qN) (Schreiber *et al.*, 1986). Photochemical quenching is proportional to the quantum yield of the linear electron transport rate and the efficiency of excitation capture by open photosystem II (PSII) reaction centers (Fv/Fm) under a wide range of physiological conditions. The rate of qN indicates regulatory adjustments in the photosynthetic membrane in response to altered external and internal conditions (Horton and Ruban, 1993).

Comparisons between sensitive and tolerant genotypes have been used for many years to assess ozone damage and to investigate sites of cellular injury (Antonielli *et al.*, 1997). Three different tobacco cultivars, Bel-B, Bel-C, and Bel-W3 have been often used in combination due to their different sensitivities Bel-W3 has been used as an indicator of ozone for decades since it is extremely sensitive to ozone and visible symptoms are easily recognizable (Ribas *et al.*, 1998; Lorenzini, 1994; Heggestad, 1991). Bel-B, ozone-tolerant, is often used with Bel-W3 in indicator studies (Krupa *et al.*, 1993). According to Heggestad (1991), ozone-induced foliar injury symptoms on Bel-W3 consist of upper surface flecking or bifacial necrosis, depending upon the severity of the ozone exposure. Under ambient ozone concentration of 50 to 150 part per billion (ppb), Bel-B does not usually show foliar symptoms. Ribas *et al.* (1998) conducted a field study to assess ozone phytotoxicity determined by leaf injury on three tobacco cultivars, Bel-W3, Bel-C, and Bel-B. The authors confirmed that Bel-W3 was the most sensitive cultivar, followed by Bel-C and Bel-B in orderly. Under appropriate environmental conditions, Bel-C also showed leaf injury and Bel-B was the most tolerant cultivar.

Although, ozone-induced declines in photosynthesis have been well documented in numerous plant species (Pell *et al.*, 1994), the central question of where oxidative damage to the photosynthetic apparatus occurs has not yet been completely resolved (Torsethaugen *et al.*, 1997). This is true with the tobacco cultivars Bel-W3 and Bel-B, where surprisingly little information on photosynthetic function and gas exchange measures available. In this present study, we examined whether a moderately high ozone concentration differentially affected net CO_2 fixation and PSII function in the cultivars Bel-W3 and Bel-B.

2. MATERIALS AND METHODS

2.1 Plant material and growth conditions

Two varieties of tobacco (*Nicotiana tabacum* L.), Bel-W3 and Bel-B, were chosen for their different sensitivities to O_3. Germination and initial growth of plants took place in a greenhouse supplied with charcoal-filtered (CF) air to reduce O_3 concentration below 25 ppb under a day temperature of 22～30°C. All plants were grown until the 7-8-leaf stage prior to O_3 exposure in 10-cm diameter pots containing potting mixture metromix 200® and supplied with certain gm of 14 : 14 : 14 (NPK).

2.2 Ozone exposure

Plants were exposed to 200 ppb of O_3 or to CF air (containing less than 10 ppb O_3) for 4 hrs in CSTR chambers in the greenhouse. O_3 was generated from oxygen by UV discharge and supplied to the chambers using a rotometers. A TECO (Thermo Electron Corporation, Waltham, MA) ozone analyzer recorded ozone concentrations continuously and data were stored electronically. During the fumigation, plants were exposed to a light level of 1100 ± 100 μmols m^{-2} s^{-1} PAR, temperatures of 25～30°C, R.H of 45～55% and 350 ppm CO_2.

2.3 Visible injury

The appearance of visible injury was evaluated 48 hr after exposure was completed. The 3rd, 4th, and 5th leaves were rated from 0 to 100, corresponding to the percentage of visible injury covering the upper leaf surface.

2.4 Net photosynthesis

Gas exchange measurements were conducted using a LiCor Li-6400 portable photosynthesis system (Li-Cor, Lincoln, NE). Net photosynthesis under saturating light conditions (Pn_{MAX}, μmol CO_2 m^{-2} s^{-1} at a light intensity of 900 μmol m^{-2} s^{-1}) and 350 ppm CO_2, and stomatal conductance (gs, mmolH_2O m^{-2} s^{-1}) were measured immediately after exposure and at 24 hr intervals for three days on the 4th leaf position (The first apical leaf 8-cm-long was designated leaf one). Assimilation-Irradiation curves were generated under ambient CO_2 concentrations. From the initial slope of these curves, the apparent quantum efficiency (φ_{CO_2}) for net CO_2 assimilation was determined.

2.5 Chlorophyll fluorescence

Chlorophyll fluorescence analysis was determined on the same leaves utilized for gas exchange. A pulse amplitude modulation fluorometer (PAM-2000, Heinz Walz, Effeltrich, Germany) was used to monitor chlorophyll fluorescence and to measure the status of the electron transport of PSII (detailed procedure review Guidi *et al.*, 1997). Leaves were dark adapted for a 30 min with dark leaf clips (DLC-8, Heinz Walz) prior to determination of chlorophyll fluorescence. The quenching coefficients qP (photochemical) and qNP (non-photochemical) were calculated as defined by Schreiber *et al.* (1986), while the actual quantum yield for PSII was calculated by Genty *et al.* (1989).

2.6 Experimental design and statistical analysis

One control and one O_3 treated CSTR containing 3 plants for each cultivar, were used for one fumigation experiment. Each plant was designated an experimental unit and represented a pseudo replicate. Fumigations were repeated three times for a total of nine replicates for each cultivar and each treatment. Data were analyzed by analysis of variance with ozone treatments and cultivars as class variables. When significance is noted, it refers to statistical significance at the $P \leq 0.05$ with a single asterisk and at the $P \leq 0.01$ with two asterisks. The microcomputer package SAS was utilized for the statistical analysis.

3. RESULTS

3.1 Photosynthetic function in control plants

The rates of net CO_2 assimilation at the 4th leaf position of the two tobacco cultivars, Bel-W3 and Bel-B, exposed to charcoal-filtered (CF) air were similar at low light intensity. However, they began to diverge as light intensity exceeded 300 μmol m^{-2} s^{-1} PAR (Figure 1). At saturating light intensity (900 μmol m^{-2} s^{-1}), the maximum net photosynthetic rate (Pn_{MAX}) was about 8.5% higher in the tolerant cultivar, Bel-B (11.4 μmol CO_2 m^{-2} s^{-1}), than in sensitive cultivar, Bel-W3 (10.5 μmol CO_2 m^{-2} s^{-1}) (Table 1). The apparent quantum yield for CO_2 fixation, φ_{CO_2},

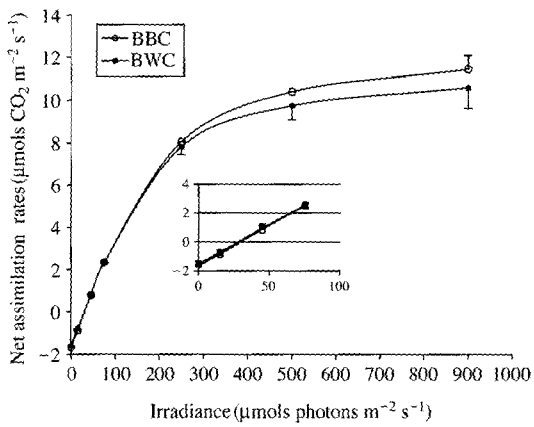

Fig. 1. Net assimilation (μmols CO_2 m^{-2} s^{-1}) -irradiance (PAR; μmols photons m^{-2} s^{-1}) on the 4th leaf position of control tobacco plants, Bel-B (BBC; opened circles) and Bel-W3 (BWC; closed squares). Insert is the initial slopes of the A-I response curves. Bars represent one standard deviation of the means and, where not apparent, are contained within the symbols.

Table 1. Maximum net photosynthetic rates (Pn_{MAX} μmol CO_2 m^{-2} s^{-1} at 900 μmols quanta m^{-2} s^{-1}) in Bel-B (tolerant) and Bel-W3 (sensitive) tobacco cultivars after exposure to 200 nl L^{-1} O_3 for 4 h. Single asterisk indicates significant differences from control plants at $P<0.05$. Values are means ± s.d.

Cultivar	Treatment	Time post-O_3-fumigation (h)			
		0	24	48	72
Bel-B	Control	11.35±0.33	10.95±0.69	10.23±0.48	8.03±0.28
	O_3	7.28±0.33*	11.23±0.13	9.76±0.50	7.31±0.10
Bel-W3	Control	10.46±0.82	11.5±0.48	10.24±1.24	8.99±0.69
	O_3	6.06±0.32*	5.83±0.68*	7.08±0.24*	5.84±0.61*

Table 2. Apparent quantum yield for CO_2 assimilation (μmol CO_2 μmol incident photons^{-1}) in Bel-B and Bel-W3 tobacco cultivars after exposure to 200 nl L^{-1} O_3 for 4 h. Single asterisk indicates significant differences from control plants at $P<0.05$, two asterisks indicate significant differences at $P<0.01$. Values are means ± s.d.

Cultivar	Treatment	Time post-fumigation (h)			
		0	24	48	72
Bel-B	Control	0.055±0.001	0.051±0.004	0.049±0. 001	0.049±0.002
	O_3	0.048±0.005**	0.047±0.004	0.048±0.001	0.048±0.003
Bel-W3	Control	0.054±0.002	0.050±0.003	0.049±0.005	0.049±0.005
	O_3	0.039±0.007**	0.041±0.006**	0.037±0.002*	0.042±0.004*

Table 3. Stomatal conductance (g_s, mmols H_2O m^{-2} s^{-1}) in Bel-B and Bel-W3 tobacco cultivars after exposure to 200 nl L^{-1} O_3 for 4 h. Single asterisk indicates significant differences from control plants at $P<0.05$. Values are means ± s.d.

Cultivar	Treatment	Time post-fumigation (h)			
		0	24	48	72
Bel-B	Control	0.225±0.053	0.250±0.072	0.212±0.036	0.148±0.046
	O_3	0.116±0.057*	0.230±0.028	0.175±0.007	0.157±0.046
Bel-W3	Control	0.165±0.040	0.218±0.042	0.176±0.024	0.178±0.652
	O_3	0.086±0.042*	0.142±0.050*	0.104±0.028**	0.108±0.024*

was very similar between the two cultivars at 0.055 and 0.054 μmoles CO_2 μmol photons^{-1} for Bel-B and Bel-W3, respectively (Figure 1, insert; Table 2). Stomatal conductance (g_s) was about 36% higher in Bel-B (225 mmols H_2O m^{-2} s^{-1}) than in Bel-W3 (165 mmols H_2O m^{-2} s^{-1}) at saturating light intensity (Table 3), indicating the potential for greater initial O_3 uptake into the leaf in the tolerant cultivar.

Since photosynthetic activity was measured over a 72-hr period after O_3 fumigation, the effect of leaf age on physiological activity was measured for 17 days beginning when a leaf attained 8 cm in length. Figure 2 shows the changes in the maximum rate of net CO_2 assimilation (A), stomatal conductance (B), and internal CO_2 concentration (C) in relation to leaf age in Bel-W3 and Bel-B. The time period designated by the arrows indicates the 6 to 8-leaf stage when plants were fumigated with O_3 and photosynthetic function measured. Maximum photosynthetic rates (Pn_{MAX}) were approximately 5 μmol CO_2 m^{-2} s^{-1} in the 8cm length leaf (day 1) reached a peak at 10 μmol CO_2 m^{-2} s^{-1} on day 7 to 10, and then declined as the leaf naturally senescenced. Stomatal conductance (g_s) attained a maximum at about 3 days and then tended to decrease during the measuring period. The internal CO_2 (Ci) concentration, reflecting

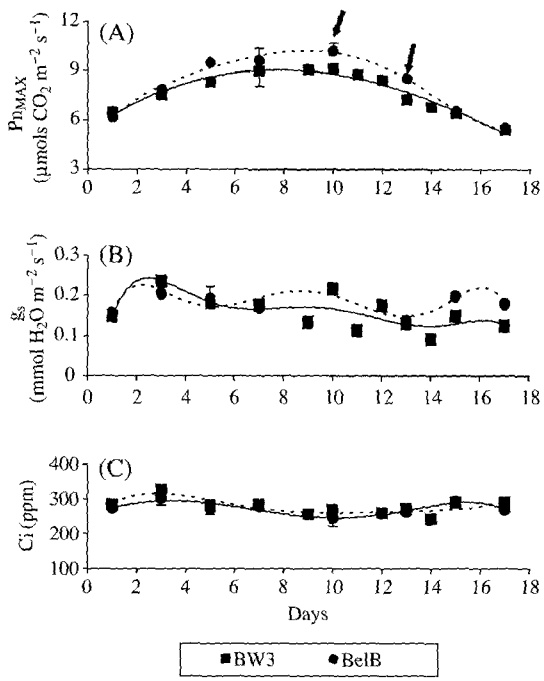

Fig. 2. Maximum net photosynthetic rate (Pn_{MAX}, μmols CO_2 m^{-2} s^{-1}) (A), stomatal conductance (g_s, mmol H_2O m^{-2} s^{-1}) (B), and internal CO_2 concentration (Ci, ppm) (C) in relation to leaf age in Bel-B (closed circles) and Bel-W3 (closed squares) (leaf 8 cm long at day 1). Arrows indicate the ozone fumigation and following experimental period. Error bars represent one standard deviation of the mean and, where not apparent, are contained within the symbols.

the net assimilation rate, was highest in young leaves, when photosynthesis was increasing, and lower in senescent leaves, when photosynthesis was declining. The time period designated by the arrows in Figure 2, corresponding to the 72-hr of O_3 fumigation and monitoring of physiological activity, corresponded to the period of maximum photosynthesis and early senescence of the 4th leaf.

3.2 Visible foliar symptoms

The sensitive cultivar, Bel-W3, developed visible injury symptoms in 48 hrs post fumigation on all leaf positions, from the 3rd and older leaves. Symptoms appeared as white, necrotic spots, principally on the upper leaf surface, confined to the tips and margins in young foliage, but covering the entire surface in older leaves. Injury was less prevalent in young foliage (20% in 3rd leaf position) than older foliage (50% in 5th leaf position) (Figure 3A). The tolerant cultivar, Bel-B showed much less symptoms with only 5% of the total area affected on the most severely injured leaf (Figure 3A). Figure 3B shows the appearance of visual foliar injury on the 4th leaf comparing the sensitive cultivar, Bel-W3 and tolerant cultivar, Bel-B.

3.3 Photosynthetic function in O_3-treated plants

Exposure to 200 nl L^{-1} of O_3 for 4 hr caused an

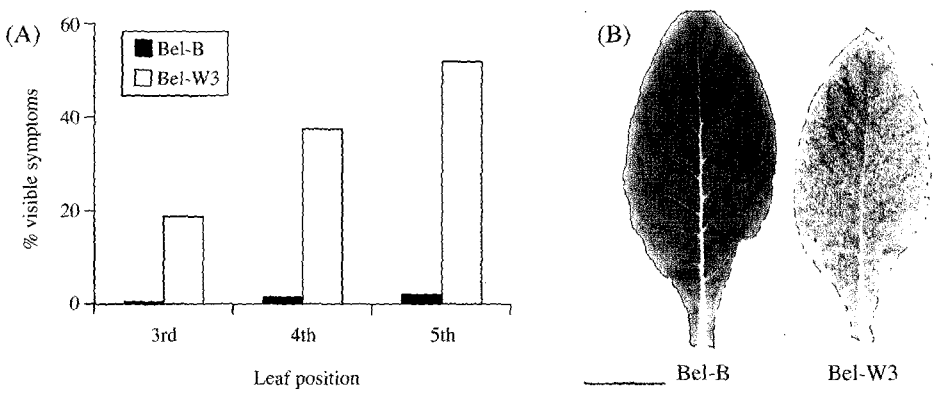

Fig. 3. Visible symptoms on the foliage 48 hrs post-O_3-fumigation (200 ppb for 4 hrs) in tobacco cultivars Bel-B (solid bars) and Bel-W3 (shaded bars), (A) Percentage of visible injury and (B) Appearance of foliar injury on the 4th leaf position.

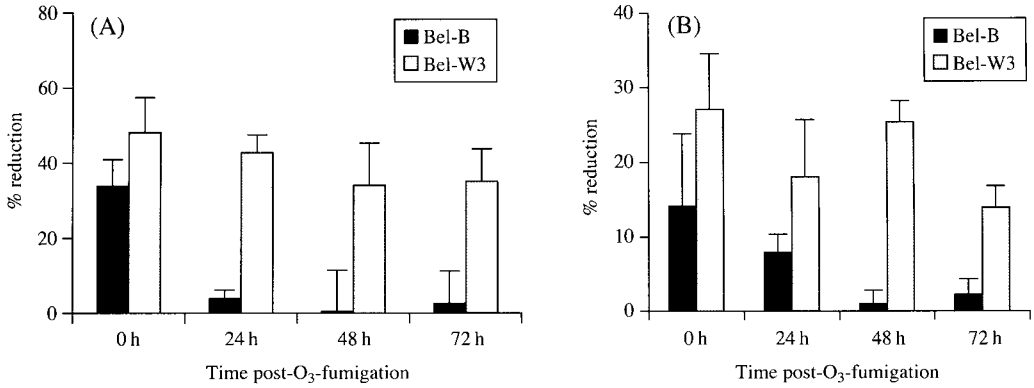

Fig. 4. Percentage reduction of (A) maximum net photosynthetic rates (μmols CO_2 m^{-2} s^{-1}) and (B) apparent quantum yield for CO_2 assimilation in Bel-B (solid bars) and Bel-W3 (shaded bars) tobacco cultivars exposed to 200 ppb of O_3 for 4 hrs. Initial rates are taken from control plants at 0hr post-fumigation. Error bars represent standard deviations.

Table 4. Fluorescence characteristics and the apparent electron transport rate (ETR) in leaves of Bel-B and Bel-W3 immediately after O_3 fumigation at 200 ppb for 4 hrs. Asterisks indicate significant differences from control plants at $P<0.05$. Values are mean ± s.D.

Parameters	Bel-B		Bel-W3	
	Control	O_3	Control	O_3
Fm	1.916±0.026	1.810±0.031*	1.952±0.043	1.258±0.396*
Fo	0.348±0.017	0.439±0.015*	0.353±0.004	0.388±0.024*
Fv	1.568±0.036	1.371±0.031*	1.598±0.044	0.870±0.415*
Fv/Fm	0.818±0.010	0.758±0.009*	0.819±0.004	0.670±0.105*
Φ_{PSII}	0.364±0.001	0.158±0.007	0.41±0.052	0.102±0.012
ETR	123±6.0	54±3.0*	139±19.8	33±3.5*
qP	0.449±0.011	0.215±0.060	0.495±0.062	0.191±0.001
qN	0.578±0.046	0.463±0.040	0.560±0.135	0.615±0.008

immediate depression in maximum photosynthetic rate (Pn_{MAX}) in both tolerant and sensitive cultivars (Table 1). The Pn_{MAX} of O_3-treated plants was reduced 36% compared to control plants immediately after fumigation in Bel-B; however, CO_2 fixation recovered to near control rates within 24 hrs post fumigation and this recovery persisted through the 72-hr measuring period (Table 1, Figure 4A). On the other hand, O_3 exposure reduced Pn_{MAX} up to 50%, with no recovery, in Bel-W3, apparently causing permanent damage to the photosystems (Figure 4A). The Pn_{MAX} in both control and fumigated plants gradually decreased as a function of the aging process (Table 1) and was evident as the incremental decrease in reduction of PnMAX with time (Figure 4A).

Apparent quantum yield for CO_2 fixation, φ_{CO_2}, was affected by O_3 fumigation in a pattern similar to maximum photosynthetic rates (Table 2). Reductions in φ_{CO_2} calculated from the assimilation-irradiation curves, differed between tolerant and sensitive cultivars (Figure 4B). Bel-B showed an immediate depression of 14% compared to controls after fumigation, whereas, Bel-W3 showed a 27% decline. φ_{CO_2} began to recover in Bel-B within 24 hr and by 48 hr-post fumigation, φ_{CO_2} was similar to controls. However, no apparent trend in recovery was evident in Bel-W3 (Figure 4B). φ_{CO_2} in Bel-W3 was still

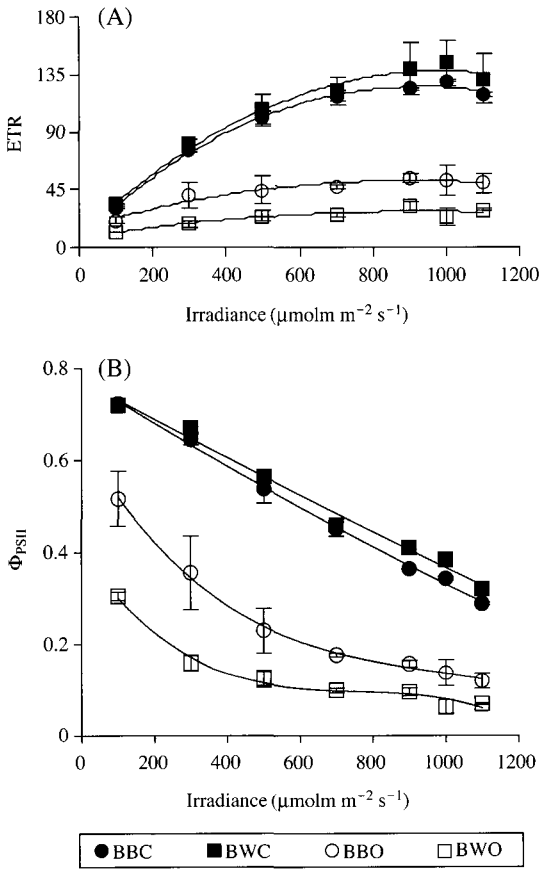

Fig. 5. (A) electron transport rates of PSII (ETR, μmole electrons m^{-2} s^{-1}) and (B) quantum efficiency for PSII (Φ_{PSII})-Irradiance (PAR μmols photons m^{-2} s^{-1}) response curves on the 4th leaf in Bel-B (BBC, control plants and BBO, O_3 treated plants) and Bel-W3 (BWC, control plants and BWO, O_3 treated plants) tobacco cultivars immediately after O_3 fumigation with 200 ppb of O_3 for 4 hrs.

25% less than controls at 72 hours after fumigation.

Stomatal conductance, g_s, also decreased after O_3 fumigation in both cultivars (Table 3). In Bel-B and Bel-W3, g_s was 48% lower than in the controls immediately after fumigation, but recovered within 24 hrs post fumigation in the tolerant cultivar. In Bel -W3, recovery was less apparent, and g_s was only 61% of the control plants 72 hrs after O_3 fumigation ended.

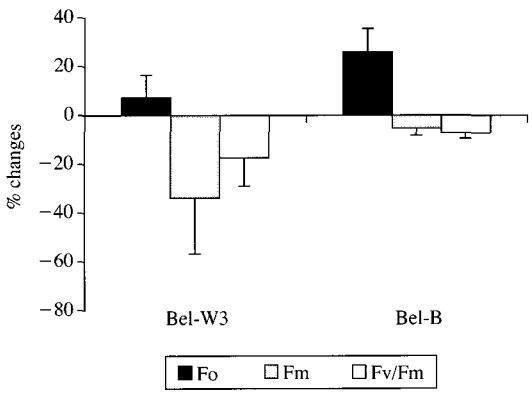

Fig. 6. Percentage changes in dark adapted state chlorophyll fluorescence parameters, Fo; minimum chlorophyll fluorescence (black bars), Fm; maximum chlorophyll fluorescence (grey bars), and Fv[Fm-Fo]/Fm; maximum photochemical efficiency of PSII (white bars) in Bel-B and Bel-W3 tobacco cultivars immediately after O_3 fumigation of 200 ppb O_3 for 4 hrs. Error bars represent standard deviations.

3.4 Chlorophyll fluorescence in control and O_3-treated plants

Immediately after 4-hr of ozone fumigation, various chlorophyll fluorescence parameters were measured and compared between sensitive and tolerant tobacco (summarized in Table 4).

Photochemical efficiency (Fv/Fm), measured in the dark-adapted state immediately after fumigation, decreased 7% and 18% in Bel-B and Bel-W3, respectively (Figure 6). However, the contributions to the decreased ratio of Fv/Fm were different between cultivars. The maximal level of chlorophyll fluorescence (Fm) decreased more in Bel-W3 than Bel-B, whereas minimal fluorescence (Fo) increased more in Bel-B than Bel-W3 (Figure 6).

Electron transport rates (ETR), in both cultivars, decreased at all irradiance levels immediately after O_3 exposure. At saturating light intensity, ETR decreased 58% and 80% in Bel-B and Bel-W3, respectively (Figure 5A). The quantum yield for transporting electrons from PSII, φ_{PSII}, measured by the chlorophyll fluorescence method, decreased immediately post-fumigation by 28% and 36% in Bel-B and Bel-

W3, respectively (Figure 5B).

Figure 1. Net assimilation (μmols CO_2 m^{-2} s^{-1})-irradiance (PAR; μmols photons m^{-2} s^{-1}) on the 4th leaf position of control tobacco plants, Bel-B (BBC; opened circles) and Bel-W3 (BWC; closed squares). Insert is the initial slopes of the A-I response curves. Bars represent one standard deviation of the means and, where not apparent, are contained within the symbols.

4. DISCUSSION

The decrease in Pn_{MAX} was greater in the sensitive cultivar, Bel-W3, than in the tolerant cultivar Bel-B. The decline in Pn_{MAX} in Bel-W3 appears due to permanent damage in the PSII reaction center which results in more than a 2X drop in Fv/Fm ratio with a substantial reduction in Fm. The minimum increase of Fo may also be responsible for this reduction of the Fv/Fm ratio in Bel-W3. The fact that the maximum level (Fm) was suppressed before any effect on the initial level (Fo) occurred showed that initial damage to the PSII donor site took place prior to any decrease in energy transfer efficiency within the pigment system. However, the response of the tolerant cultivar was different. Bel-B also showed a decrease in the Fv/Fm ratio after O_3 exposure which was mostly due to an increase in Fo with unchanged Fm. The decrease in the Fv/Fm ratio may be associated with photoinhibitory damage indicating an altered electron transport rate of PSII to PSI by the increasing Fo. The maximum photochemical efficiency (Φ_{PSII}) was reduced dramatically in both cultivars. This was due to the decrease in qP and consequently an increase in the 1-qP parameter. The decrease in Φ_{PSII} was greater in sensitive cultivar. According to Van Burren *et al.* (2002) 1-qP can be a measure of the reduction state of the primary quinone acceptor. In this instance, an increase in 1-qP indicates a less effective re-oxidation of this electron acceptor suggesting in turn, that some fraction of the PSII traps were closed during actinic illumination. These closed traps lead to decreased quantum efficiency of PSII. The apparent quantum yield also decreased to a greater extent in the sensitive cultivar compared to the tolerant one. However, the concentration of O_3 used in this fumigation study may have been sufficiently high that not only did visual injury symptoms develop, but photosynthetic activity decreased in the tolerant cultivar. Gupta *et al.* (1991) found that a reduction in CO_2 fixation was least sensitive to O_3 and the decline in photosynthesis may be a secondary effect due to less reductant available for carbon reduction. If CO_2 fixation cannot keep pace with NADPH production, the NADP pools become reduced with an excess reduction of PSII and PSI. Under these conditions, O_2 can compete for electrons from PSI leading to the generation of reactive oxygen intermediates through the Mehler reaction (Allen, 1995). The increases in the non-photochemical quenching coefficient of fluorescence, qN, also dissipates extra absorbed energy, that not consumed by carbon metabolism, as heat and prevents over-reduction of the electron transport chain (Harbash *et al.*, 1996). Mechanisms of qNP are still unknown; however, many of its characteristics are generally accepted. One of them is the xanthophyll cycle, converting violaxanthin to zeaxanthin via the intermediate of antheraxantin through increasing the trans-thylakoid pH gradient and activating the high light-triggered violaxanthin de-epoxidase enzyme (Dall'Osto *et al.*, 2005). The lower assimilation rate was reflected in the reduction in photochemical quenching, qP, which is an estimate of the number of open or oxidized PSII centers The difference between total linear electron transport and electrons being used for carbon assimilation may indicate the existence of alternative electron sinks such as photorespiration, Mehler reaction, and nitrite reduction (Harbash *et al.*, 1996).

Ozone causes unspecific changes in fluorescence parameters. The effects of O_3 on whole plants can be determined with the chlorophyll fluorescence induction assay. In 1978, Schreiber *et al.* reported that the way in which fluorescence induction is affected may suggest the sites of O_3 damage within photosynthetic apparatus. The fact that the maximum level (Fm) is suppressed before any effect on the initial level (Fo) occurs indicates initial damage

to the PSII donor site prior to any decrease in energy transfer efficiency within the pigment system. The electron transport from PSII to PSI also becomes inhibited as indicated by increasing Fo.

In summary, in both cultivars with decreased maximum net photosynthesis and stomatal conductance, quantum yield for CO_2 fixation decrease immediately following O_3 fumigation. Maximum electron transport rate through PSII to PSI and quantum yield of PSII decrease in both cultivars immediately following O_3 fumigation. Fumigation at 200 ppb concentration for 4 hr was severe enough to cause reductions in physiological function in Bel-B, the tolerant cultivar. Ozone caused a greater relative decrease in linear electron transport than maximum net photosynthesis, suggesting greater damage to PSII than the carbon reduction cycle in the sensitive cultivar. The impairment of physiological functions in the tolerant cultivar was temporary and fully recovered by 24 hr-post-fumigation. However, the damage caused by the high concentration of O_3 in sensitive cultivar was permanent and no recovery was observed.

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