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The Absence of Cross Tolerance Between Ozone and Paraquat: The Case of *Conyza bonariensis*

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S u m m a r y

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The behavior of two biotypes of the composite weed *Conyza bonariensis*, resistant and sensitive to paraquat (PQ), exposed to PQ (10 ppm) or to ozone (O_3, 50-250 ppb, 5 h) was investigated by examining the fluctuation in gas exchange parameters and chlorophyll content, as well as membrane integrity and antioxidant enzyme activities. The two biotypes proved not to be different for the variables tested under undisturbed conditions, with the exception of intercellular CO_2 concentration, the resistant biotype showing the highest constitutive values. Even thought necrotic lesions developed similarly in both biotypes upon treatment with PQ or O_3, changes in malondialdehyde formation, superoxide dismutase and peroxidase system activities were significantly different.

I n t r o d u c t i o n

Under natural conditions, living organisms are exposed to a number of stress factors, both biotic and abiotic. The evolution of permanent forms of resistance/tolerance to these adverse agents is a common phenomenon; so, individuals may develop biochemical and/or physiological mechanisms in order to withstand more stress factors, if their aggression pathways share relevant steps.

The oxidative stress is very frequent in nature and is due to an increase of the reactive oxygen species (ROS); it is induced by several phytotoxic chemical agents, including non-selective contact herbicides (such as paraquat, 1,1'-dimethyl-4,4'-bipyridinium, PQ) and air pollutants (such as the most common of them,

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ozone, O_3). Organisms counteract ROS by means of antioxidant compounds ("scavengers") as well as of specific enzymes. The major target of PQ seems to be the chloroplast; PQ can accept one electron from photosystem I and the formed PQ radicals rapidly react with molecular oxygen (O_2) forming ROS. O_3-induced effects are also the result of ROS action: symptoms of O_3 and PQ damage really share a number of characteristics, such as perturbation of cell membranes, reduction in CO_2 uptake and degradation of chloroplasts and pigments (KIRTIKARA & TALBOT 1996).

There are populations of hairy fleabane (*Conyza bonariensis* (L.) Cronq.), an annual composite weed, which have become insensitive to PQ under field conditions (LEBARON & GRESSEL 1982). In this study, the behavior of two biotypes (one PQ-resistant, PQ-R, and one PQ-sensitive, PQ-S) exposed to PQ or to O_3 was investigated under experimental conditions.

Material and Methods

Achenes of a wild type were originally gathered near Alexandria, while achenes of the resistant type came from the Tahrir irrigation district, also in Egypt, where PQ resistance has evolved (YE & GRESSEL 1994). Plants were raised in containers in a controlled-environment chamber, using a standard substrate; they were selected for homogeneity and treated when 8-weeks old (rosette stage).

All treatments were performed on intact plants. PQ was sprayed with a hand glass atomizer onto the leaves until drainage, using a commercial formulation having a concentration of 10 ppm in distilled water, to which a drop of surfactant Tween 20 had been added. Plants were then kept in the dark for 4 h to improve absorption. Exposures to O_3 (50-250 ppb, 5 h in the form of a square wave) were performed in a fumigation facility, as described before (LORENZINI & al. 1994). The "Accumulated exposures Over the Threshold of 40 ppb" (the so-called "AOT40", sensu DE LEEUW & VAN ZANTVOORT 1997) were 50 and 1050 ppb.h respectively, for 50 and 250 ppb concentrations.

Measures in vivo and samples were collected 24 h after the treatments. Chlorophylls (Chl) were determined from leaf discs according to MORAN 1982; leaf greenness was assessed by means of a Minolta SPAD-502 Meter, and results were converted into absolute Chl values through a calibration curve. Production of malondialdehyde (MDA), a breakdown product of the unsaturated fatty acid hydroperoxides and a membrane damage marker, was evaluated using a method based on their reaction with thiobarbituric acid (RANIERI & al. 1996). Relative water content (RWC) and electrical conductivity (EC) of leachates from foliar discs were determined according to GUIDI & al. 2001. CO_2 and water vapor exchange of leaves was measured with an open infra-red gas exchange system (CIRAS-1 PP-Systems) (CASTAGNA & al. 2001). The following enzyme activities were assayed: superoxide dismutase (SOD) and peroxidase systems which use guaiacol (GuPX) and ascorbate (APX) as electron donors, according to the methods described by DIOP & al. 1997.

Experiments were performed three times, with at least three plants for each variable. Data were analyzed collectively using MANOVA, and two-factors ANOVAs were used to assess which factors significantly influenced the variables measured. Differences between averages were evaluated with a multiple comparisons procedure (SNK test, $P \leq 0.05$). Data population was analyzed by means of canonical discriminant analysis, as described by DERKSEN & al. 1993.

Results and Discussion

When undisturbed, the two biotypes proved not to be different for the variables tested, with the exception of Ci, PQ-R showing the highest constitutive values (+19 % compared to PQ-S) (Fig. 1, white columns). At odds with SHAALTIEL & GRESSEL 1986, but in accordance to POWLES & CORNIC 1987, CARROLL & al. 1988 and PRESTON & al. 1991, PQ resistance was not associated with higher levels of SOD and APX activity than for in the wild-type.

Table 1. Results of multiple analysis of variance for paraquat and ozone treatment effects (AOT40 50 or 1050 ppb.h) and genotype on overall biochemical and physiological parameters of *Conyza bonariensis* biotypes. D.f. (H) and d.f. (E) represent the degrees of freedom for the hypothesis and error sum of squares cross product matrices, respectively.

Source	d.f. (H)	d.f. (E)	Wilk's Lambda Test	P
Treatment	30	21	0.000002	≤ 0.001
Biotype	10	7	0.008968	≤ 0.001
Treatment *x* biotype	30	21	0.000038	≤ 0.001

Following O_3 treatment, the two biotypes showed the same phenomenological response: foliar chlorotic mottles, which enlarged with time and resulted in small, whitish necrotic lesions were observed on the adaxial surface 6-7 days after fumigation with AOT40 above 550 ppb.h. Table 1 shows that the biochemical and physiological variables investigated are significantly affected by PQ and O_3 though differently according to the biotype. Separate two-way ANOVAs (Table 2) describe the influence of factors on specific parameters: both factors (chemical treatments and biotypes, respectively) significantly affected all variables, with the exception of MDA for chemical stress and APX for biotype; all "treatment x biotype" interactions were significant.

As shown in Fig. 1, PQ induced inhibition of CO_2 uptake (-85 and -21 % in PQ-S and PQ-R, respectively) in both biotypes, due to a reduced Gw (-79 and -34 % in PQ-S and PQ-R, respectively), a decrease in Chl content (-54 and -24 % in PQ-S and PQ-R, respectively) and to the mesophyll being unable to fix CO_2 in PQ-S (+42 % in Ci). PQ also caused lipid peroxidation (in PQ-S treated plants, MDA content was three times higher than in PQ-R), resulting in electrolyte leakage for the wild-type (+248 % compared to the control) and in a RWC decrease in both biotypes (-6 and -25 % in PQ-R and PQ-S, respectively). SOD, APX and GuPX activities increased similarly in both biotypes (+211, +233 and +67 %, in PQ-R, and +184, +212 and +75 %, in PQ-S, for SOD, APX and GuPX, respectively).

Table 2. F values of two-factor analyses for effects of paraquat or ozone (AOT40 50 or 1050 ppb.h) treatment and role of genotype on two *Conyza bonariensi* biotypes. For abbreviations, see Fig. 1. D.f. (E) represents the degrees of freedom * = P≤0.05, ** = P≤0.01, *** = P≤0.001.

Source	d.f.	Photosynthetic parameters				Membrane state			Antioxidant enzymes		
	(E)	A_{max}	Gw	Ci	Chl	CE	MDA	RWC	APX	GuPX	SOD
Treatment		113 ***	156 ***	24.4 ***	74.1 ***	88.4 ***	331	52.7 ***	76.9 ***	129 ***	243 ***
Biotype	1	161 ***	103 ***	11.4 **	33.2 ***	64.9 ***	79.2 ***	22.1 ***	0.36	9.61 **	39.4 ***
Treatment x biotype	3	24.1 ***	22.4 ***	51.7 ***	19.9 ***	79.2 ***	95.3 ***	20.6 ***	3.88 *	22.7 ***	9.98 ***

A_{max} after fumigation showed that the PQ-R biotype was unaffected by the AOT40 of 50 ppb.h, but severely injured by 1050 ppb.h; on the contrary, PQ-S was significantly affected by 50 ppb.h as well; the very same trend was observed for Gw. Chl content was strongly depressed (-40 %) in PQ-S by treatment at 1050 ppb.h. Under the same conditions, Ci increased in PQ-S (+39 %). Evidence of membrane damage was detected in both biotypes (in terms of EC increase and RWC decrease) following treatment with 1050 ppb.h, but the inner causes were not found in lipid peroxidation, as shown by the lack of MDA formation, suggesting that O_3 caused a different membrane damage from that induced by PQ. In *C. bonariensis*, MDA (marker of lipid peroxidation) formation seems to be a peculiar trait of PQ toxicity, but not of O_3. As suggested by ASHTON & CRAFTS 1981, the $OH^{\bullet}$ radical, resulting from a reaction between excited Chl and O_2, seems to be responsible for PQ-induced lipid peroxidation and Chl destruction, rather than O_2^- or H_2O_2.

Even thought necrotic lesions developed similarly in plants upon treatment with PQ and O_3, changes in SOD and peroxidase systems were significantly different. SOD activity increased similarly in both biotypes, following PQ treatment. This suggests that SOD may not be regarded as a detoxification mechanism in the case of PQ resistance, as already described by KIRTIKARA & TALBOT 1996 in tomato plants. The complexity of the issue is shown by the fact that these authors did not describe a parallel increase in APX activity, which was clearly apparent in this study. It can be assumed without doubt that, if PQ causes a massive oxidation of ascorbate, a higher level of APX will be required to regenerate ascorbate from dehydroascorbate. If targets between PQ and O_3 toxicity were the same, both stresses would be expected to lead to the same changes at cellular level.

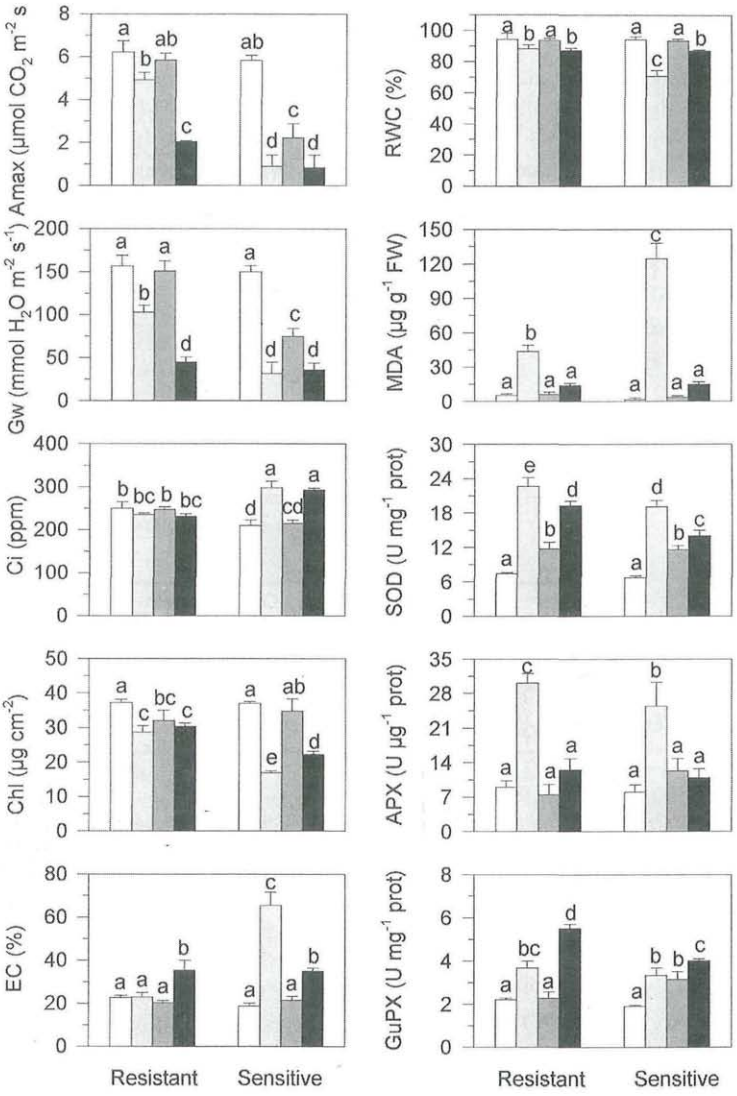

Fig. 1. Effect of paraquat and ozone treatment and of genotypical variation on photosynthetic parameters, membrane state and antioxidant enzymes of the two biotypes of *Conyza bonariensis*. Legend: white = control; grey = PQ-treated plants; dark grey = O_3-treated plants (AOT40 50 ppb.h); black = O_3-treated plants (AOT40 1050 ppb.h). Abbreviations: A_{max} = photosynthetic activity at saturating light; APX = ascorbate peroxidase; Chl = total chlorophylls; Ci = intercellular CO_2 concentration; EC = electrical conductivity of leachates from foliar discs, expressed in percentage of control discs which have been frozen to disrupt membrane integrity; GuPX = guaiacol peroxidase; Gw = stomatal conductance to water vapor; MDA = malondialdehyde; RWC = relative water content; SOD = superoxide dismutase. Vertical bars over each column indicate standard deviation; in each graph, columns marked with the same letters are not different for $P \leq 0.05$.

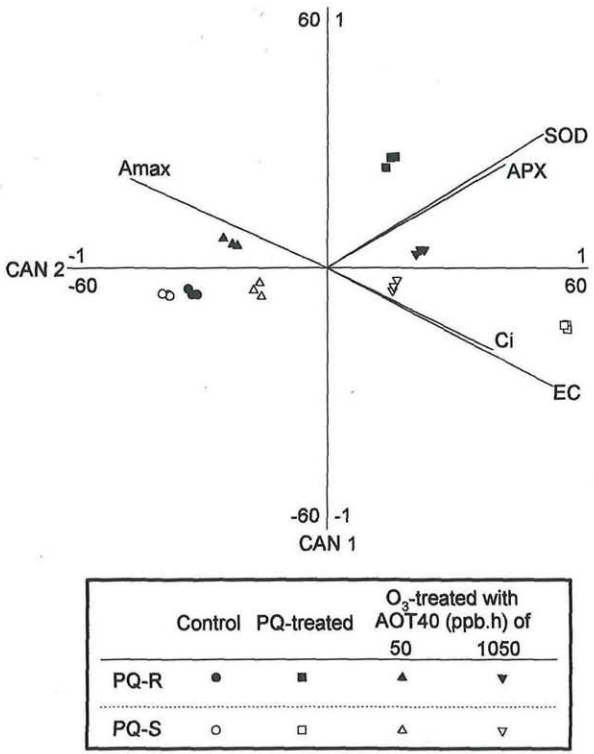

Fig. 2. Ordination biplot of biochemical and physiological parameters (vectors) under four paraquat and ozone treatments (one control, two ozone regimes, one paraquat spray) of the two *Conyza bonariensis* biotypes. CAN 1, first canonical variable; CAN 2, second canonical variable. Each point represents the mean value of each replicate test. For abbreviations, see caption of Fig. 1.

O_3 exposure and PQ treatment caused the same variation (i.e. stimulation) of SOD activity in both PQ-R and PQ-S. On the contrary, in ozonated plants APX maintained a similar activity to that in controls, regardless of the Conyza biotype. The measured APX levels represent the combined activities of chloroplastic and non-chloroplastic isoforms: the regeneration of ascorbate from dehydroascorbate stabilizes the ascorbate level and APX activity in both chloroplasts and cytosol (NAKANO & ASADA 1981).

In the whole data population, the degree of homogeneity observed within the two biotypes differed depending on O_3 or PQ treatment (Fig. 2). The distribution of points, as defined by the discriminant functions (CAN 1 and CAN 2), identified three distinctive populations: (i) controls and plants fumigated with O_3 at the lower AOT40 (50 ppb.h); (ii) PQ-treated plants of the PQ-R biotype; (iii) PQ-treated plants of the PQ-S biotype and those fumigated with O_3 at AOT40 1050 ppb.h, of both biotypes. A_{max} was highly associated with the first group; APX and

especially SOD activities were noticeably associated with the second group; Ci and EC were distinctive of the third one.

A lack of cross resistance of PQ-R Conyza to other ROS generators has already been described (VAUGHN & al. 1989) and attributed to PQ compartmentalization and subsequent inability to reach the chloroplast. Only one of the four *Nicotiana tabacum* O_3-tolerant strains tested did show PQ tolerance (SHAALTIEL & al. 1988). Our results support the assumption that there is no evidence of O_3 co-tolerance in PQ-R plants, their behavior when exposed to this pollutant being similar to that of PQ-S individuals. The different stimulation of the antioxidant systems suggests, instead, that the initial target of O_3 damage is different from that of PQ, and metabolic events in response to PQ and O_3 stress are different.

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