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Oxidative stress in herbicide-treated broad bean and maize plants

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Abstract

Treatments of broad bean and maize seedlings with fluometuron, atrazine or rimsulfuron affected some parameters of oxidative stress. Fluometuron significantly reduced activity of Hill reaction (PSII), chlorophyll a+b contents and activity of ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco, EC 4.1.1.39) in leaves of both species and significantly increased contents of H_2O_2, lipid peroxides and carbonyl groups during the whole experiment. There were, moreover, significant inhibitions in activities of superoxide dismutase (SOD; E.C. 1.15.1.1), catalase (CAT; E.C. 1.11.1.6), ascorbate peroxidase (APX; E.C. 1.11.1.11) and guaiacol peroxidase (GPX; E.C. 1.11.1.7). Response to atrazine was, to some extent, similar to fluometuron throughout the entire experiment in broad bean and up mostly to the 12^{th} day of the experiment in maize. The herbicide effect was more pronounced in broad bean than maize. These results point to indicate an occurrence of oxidative stress in both species by fluometuron and only in broad bean by atrazine. The increase in H_2O_2 content accompanied with drop in activities of SOD, CAT and peroxidases indicates a decline in its detoxification rather than increase in its synthesis. On the contrary, rimsulfuron seemed to have no effect on most of the tested parameters although there were transient significant increases in H_2O_2, lipid peroxides and carbonyl groups as well as activities of SOD, CAT, APX and GPX. These findings, based on the recovery in oxidative stress, indicate that fluometuron is involved in oxidative stress generation in both species but atrazine only in broad bean while rimsulfuron is not in both species.

Introduction

Plants, as other aerobic organisms, require oxygen for the efficient production of energy. During the reduction of oxygen to water, active oxygen species (AOSs) such as superoxide radicals ($O^{\cdot -}_2$), hydrogen peroxide (H_2O_2) and hydroxyl radicals ($OH^{\cdot -}$) can be formed. Among other AOSs, singlet oxygen ($^1O_2$) is also capable of damaging important plant cell constituents (Foyer *et al*. 1994). Singlet oxygen sources in biological systems include light-dependent reactions and chemical generations in dark reactions (Briviba *et al*. 1997, Gulner and Dodge 2000, Wakabayashi and Boger 2004). The negative effect of stress is often mediated by AOSs-initiated oxidative damage (Kuzniak 2002). Under optimal environmental conditions, the antioxidant system in plant cells effectively protects them from potentially deleterious effects of AOSs. However, under environmental stress conditions, AOSs generation is enhanced, thus the cellular antioxidant capacity can be over-whelmed and oxidative stress occurs (Hippeli and Elstner 1996, Pasqualini *et al*. 2001, Noctor and Foyer 1998, Hernández *et al*. 2000). AOSs react with lipids, proteins, pigments and nucleic acids and cause lipid peroxidation, membrane damage, inactivation of enzymes, thus affecting cell viability. Some herbicides produce oxidative stress (Van Camp *et al*. 1994). The phenylurea herbicide fluometuron

[l,l-dimethy1-3-(α,α,α-trifluoro-m-tolyl)urea] and the S-triazine herbicide atrazine [2-chloro-4-ethylamino-6-isopropyl-amino-1,3,5-triazine] controls weeds and grasses by blocking the electron flow between the photosystems (Kirkwood 1987, Dodge 1994). On the other hand, the sulfonylurea herbicide rimsulfuron [1-(4,6-dimethoxypyrimidin-2-y1)-3-(pyridylsulfonyl)urea] acts by inhibiting acetohydroxyacid synthase [AHAS, EC 4.1.3.18], the first enzyme in valine, leucine, and isoleucine biosynthesis (Martinetti *et al*. 1995, Jiménez *et al*. 1997). Excess electrons are donated to molecular oxygen to generate superoxide radicals. Superoxide dismutase (SOD; E.C. 1.15.1.1) catalyzes the conversion of superoxide radicals to H_2O_2 and O_2. Hydrogen peroxide is reduced to water by the action of catalase (CAT; E.C. 1.11.1.6), or by ascorbate peroxidase (APX; E.C. 1.11.1.11) and guaiacol peroxidase (GPX; E.C. 1.11.1.7). There are basic differences in dark phase of photosynthesis between broad bean (C3) and maize (C4) plants. However, some other common parameters in both species were checked to investigate their interference by a given herbicide. Therefore, this work was aimed to study the differential effects of herbicides belonging to different groups, on some physiological parameters of oxidative stress in broad bean and maize to elucidate which of these herbicides is possibly involved in the generation of oxidative stress in C3 and/or C4 plants.

Materials and Methods

Plant materials and growth conditions

Broad bean (*Vicia faba* L., Giza 2) and maize *(Zea mays* L., hybrid 351) seeds were surface sterilized and germinated in sand/clay soil (1/1, v/v) in pots (25 cm diameter x 20 cm height). Pots, after being left in dark for 3 days, were transferred to a 14-h photoperiod with 450-500 $\mu mol \cdot m^{-2} \cdot s^{-1}$ photosynthetic photon flux density, 75-80 % relative humidity, and 22/10 °C or 28/14 °C day/night regime for broad bean or maize, respectively. When the seedlings were ten days old, the pots were divided into four groups; one to serve as a control and one for each herbicide treatment at a dosage equivalent to the field rates (2.98, 1.79 and 0.015 kg $ai \cdot ha^{-1}$ for fluometuron, atrazine and rimsulfuron, respectively). The herbicide treatments were applied only once. The rate per hectare was calculated according to the surface area per pot and then the herbicide quantity was solubilized in a suitable amount of water. Seedlings in each pot were sprayed twice, in one direction and crosswise without avoiding that the sprays reach the soil. Shoots were collected just before herbicide application (zero time) and also after 2, 4, 8, 12, 16 and 20 days from the treatment. Samples were washed with copious amounts of water and blotted dry with paper towels before the subsequent analyses.

Determination of Hill reaction activity and chlorophylls content

Leaf tissues were ground in 50 mM Na-tricine (N-tris hydroxymethyl methyl glycine, pH 7.8), 0.3 M sucrose and 3 mM $MgCl_2$. The resulting homogenate was centrifuged at 2,000 x g for 10 min. The pellets were resuspended in 0.1 M NaCl and then centrifuged again at 5,000 x g for 5 minutes. The resulting pellets were resuspended in 1 mM Na tricine (pH 7.8), 10 mM NaCI and 10 mM $MgCI_2$. Photosystem II activity was monitored at 600 nm in 200 mM Na-phosphate (pH 6.7), 2 mM $MgCl_2$ and 0.5 mM 2,6-dichlorophenolindophenol (DCPIP), as indicated by the rate of 2,6-DCPIP photoreduction (Trebst 1972).

Contents of chlorophylls were determined in the fresh tissues after extraction with 85 % acetone according to the spectrophotometric method described by Metzner *et al*. (Metzner *et al*. 1965).

Assay of (Rubisco, EC 4.1.1.39)

According to Keys and Parry (1990), leaf tissues were homogenized in 20 mM Tris-HCl (pH 8.0), 10 mM $NaHCO_3$, 10 mM $MgCl_2$, 1 mM EDTA, 5 mM dithiothreitol (DTT), 0.08 mM chlorohexidine diacetate, and 1 % (w/v) acid-washed insoluble polyvinylpyrrolidone. The homogenate was centrifuged at 5,000 x g for 15 min and the supernatant was treated with solid $(NH_4)_2SO_4$ to give 35 % saturation. After 20 min, the suspension was centrifuged at 20,000 x g for 15 min and the supernatant was raised to 55 % saturation. After centrifugation at 20,000 x g for 15 min, the pellet was dissolved in

a solution contains 20 mM Tris, 1 mM DTT, 1 mM $MgCl_2$ and 0.08 mM chlorohexidine diacetate and adjusted at pH 8. Rubisco activity was assayed in 50 mM 4-(2-hydroxymethyl)1-piperazine ethane sulfonic acid (HEPES, pH 7.8), 10 mM $NaHCO_3$ 20 mM $MgCl_2$, 0.66 mM ribulose-1,5-bisphosphate (RuBP), 0.2 mM NADPH, 5 mM ATP, 5 mM creatine phosphate, 2.0 U creatine phosphokinase, 2.8 U glyceraldehyde-3-phosphate dehydrogenase, and 2.0 U phosphoglycerate kinase. The reaction was started by addition of the enzyme, and the contents were allowed to react at 30 °C for 30 min, during which the decrease in absorbance at 340 nm was measured. One unit was defined as the oxidation of μmol NADPH per minute

Determination of lipid peroxidation and carbonyl groups in proteins

Lipid peroxidation was determined by measuring the concentration of thiobarbituric acid- (TBA-) reacting substances as described by Buege and Aust (1972). Plant tissues were homogenized in 150 mM KCl, centrifuged at 7,000 x g for 15 min and 1 ml of the supernatant was incubated at 37 °C for 2 h after which 1 ml of 0.6 M trichloroacetic acid was added. After thorough mixing, the reaction mixture was centrifuged at 5,000 x g for 10 min. One ml of supernatant was taken with an equal volume of 2-thiobarbituric acid and placed in a boiling water bath for 10 min, cooled, and diluted with 1 ml distilled water. Spectrophotometric readings at 535 nm were calculated as nmoles malonaldehyde (MDA)·g^{-1} plant tissue and then converted to nmol MDA·mg^{-1} protein.

Carbonyl groups were assayed using the dinitrophenyl hydrazine method (Levine *et al*. 1991). Proteins were extracted from 0.5 g of plant tissue with 100 mM potassium phosphate (pH 7.0), 0.1 % Triton X-100, 1 mM Na_2EDTA. After precipitation of the possible contaminating nucleic acids in the sample with 1 % (w/v) streptomycin sulfate, an aliquot of 0.8 ml of the extract was reacted with 0.2 ml of 20 mM dinitrophenyl hydrazine (in 2 M HCl) for 1 h, with vigorous shaking. Proteins were then precipitated with 0.6 M TCA, washed with 1:1 (v/v) ethanol:ethyl acetate, solubilized in 6 M guanidine-HCl (pH 4.5) and the absorbance of the hydrazones (derivatized carbonyls) was measured at 370 nm.

Determination of H_2O_2

Plant tissues were homogenized in 200 mM perchloric acid, centrifuged at 5,000 x g for 10 min and the supernatant was neutralized with 4 M KOH. After centrifugation at 3,000 x g for 5 min, 0.2 ml of the supernatant was loaded on 1 ml column of Dowex 1X8-100 anion exchange resin and eluted with 0.8 ml of distilled water. According to Okuda *et al*. (1991), the assay mixture contained 1 ml extract, 0.4 ml 12.5 mM 3-dimethylaminobenzoic acid in 375 mM phosphate buffer pH 6.5, 0.08 ml 1.3 mM 3-methyl-2-benzothiazolinone hydrazone, and 0.02 ml (0.25 units) horseradish peroxidase. The reaction was initiated by the addition of peroxidase and the increase in absorbance at 590 nm was monitored for 3 min.

Assay of superoxide dismutase (SOD; E.C. 1.15.1.1)

SOD activity was assayed by using the photochemical nitroblue tetrazolium (NBT) method in terms of SOD's ability to inhibit reduction of NBT to form formazan by superoxide (Beyer and Fridovich 1987). Plant tissues were homogenized in 50 mM phosphate, pH 7.8, 0.1 % (w/v) BSA, 5.5 mM ascorbate, and 8 mM β-mercaptoethanol. SOD was assayed in 50 mM phosphate, pH 7.8, 9.9 mM L-methionine, 0.057 mM NBT, 0.025% (w/v) Triton X-100, and 0.1 mM riboflavin. The photoreduction of NBT (formation of purple formazan) was measured at 560 nm and an inhibition curve was made against different volumes of extract. One unit of SOD was defined as that being present in the volume of extract that caused inhibition of the photoreduction of NBT by 50 % of control (0.025 absorption units at 560 nm·min^{-1}).

Assay of catalase (CAT; E.C. 1.11.1.6)

Catalase activity was determined by measuring H_2O_2-dependent oxygen evolution at room temperature with an oxygen electrode unit. Plant tissues were homogenized in 50 mM potassium phosphate buffer, pH 7, and 1 mM DTT. Catalase activity was assayed in 50 mM phosphate buffer, pH 7

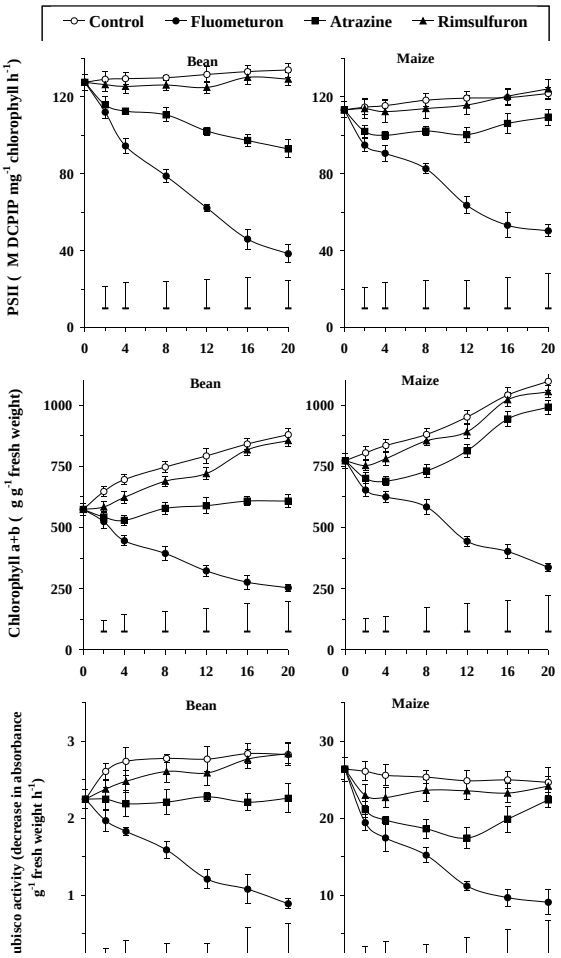

Fig. 1. Effect of herbicides on 2,6-dichlorophenolindophenol (2,6-DCPIP) photoreduction, chlorophyll a+b contents and Rubisco activity in leaves of broad bean and maize. The data presented are means (±SD) of at least six biological replications from two independent experiments. Vertical bars represent LSD at 5 % level.

and 33.5 mM H_2O_2 by monitoring the production of dioxygen using an oxygen electrode (del Rio *et al*. 1977). One unit was defined as the decomposition of μmol H_2O_2 per minute.

Assay of ascorbate peroxidase (APX; E.C. 1.11.1.11)

Plant tissues were homogenized in 0.1 M Tricine--KOH buffer, pH 8, 1 mM DTT, 10 mM $MgCl_2$, 50 mM KCl, 1 mM EDTA, 0.1 % (w/v) Triton X-100, and 0.28 mM phenylmethylsulfonyl flouride (PMSF) and centrifuged at 12,000 x g for 30 min at 4 °C. The reaction medium contained 0.5 mM ascorbic acid, 0.1 mM H_2O_2, 1 mM EDTA, and 0.1 mM HEPES-KOH buffer, pH 7.8 (Ranieri *et al*. 1996). The enzyme activity was determined following the decrease in absorbance at 290 nm in the first 30 s from the start of the reaction using the extinction coefficient of 2.8 $mM^{-1} \cdot cm^{-1}$ for ascorbate. One enzymatic unit is equivalent to 1 μmol of ascorbic acid oxidized·min^{-1}.

Assay of guaiacol peroxidase (GPX; E.C. 1.11.1.7)

Plant tissues were homogenized in 220 mM Tris-HCl, pH 7.4, 250 mM sucrose, 50 mM KCl, 1 mM $MgCl_2$, 160 mM β-mercaptoethanol, and 0.57 mM PMSF and centrifuged at 12,000 x g for 30 min at 4 °C. The reaction mixture contained 20 mM Na acetate, pH 5, 30 mM H_2O_2, 2 mM guaiacol and plant extract. The rate of guaiacol oxidation was recorded at 470 nm and the activity was calculated using the extinction coefficient of 26.6 $mM^{-1} \cdot cm^{-1}$ for tetraguaiacol (Ranieri *et al*. 1997). The peroxidase activity was converted to peroxidase units using horseradish peroxidase standards and assayed under identical conditions as samples.

Protein content was determined spectrophotometrically by reaction with Commassie Brilliant Blue G according to Bradford (1976). All values reported herein are means (±SD) of at least six biological replications from two independent experiments. The full data were statistically analyzed using the least significant difference (LSD) test at 5 % level (Snedecor and Cochran 1980).

Results and Discussion

Figure 1 represents the changes in the activity of Hill reaction (Photosystem II) of broad bean and maize leaves, as indicated by the rate of photoreduction of 2,6-dichlorophenol indophenol (2,6-DCPIP). As compared with controls, fluometuron induced significant reduction in the rates of Hill reaction activity in both species throughout the experiment. Atrazine, on the other hand, significantly reduced Hill reaction activity in broad bean during the whole experiment and in maize up to the 12th day of treatment, thereafter the herbicide became of no significant effect. The greater reduction

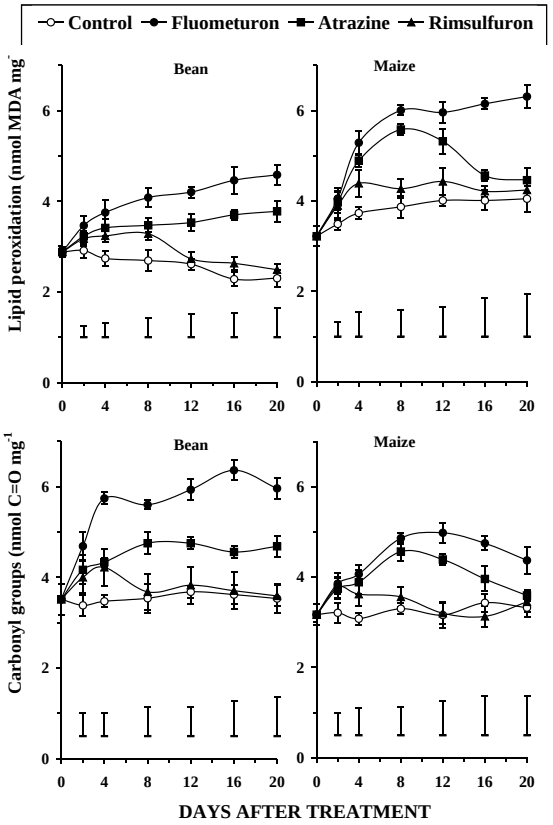

Fig. 2. Effect of herbicides on lipid peroxidation products and carbonyl groups contents in leaves of broad bean and maize. The data presented are means (±SD) of at least six biological replications from two independent experiments. Vertical bars represent LSD at 5 % level.

in Hill reaction activity due to fluometuron or atrazine was observed in broad bean than in maize. On the contrary there were no changes at all in Hill reaction activity in leaves of both species as a result of treatment with rimsulfuron during the entire experimental period.

In addition, fluometuron significantly and progressively decreased the contents of chlorophyll a+b of broad bean and maize seedlings during the entire experiment as compared with the respective controls (Fig. 1). The magnitude of decrease augmented with the elapse of time. Atrazine inhibited chlorophyll accumulation in broad bean during the whole experiment and in maize only during the first 12 days of treatment, thereafter no significant differences from control were found. The decrease in chlorophyll a+b by either herbicide with respect to control was greater in broad bean than in maize. On the other hand, rimsulfuron had no significant effects on chlorophyll a+b of both species although a

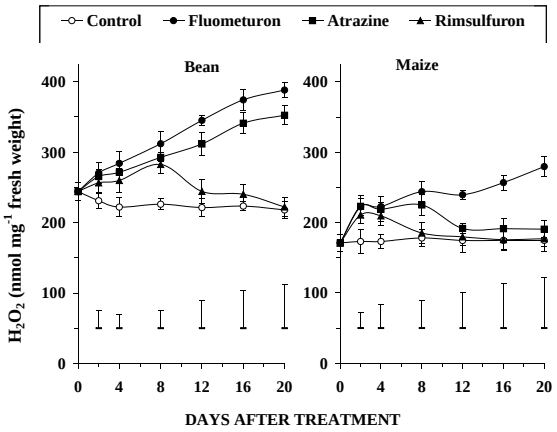

Fig. 3. Effect of herbicides on H_2O_2 contents in leaves of broad bean and maize. The data presented are means (±SD) of at least six biological replications from two independent experiments. Vertical bars represent LSD at 5 % level.

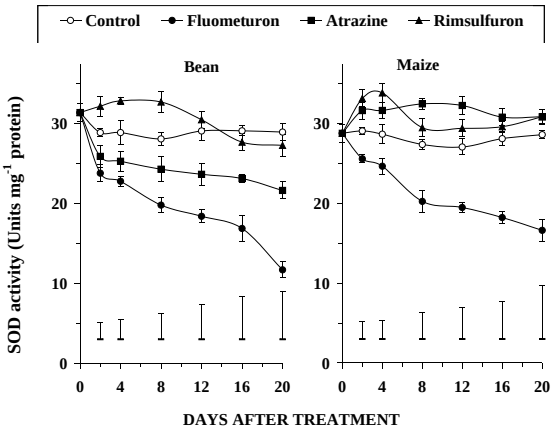

Fig. 4. Effect of herbicides on superoxide dismutase activity in leaves of broad bean and maize. The data presented are means (±SD) of at least six biological replications from two independent experiments. Vertical bars represent LSD at 5 % level.

significant decrease was observed in broad bean only during the first 4 days of treatment.

The observed depression in chlorophyll contents and the activity of Hill reaction in both species in response to treatment with fluometuron, and to some extent with atrazine, appears to coincide with a decrease in activity of Rubisco, the key enzyme in photosynthetic efficiency (Fig. 1). The Figure shows that the activity of Rubisco in shoots of both species was significantly and consistently inhibited by fluometuron treatment during the entire experi-

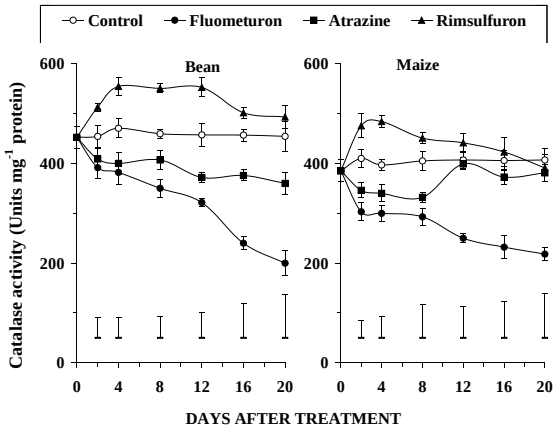

Fig. 5. Effect of herbicides on catalase activity in leaves of broad bean and maize. The data presented are means (±SD) of at least six biological replications from two independent experiments. Vertical bars represent LSD at 5 % level.

mental period as compared with control values at each interval. The enzyme inhibition increased with time. Atrazine slowed down the activity increase observed in the controls. In comparison to control levels at each interval, atrazine resulted in a significant inhibition in the enzyme activity of broad bean during the whole experiment and of maize up to the 12th day of treatment. The magnitude of inhibition in activity of the enzyme activity was higher in broad bean than in maize. The effect of rimsulfuron on the enzyme activity was insignificant from the 2nd day of treatment onward either in broad bean or in maize.

During periods of severe stress, the increased rate of AOSs production results in damage to the photosynthetic apparatus (Van Breusegem *et al*. 1999). In accordance, PSI and PSII were decreased in many plant species in consequence to several stress stimuli (Allakhverdiev *et al. 20*00, El-Shahaby *et al*. 2002). Therefore, AOSs generation due to the disturbances in the electron transport rate of PSI and PSII could subsequently cause degradation of photosynthetic pigments and inhibition of their biosynthesis with a consequence decrease in photosynthesis. In addition, some enzymes of photosynthetic carbon reduction cycle could also be responsible for photosynthesis decreases. Of the important enzymes in driving photosynthesis in both C3 and C4 plants, Rubisco being the most essential one; it is by far the most abundant protein present in

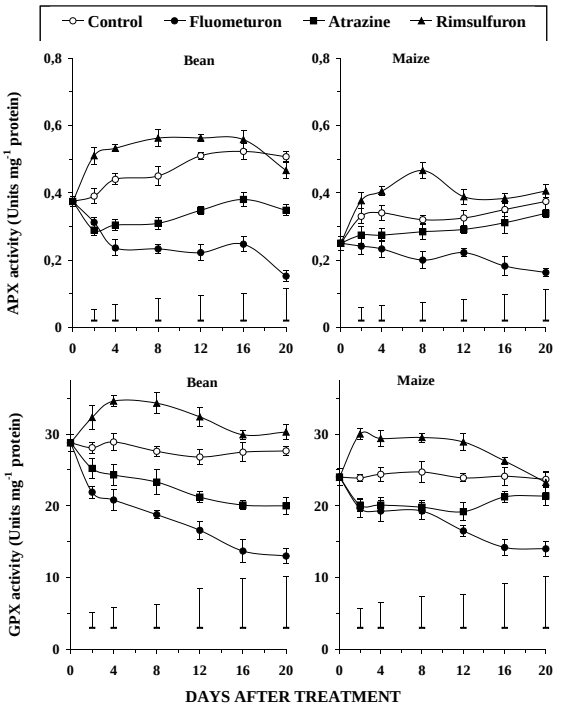

Fig. 6. Effect of herbicides on ascorbate peroxidase and guaiacol peroxidase activities in leaves of broad bean and maize. The data presented are means (±SD) of at least six biological replications from two independent experiments. Vertical bars represent LSD at 5 % level.

green leaves of higher plants (Spreitzer 1993). Vecchia *et al* (2001) reported that bleaching herbicides caused severe photo-oxidative damage to maize chloroplasts. They, moreover, stated that unlike C3 species, C4 species can express a strong tolerance to herbicides. This suggestion could support the present observations that fluometuron and atrazine had more severe effects on broad bean than in maize. On the other hand, Kingston-Smith and Foyer (2000) found that paraquat treatment to maize modifies PSII reaction center polypeptide, D1, increased the number of polypeptides arising from breakdown of Rubisco and increased carbonyl groups in bundle sheath. These effects might result from the generated AOSs that would react with lipids, proteins and pigments causing lipid peroxidation and membrane damage. Consequently, the increased accumulations of lipid peroxides and carbonyl groups due to lipid peroxidation and protein oxidation respectively were checked.

Figure 2 clearly indicates that levels of TBA- reacting substances and carbonyl groups production increased in broad bean and maize following herbicide treatments. The greatest increase in MDA and carbonyl groups occurred in fluometuron-treated plants in both species during the entire period of the experiment. Atrazine also resulted in significant increased lipid peroxides and carbonyl groups production with respect to control values either in broad bean throughout the whole experiment or in maize only during the first 12 days of treatment. The effect of fluometuron and atrazine on the accumulation of MDA and carbonyl groups was greater in broad bean than in maize. Rimsulfuron treatment to both species induced slight increases in both parameters only at the start in comparison to control values. In broad bean and maize, lipid peroxides increased significantly during the first 8 and 4 days of treatment, respectively and carbonyl groups during the first 4 and 2 days, respectively.

The increased rate of MDA and carbonyl groups gives an index of lipid peroxidation and protein oxidation and therefore, of oxidative stress. Increasing in TBA-reacting substances were observed in many plant species due to several factors (Dixit *et al*. 2001, Sandalio *et al*. 2001). Moreover, Halliwell and Gutteridge (1989) confirmed that the peroxidation of cell membranes severely affects its functionality and integrity and can produce irreversible damage to the cell function. On the other hand, Reinheckel *et al*. (1998) reported that the functionality of proteins can be affected by ROSs either by oxidation of amino acid side chains or by secondary reactions with aldehydic products of lipid peroxidation. Both primary and secondary reactions can introduce carbonyl groups into proteins. Such conditions of oxidative stress can also be enhanced by a decrease of enzymatic antioxidants involved in the detoxification of AOSs. These enzymes catalyze the formation and scavenging of H_2O_2 which is considered a potent reactive substance that can inactivate enzymes by oxidizing their thiol groups (Bowler *et al*. 1994).

In Figure 3, H_2O_2 content in broad bean showed a significant increase following treatment with fluometuron or atrazine during the whole experimental period and with rimsulfuron during the first 8 days of treatment. In maize, H_2O_2 content was significantly higher in fluometuron-treated samples than in controls during the whole experiment. Atrazine and rimsulfuron significantly increased these contents only during the first 8 and 4 days of treatment, respectively. The magnitude of increase in H_2O_2 content in treated samples was more apparent in broad bean than in maize. However, there were no significant changes relative to controls either in atrazine-treated broad bean or in both species treated with rimsulfuron. The formation of H_2O_2 or its scavenging might depend on the enzymes SOD or CAT, APX and GPX, respectively. Therefore, the activities of these enzymes were checked.

SOD activity was significantly inhibited in broad bean treated with either fluometuron or atrazine during the entire experiment as well as in maize treated with fluometuron (Fig. 4). The inhibition of SOD by fluometuron at the end of the experiment was greater in broad bean than in maize. In contrast, in maize treated with atrazine the activity of SOD significantly increased up to the 12^{th} day of treatment. A similar response was observed by rimsulfuron up to the 8^{th} and the 4^{th} day of treatment in broad bean and maize, respectively and then it decreased to control levels.

A reduction in SOD activity due to fluometuron and atrazine might be attributed to inactivation of the enzyme by H_2O_2 that is produced in different cellular compartments where SOD catalyses the disproportionation of superoxide radicals (Bowler *et al.* 1994, Van Camp *et al*. 1994, 1996, Sandalio *et al*. 2001). Moreover, enhanced levels of SOD in plants has been correlated with tolerance to oxidative stress (Van Breusegem *et al*. 1999). In addition, overproduction of SODs in plant chloroplasts has been found to increase protection against herbicides (Van Camp *et al*. 1996, Iannelli *et al*. 1999). SOD and APX are responsible, respectively, for H_2O_2 production and its scavenging (Yamaguchi *et al*. 1995, Jimenez *et al*. 1997). Therefore, the decreased activity of SOD in response to treatment with fluometuron and atrazine point out that the high H_2O_2 levels might not be a result of an active production but probably due to a decreased capacity of its scavenging. However, there was a retraction in H_2O_2 content in both species treated with rimsulfuron after an incipient rise. Similar retrac-

tions were also observed in atrazine-treated maize probably due to the efficient degradation of the herbicide by GSH and GST mechanism in maize relative to broad bean (Farago *et al*. 1993, Nemat Alla 1995, 2000, Nemat Alla and Hassan 1996, Hassan 2000). In spite of the high SOD activity in both species treated with rimsulfuron and in maize treated with atrazine, H_2O_2 contents remained lower than in fluometuron-treated samples. These observations could indicate that a decrease in peroxide detoxification was set in by fluometuron. On the other hand, the decreased capacity to decompose H_2O_2 may be the result of an unsuccessful activation of CAT and peroxidase enzymes.

As shown in Figure 5, fluometuron treatment resulted in a significant inhibition in CAT activity of both species throughout the entire experiment. The inhibition of CAT activity was greater in broad bean than in maize. Atrazine also induced a significant inhibition in CAT activity in broad bean during the whole experiment. In maize, the effect was observed up to the 8th day of treatment then it became non significant. Rimsulfuron, on the contrary, significantly stimulated the CAT activity up to the 12th day in broad bean and up to the 4th day of treatment in maize; the enzyme activity became thereafter comparable to control levels.

There was a decrease in CAT concomitant with an enhanced production of carbonyl groups probably because CAT is known to be a target of peroxisomal protease activity (Distefano *et al*. 1999). Thus the decreases in CAT activity in the present study by fluometuron and atrazine could explain the accumulated levels of H_2O_2. Because APX and GPX are efficient in preventing the accumulation of H_2O_2, their activities were checked (Fig. 6). Both enzymes responded similarly to each herbicide. Fluometuron resulted in a significant decrease in APX and GPX activities in both species during the whole experiment; the effect was greater in broad bean than maize. In response to atrazine, activities of APX and GPX were inhibited either in broad bean during the entire experiment or in maize during the first 4 and 12 days of treatment, respectively. On the contrary, rimsulfuron induced significant increases in APX activity of both species up to the 8th day of treatment and in GPX up to the 12th day. Thereafter, activities of both enzymes decreased to control values.

Under these conditions, where CAT, APX and GPX are diminished, the cell is not fully competent to remove H_2O_2 which would accumulate to toxic levels. Moreover, the decrease in SOD activity would delay the elimination of superoxide radicals whereof would decrease plant tolerance to herbicides. In this context, Iannelli *et al*. (1999) found that an increased activity of SOD and APX is associated to the increased tolerance of maize to paraquat. In addition, Pyon *et al*. (2004) reported that the activities of SOD, CAT and peroxidases were higher in paraquat-resistant *Erigeron canadensis* than in susceptible biotype. Therefore, the inhibition in CAT, APX and GPX activities might confirm a deficiency in H_2O_2 detoxification with a consequent rise in its contents.

In conclusion, treatment of both species with fluometuron declined activities of SOD, CAT, APX and GPX, PSII activity, chlorophyll contents and Rubisco activity. On the other hand, there were increases in H_2O_2 contents, lipid peroxides and carbonyl groups. Similar responses were observed in broad bean treated with atrazine. However, rimsulfuron treatment to both species induced incipient increases in H_2O_2, lipid peroxides, carbonyl groups, and decreases in PSII activity, chlorophyll contents and Rubisco activity accompanied with increases in activities of SOD, CAT, APX and GPX. Thereafter, a retraction in all tested parameters to reach control levels was mostly set in indicating that rimsulfuron seemed insignificant upon oxidative stress induction. The fast recovery in oxidative stress in both species treated with rimsulfuron and, to some extent, maize treated with atrazine might be owing to an increase in herbicide detoxification. In contradiction, more persistent fluometuron in both species and also atrazine in broad bean would result in constantly occurrence of oxidative stress. These findings might indicate that fluometuron is involved in the generation of oxidative stress in both species but atrazine only in broad bean while rimsulfuron is not in both species.

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