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Selective Herbicide Activity of 2,5-Di(benzylamine)-*p*-benzoquinone against the Monocot Weed *Echinochloa crusgalli*. An in Vivo Analysis of Photosynthesis and Growth

MINERVA GONZÁLEZ-IBARRA,[†,‡] NORBERTO FARFÁN,[§] CARLOS TREJO,[||] SALVADOR URIBE,[⊥] AND BLAS LOTINA-HENNSEN*[,†]

Departamento de Bioquímica, Facultad de Química, Universidad Nacional Autónoma de México (UNAM), Ciudad Universitaria, 04510 México DF, Departamento de Química, Centro de Investigación y Estudios Avanzados del IPN, Especialidad de Botánica, IRENAT, Colegio de Posgraduados, Montecillos, and Instituto de Fisiología Celular, UNAM, Mexico

Six 2,5-diamino-*p*-benzoquinone derivatives previously characterized as photosystem I electron acceptors were tested for their postemergence herbicide activity. By induction kinetics of chlorophyll *a* fluorescence performed in vivo it was determined that 2,5-di(benzylamine)-*p*-benzoquinone diverted electrons at the reducing side of the chloroplast photosystem I. This derivative decreased the efficiency of photosystem II as evidenced by the decrease in the F_v/F_m change in *Echinochloa crusgalli* leaf disks. In addition, 2,5-di(benzylamine)-*p*-benzoquinone was a CO_2 assimilation inhibitor to Rubisco: the A/C_i curve analysis indicates that 2,5-di(benzylamine)-*p*-benzoquinone affected both the carboxylation reaction itself and the regeneration of RuBP. 2,5-Di(benzylamine)-*p*-benzoquinone did not exhibit any effect on the dicot plants *Phaseolus vulgaris* and *Physalis ixocarpa* or the monocot *Zea mays*. These species may have metabolized the herbicide to an inactive compound. Thus, 2,5-di(benzylamine)-*p*-benzoquinone was found to be a selective herbicide against the monocot weed *E. crusgalli*.

KEYWORDS: 2,5-Di(benzylamine)-*p*-benzoquinone; monocot weed herbicide; leaf gas exchange; *Echinochloa crusgalli*; chlorophyll *a* fluorescence

INTRODUCTION

Inhibitors may block photosynthesis at different sites along the electron transport chain (*1*). Electron acceptors may divert electron flow at the level of different redox enzymes. In isolated chloroplasts, many extensively studied commercial herbicides inhibit the reducing side of photosystem II (PSII) or accept electrons at the reducing side of photosystem I (PSI). Bipyridinium derivatives have been studied both as herbicides and as electron acceptors (*2*, *3*). Bipyridinium derivatives cause membrane lipid peroxidation leading to leaf wilting. Peroxidation results from a cycle of photosynthetic electron transport chain-mediated photoreduction of the herbicide followed by its reoxidation by molecular oxygen. During this cycle, reactive oxygen species (ROSs) are released.

Among the many herbicides acting as PSI electron acceptors, there are also nonbipyridinium molecules such as quinones and quinonoid derivatives (not derived from benzoquinones) which are less prone to produce ROSs because they possess high reactivity toward each other (*4*). Thus, instead of combining with O_2 to produce ROSs, when two quinone derivatives accept electrons from PSI, the two resulting semiquinones react with each other to yield an oxidized quinone plus one quinol (*4*).

The efficiency of electron acceptor herbicides does not depend solely on their affinity for the proteins they inhibit. To gain access to their target(s) in thylakoids they need to traverse many barriers including the plant epidermis. In addition, biological detoxification may modify the inhibitor, yielding inactive molecule(s). Consequently, molecules which are potent electron acceptors in isolated chloroplasts may be completely inactive when tested on the whole plant. Therefore, experiments with whole plants are required to check whether a chloroplast electron acceptor is a herbicide.

In the search for potential agrochemicals, six different 2,5-diamino-*p*-benzoquinone derivatives have been tested in isolated chloroplasts. The electron acceptor activity in PSI and the redox potential of each derivative were determined (*5*). These deriva-

* To whom correspondence should be addressed. Phone: +5255 56225294. Fax: +5255 56225329. E-mail: blas@servidor.unam.mx.
[†] Facultad de Química, UNAM.
[‡] Permanent address: Universidad Autonóma Metropolitana-Xochimilco, Calzada del Hueso1100, Mexico 04960 DF, Mexico.
[§] Centro de Investigación y Estudios Avanzados del IPN.
[||] Colegio de Posgraduados.
[⊥] Instituto de Fisiología Celular, UNAM.

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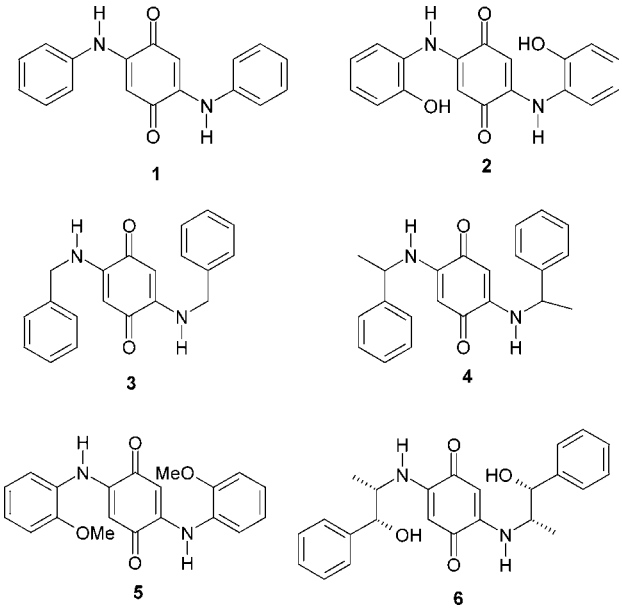

Figure 1. Structures of diamino-*p*-benzoquinone derivatives (**1**) 2,5-di(phenylamine)-*p*-benzoquinone, (**2**) 2,5-di(*o*-aminophenol)-*p*-benzoquinone, (**3**) 2,5-di(benzylamine)-*p*-benzoquinone, (**4**) 2,5-di((*R*,*S*)-(+)-α-methylbenzylamino)-*p*-benzoquinone, (**5**) 2,5-di(*o*-anisidine)-*p*-benzoquinone, and (**6**) 2,5-*N*,*N*-(1′*R*,2′*R*)-norpseudoephedrine-*p*-benzoquinone.

tives increase oxygen consumption in illuminated thylakoids, as does paraquat, a commercial herbicide. For each 2,5-diamino-*p*-benzoquinone derivative, the 50% concentration needed to accept electrons at PSI (AC_{50}) was determined (*5*). The most active molecule is derivative **3**, 2,5-di(benzylamine)-*p*-benzoquinone ($AC_{50} = 0.23$ μM), which exhibits an affinity 478 times higher than that observed for paraquat ($AC_{50} = 110$ μM). The PSI electron-accepting properties of **3** are similar to those of the bipyridinium herbicides (*4*, *5*). Thus, it was considered of interest to test the 2,5-diamino-*p*-benzoquinone derivatives as postemergence herbicides on whole plants and to determine their mechanism of action.

Each 2,5-diamino-*p*-benzoquinone derivative **1**−**6** (**Figure 1**) previously tested in isolated chloroplasts (*5*) was evaluated for its postemergence herbicide potential. The effects of derivatives **1**−**6** on CO_2 assimilation, stomatal conductance, biomass production, leaf chlorophyll (Chl) content, membrane permeability, and leaf chlorophyll *a* fluorescence were measured. The herbicide effects were tested on four plant species: two crop plants (*Zea mays* L. and *Phaseolus vulgaris* L.) and two common weeds *(Echinochloa crusgalli* L., P. Beauv. and *Physalis ixocarpa* L.).

MATERIALS AND METHODS

2,5-Diamino-*p*-benzoquinone Derivatives. All 2,5-diamino-*p*-benzoquinone derivatives **1**−**6** (**Figure 1**) were prepared according to ref *5* using the method described by Ross (*6*). These were (**1**) 2,5-di(phenylamine)-*p*-benzoquinone, (**2**) 2,5-di(*o*-aminophenol)-*p*-benzoquinone, (**3**) 2,5-di(benzylamine)-*p*-benzoquinone, (**4**) 2,5-di((*R*,*S*)-(+)-α-methylbenzylamino)-*p*-benzoquinone, (**5**) 2,5-di(*o*-anisidine)-*p*-benzoquinone, and (**6**) 2,5-*N*,*N*-(1′*R*,2′*R*)-norpseudoephedrine-*p*-benzoquinone.

Plant Material. The seeds of two weed species (*E. crusgalli* L., P. Beauv. and *P. ixocarpa* L.) and two crop species (*Z. mays* L. and *P. vulgaris* L.) were sown in 12 cm diameter pots containing a mixture of 2/1 v/v soil/sand. All pots were watered daily and maintained close to field capacity. In the greenhouse, $T = 25-30$ °C and there is normal day/night illumination. After 30 and 18 days of emergence for weed and crop species, respectively, plants were selected for uniformity. Plants of similar size were divided into two groups: the control and the experimental, which was sprayed manually with a 40 μM solution of a 2,5-diamino-*p*-benzoquinone derivative. In preliminary experiments this concentration mediated maximum growth inhibition (data not shown). Each derivative was dissolved in DMSO to 20 mM. An aliquot of this solution was taken to obtain the desired concentration in an aqueous suspension containing maize oil (0.1% w/v, as an adjuvant) and 0.25% v/v Tween 20. Tween 20 was added to reduce the surface tension of the suspension. The control group was sprayed with distilled water containing the same amount of DMSO, Tween 20, and maize oil. The plants were arranged in a completely randomized design, and then the CO_2 assimilation rate, stomatal conductance, chlorophyll *a* fluorescence, total chlorophyll content, electrolyte leakage, and biomass production were determined.

Gas Exchange Measurements. The youngest fully expanded leaf 1, 24, and 120 h after treatment was used. Measurements were made with a portable open infrared gas analyzer system (CIRAS-1, PP Systems, Amesbury, MA) in eight different plants. Measurements were taken at 25−30 °C over a photosynthetic photon flux density (PPFD) range of 800−1000 μmol m^{-2} s^{-1}.

Chlorophyll fluorescence determination was performed for control plants and plants sprayed with a 40 μM concentration of each derivative. After being kept in the dark for 15 min, the leaves were excited by saturating light from an array of six light-emitting diodes delivering 3000 μmol of photons m^{-2} s^{-1} of red light (peak at 650 nm). Chlorophyll *a* fluorescence induction curves were measured at room temperature with a portable shutterless apparatus (plant efficiency analyzer, Kings Lynn, U.K.) (*7*).

Dry Biomass. After 15 days of treatment with the derivatives, all plants were harvested at ground level and dried in an oven at 65 °C for at least 72 h (or until constant weight). Then the dry mass was determined.

Electrolyte Leakage Assay. Four leaf disks of *E. crusgalli* ($d = 7$ mm) were washed in 20 mL of double-distilled water. Five days after the derivatives were applied, the changes in cell membrane permeability were determined by measuring the solution conductivity at 1 h intervals for 10 h, using a Horiba B-173 conductivity meter. Experiments were conducted at 25 °C in the absence of light.

Leaf Chlorophyll Determination. Four 7 mm diameter leaf disks were collected from the *E. crusgalli* control or *E. crusgalli* treated with derivative **3**, and the weight was determined. Each measurement was repeated three times. The disks were placed into test tubes and incubated in 5 mL of *N*,*N*-dimethylformamide at 4 °C for 24 h in the dark for chlorophyll extraction. The absorbance of a 1.0 mL sample was determined at 664.5 nm for chlorophyll *a* and at 647 nm for chlorophyll *b* using a spectrophotometer (Beckman model DV-650). The extinction coefficients assigned by Inskeep and Bloom (*8*−*10*) were used to estimate the chlorophyll concentration.

RESULTS

Gas Exchange Measurements. CO_2 assimilation and stomatal conductance were measured 1, 24, and 120 h after foliar application of each 2,5-diamino-*p*-benzoquinone derivative **1**−**6** (**Figures 2** and **3**). The effect of the derivatives on the dicot plants *P. vulgaris* and *P. ixocarpa* was minimal (**Figure 2**), while the effects on the monocots *Z. mays* and *E. crusgalli* were much higher (**Figure 3**); e.g., after application of 40 μM derivative **3**, high effects were observed on *E. crusgalli* and *Z. mays* (**Figure 3**), while almost no effects on the dicot plants were observed (**Figure 2**).

In *E. crusgalli* 1 h after application, up to 80% inhibition in CO_2 assimilation (**Figure 3A**) and 45% inhibition of stomatal conductance (**Figure 3B**) were observed. Five days after application this effect was maintained with only slight changes for derivatives **3** and **4**. In contrast, after 5 days the effects of derivatives **2**, **5**, and **6** were reverted to different extents (**Figure 3A,B**). The effect of derivative **1** on CO_2 assimilation was negligible, while the effect on stomatal conductance was similar to those observed for derivatives **3** and **4** (**Figure 3A,B**).

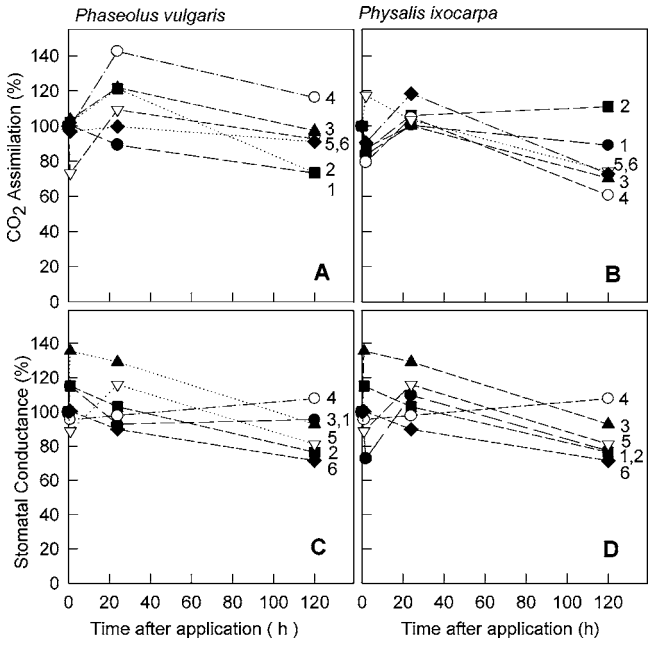

Figure 2. Rate of CO_2 assimilation and stomatal conductance of *P. vulgaris* (**A**, **C**) and *P. ixocarpa* (**B**, **D**) in the presence and absence of different 2,5-diamino-*p*-benzoquinone derivatives. Each 2,5-diamino-*p*-benzoquinone derivative was sprayed on the leaves of each plant from a 40 μM concentration solution. Measurements were made at the indicated times. (**A**, **B**) CO_2 assimilation rates after 1, 24, and 120 h of exposure. (**C**, **D**) Stomatal conductance of the uppermost fully expanded leaf 1, 24, and 120 h after exposure. Results are percentages of the control. Data are the means of eight determinations. For assimilation rates, SEs were always below 1.2 μmol m^{-2} s^{-1} (see **Table 1**), and for stomatal conductance, SEs were equal to or less than 36 mmol m^{-2} s^{-1}.

In *Z. mays* the effect of each of these six derivatives reached maximum inhibitory effects after 24 h, exhibiting 84% inhibition of CO_2 assimilation (**Figure 3C**). Afterward the effect diminished for derivatives **3**−**6**, while derivatives **1** and **2** maintained the maximum inhibition after 5 days (**Figure 3C**). In regard to stomatal conductance, 62% inhibition was observed at 20 h of application, and only the effect of derivative **3** was slightly reverted after 120 h (**Figure 3D**). These results indicate that derivative **3** is a postemergence herbicide, highly selective against the monocot *E. crusgalli.* In the dicot plants tested (Figure 2), derivative **3** mediated a small inhibition (3−27%), a small stimulation (6−42%), or no effect, both on CO_2 assimilation and on stomata conductance. Derivatives **1** and **2** were not toxic to the weeds tested, while they were toxic to *Z. mays* and thus were not studied further.

Intercellular CO_2 Concentration. The effects of derivative **3** on stomatal conductance and on the rate of CO_2 assimilation were observed at the same derivative concentrations, and thus, it was decided to test whether these effects were related, i.e., that stomatal closure limited CO_2 availability, leading to decreased assimilation. To test this, the concentration of CO_2 within the tissue was measured (**Table 1**), and it was determined it was not affected by stomatal closure. In *E. crusgalli* derivative **3** inhibited CO_2 assimilation (**Figure 4**, **Table 1**), while in *P. ixocarpa*, *Z. mays*, and *P. vulgaris* less or no inhibition of CO_2 assimilation was observed (**Figure 4**).

To determine the intercellular CO_2 concentration (C_i) dependence of the CO_2 assimilation (A) by *E. crusgalli* and its inhibition by **3**, the rate of CO_2 assimilation was determined in the presence of increasing internal CO_2 concentration (C_i) on leaves of the control and **3**-treated *E. crusgalli* (**Figure 5**).

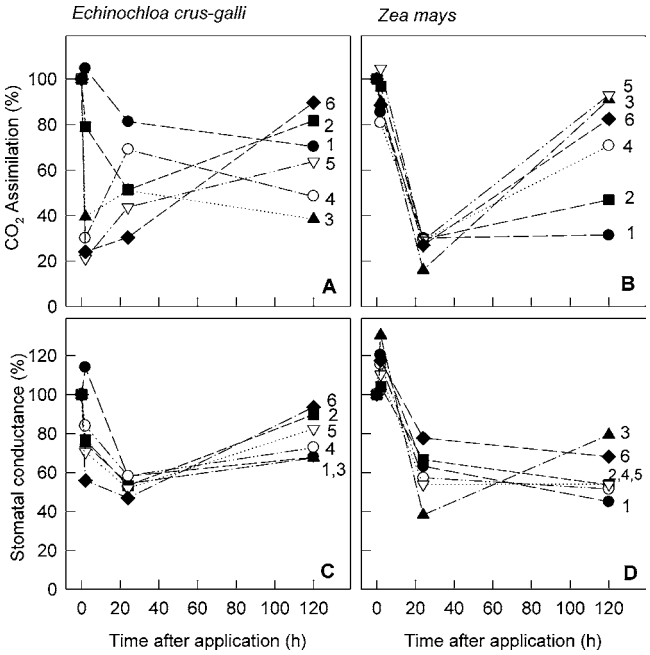

Figure 3. Rate of CO_2 assimilation and stomatal conductance of *E. crusgalli* (**A**, **C**) and *Z. mays* (**B**, **D**) in the presence and absence of different 2,5-diamino-*p*-benzoquinone derivatives. Experimental conditions are as described in **Figure 2**. (**A**, **B**) CO_2 assimilation rates after 1, 24, and 120 h of exposure. (**C**, **D**) Stomatal conductance of the uppermost fully expanded leaf 1, 24, and 120 h after exposure. For assimilation rates, SEs were always below 1.2 μmol m^{-2} s^{-1}, and for stomatal conductance, SEs were equal to or less than 18 mmol m^{-2} s^{-1} (see **Table 1**).

Table 1. CO_2 Assimilation Rate (A), Stomatal Conductance (G_s), and Intercellular CO_2 Concentration (C_i) in the Control and 40 μM Derivative **3**-Treated Plants[a]

species	A (μmol m^{-2} s^{-1})	G_s (mmol m^{-2} s^{-1})	C_i (μL L^{-1})
Z. mays			
control	8.0 ± 0.4	103.9 ± 6.5	196.1 ± 4.9
3-treated	7.2 ± 0.6	82.4 ± 18.0	212.0 ± 21.6
E.crusgalli			
control	10.7 ± 1.1	100.7 ± 8.6	171.0 ± 23.1
3-treated	4.2 ± 0.6	75.3 ± 7.4	221.0 ± 21.6
P. vulgaris			
control	7.2 ± 0.3	161.8 ± 10.0	256.0 ± 5.8
3-treated	7.0 ± 0.4	150.0 ± 14.8	254.0 ± 5.3
P.ixocarpa			
control	6.3 ± 0.6	184.6 ± 36.6	254.0 ± 8.6
3-treated	5.6 ± 1.1	151.2 ± 31.1	254.3 ± 6.3

[a] Data are the mean ± SE of eight measurements (n = 8).

Treatment of *E. crusgalli* with **3** resulted in a reduction both in the initial A slope and in A_{sat} (**Figure 5**). Five days after treatment with **3**, in *E. crusgalli* leaves $V_{c,max}$ decreased to 0.043 μmol m^{-2} s^{-1}, while in control plants $V_{c,max}$ was 0.21 μmol m^{-2} s^{-1}. Derivative **3** significantly affected A_{sat}, but the CO_2 compensation point was not affected; for both treatments the average value was 25 μL L^{-1}. These results suggest that, in *E. crusgalli* treated with **3**, there is an inhibition of both the carboxylation reaction and those factor(s) responsible for regenerating acceptors for CO_2 fixation. The effects on CO_2 assimilation kinetics are similar to those promoted by other factors published previously (*11*−*13*).

Chlorophyll Fluorescence Determination. In the case of herbicides that block photosynthetic electron transport at the

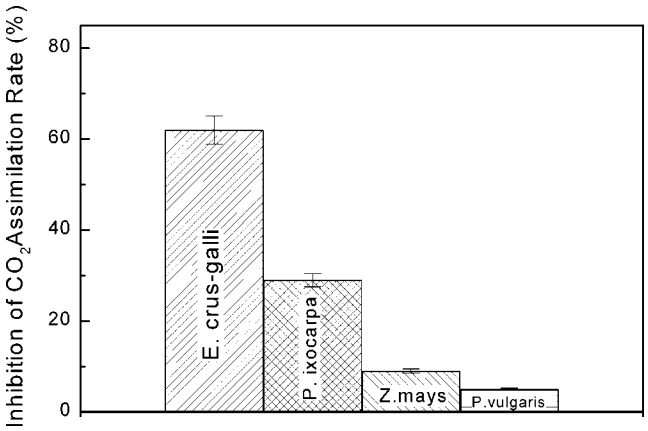

Figure 4. Derivative **3**-mediated inhibition of the rate of CO_2 assimilation in each of the weed and crop species selected. Experimental conditions are as in **Figure 2**. The rate of CO_2 assimilation was determined 120 h after spraying and is presented as the inhibition percentage of a control sprayed with the solution without **3**.

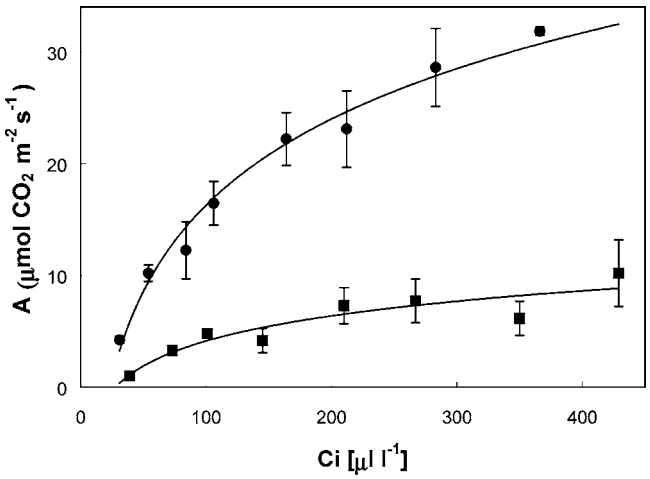

Figure 5. Relationship between the rate of CO_2 assimilation (A) and intercellular concentration of CO_2 (C_i) in leaves of *E. crusgalli.* Measurements were conducted at an irradiance of 900 μmol of quanta m^{-2} s^{-1} and a leaf temperature of 28 °C. Key: (●) untreated controls, (■) samples treated with 40 μM derivative **3**. Data are from the leaves of five different plants. Bars are SEs.

reducing side of PSII or divert electrons at the level of PSI, fluorescence emission measurements may be used as an activity monitor. To further characterize the mechanism of action of derivative **3** as a potential herbicide, its effects on chlorophyll *a* fluorescence were studied in *E. crusgalli* leaves (**Figure 6**). The herbicides diuron and paraquat were included as controls. Inhibition of electron transport at the reducing side of PSII between Q_A and Q_B is indicated by a higher fluorescence yield, which results in the formation of an O−J sequence. In an *E. crusgalli* leaf the well-known photosynthesis inhibitor diuron was added at two different concentrations (100 or 40 μM), and the resulting increase in fluorescence was recorded. The increase in fluorescence is due to the accumulation of Q_A^-, resulting in the complete closure of PSII reaction centers during the first 2 ms of the induction curve. In the absence of diuron, the leaf required 900 ms to close all PSII reaction centers, exhibiting a classical polyphasic rise, i.e., an O−J−I−P Chl *a* fluorescence transient (*7*) (Figure 6).

An increase in the relative variable fluorescence yield in the presence of both derivative **3** and paraquat with a polyphasic rise similar to that of the control was indicative of a reoxidation

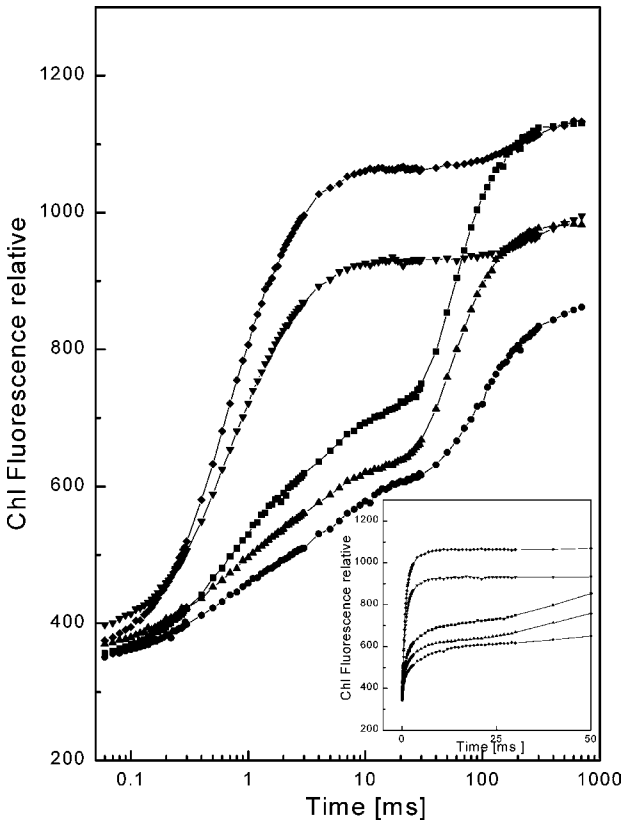

Figure 6. Effect of different herbicides on the chlorophyll fluorescence induction curves of a dark-adapted *E. crusgalli* leaf. Chlorophyl fluorescence was measured 1 h after application in the absence (control) and in the presence of different herbicides. The leaf was dark adapted for 15 min and further excited for 1 s at a light intensity of 3000 μmol of photons m^{-2} s^{-1} Key: (■) control, (●) 40 μM paraquat, (▲) 40 μM derivative **3**, (▼) 40 μM DCMU, (◆) 100 μM DCMU. Inset: linear plot of the data.

of Q_A^- to Q_A. Therefore, neither derivative **3** nor paraquat seems to promote accumulation of Q_A^-, but instead they seem to act as electron diverters. These data indicated that the main target site of derivative **3** was located in the acceptor side of PSI. Furthermore, the shape of the induction curve of chlorophyll *a* in the presence of derivative **3** at 1 is lower than that found in the control although not as much as in the presence of paraquat, suggesting that derivative **3** is indeed diverting electrons at PSI both in vivo (**Figure 6**) and in vitro (*5*). The induction curve of chlorophyll *a* fluorescence on maize, bean, and green tomato leaves in the presence of **3** was similar to that of the control in the absence of derivative **3** (data not shown); thus, these results confirm the effect of derivative **3** is selective against the monocot weed *E. crusgalli.* In the inset to **Figure 6**, the lineal time plot of fluorescence demonstrates the high fluorescence increase in the presence of diuron as compared to that of the control or the samples in the presence of derivative **3** or paraquat.

In *E. crusgalli*, the Chl *a* fluorescence results indicated that, on the first day after treatment with paraquat, no effects on the activity of PSII were detected, while it was only mildly inhibited by **3** (data not shown). However, in other plants it has been shown that, following exposure to paraquat, the activity of PSII does decrease at longer times (*14*, *15*). Thus, it was decided to determine the long-term effect of leaf exposure to derivative **3** on the activity of PSII in *E. crusgalli* (**Figure 7**). The maximal quantum yield of PSII primary photochemistry is given by $F_v/F_m = 1 - F_o/F_m$. In healthy *E. crusgalli* leaves, the value is 0.8. The F_v/F_m ratio has become an important and easily

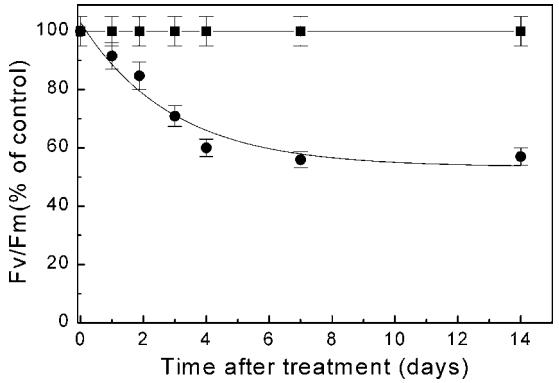

Figure 7. Time courses of the maximum quantum yield of PSII photochemistry (F_v/F_m) in intact attached leaves of *E. crusgalli* (●) untreated or (■) treated with 40 μM derivative **3**. Values are the percentage of the initial fluorescence of an untreated sample. Data are the mean ($n = 6$) ± SE.

measurable parameter of the physiological state of the photosynthetic apparatus in intact plant leaves (*14*, *15*). Environmental stresses that affect PSII efficiency lead to a characteristic decrease in the F_v/F_m ratio as found in *E. crusgalli* leaves. After 7 and 14 days of exposure, the leaf treated with **3** exhibited a decrease of 44% in the F_v/F_m ratio (**Figure 7**). These last results indicate that **3** inhibits the electron flow at PSII by 44%. These results were similar to those reported for the effect of paraquat in other plants (*14*, *15*).

Biomass production was estimated by measuring the dry weight of *Z. mays* and *E. crusgalli* plants grown in the presence or absence of herbicides (**Figure 8**). After 14 days of application of 40 μM derivative **3**, the total dry biomass was decreased by 76.5% in *E. crusgalli* plants. In contrast, the dry weight of *Z. mays*, *P. vulgaris*, and *P. ixocarpa* plants was not affected by **3** (data not shown). Thus, derivative **3** acts as a selective postemergence herbicide by reducing biomass production only in the weed *E. crusgalli*. In contrast, paraquat was not selective, as it reduced the dry weight of both *E. crusgalli* by 70% and *Z. mays* by 60%.

Membrane Permeability. To determine whether derivative **3** damages membranes, conductivity was assayed in leaf disks. In *E. crusgalli* leaf disks treated with derivative **3** no electrolyte leakage from the cells was detected 5 days after application (**Table 2**). This was in contrast to the marked effect of paraquat, which did promote electrolyte leakage 4 times higher than in the control (**Table 2**).

Chlorophyll Concentration. To rule out whether **3** inhibits chlorophyll synthesis and/or increases degradation, its effect on chlorophyll concentration was measured. Derivative **3** did not affect the total chlorophyll contents of *E. crusgalli* leaves after 120 h of treatment (**Table 2**). However, in the presence of the positive control paraquat, the chlorophyll concentration decreased by almost 50%.

DISCUSSION

In isolated chloroplasts, one of the most important primary effects of molecules such as triazines, pyradiazines, phenylureas, and uracils is inhibition of photosynthesis. However, photosynthesis inhibition in isolated chloroplasts is no guarantee that a given molecule will be a herbicide in vivo.

Many commercial herbicides inhibit the Hill reaction at the reducing side of PSII (*2*). In addition, bipyridinium derivatives divert electrons at PSI (*3*). The 2,5-diamino-*p*-benzoquinone derivatives tested here are PSI electron acceptors (*5*). Furthermore, all these derivatives possess midpoint potential values between −0.805 and −0.654 V (*5*), well within the margins of −1.0 to −0.7 V established for electron carriers at the reducing side of PSI (*16*).

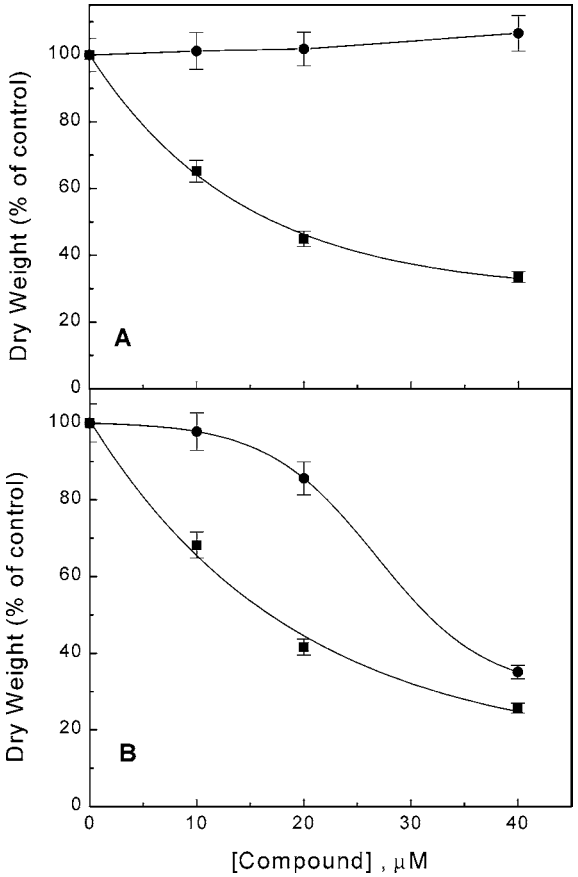

Figure 8. Comparison of the effect of **3** (**A**) and paraquat (**B**) on growth of *E. crusgalli* (■) and *Z. mays* (●) measured as dry weight (percentage of the control) after 14 days of treatment. Data are the mean ($n = 8$) ± SE.

Table 2. Response of *E. crusgalli* Disks to Exposure to 40 μM Derivative **3** or Paraquat after 5 Days of Application

	cellular toxicity at 120 h (%)	
derivative	electrolyte leakage increase	chlorophyll loss
control	100.0	0
3	135.6	3.5
paraquat	438.59	44.3

[a] Results are percentages of the control. All data are the mean of three replicates.

In isolated spinach thylakoids, 2,5-diamino-*p*-benzoquinone derivatives behave as PSI electron acceptors similarly to the bipyridinium herbicide paraquat (*4*). However, when each of the derivatives **1**−**6** was sprayed in vivo on the leaves of four different species, only derivative **3** was active as a postemergence, highly selective herbicide on the monocot weed *E. crusgalli* (**Figure 8**). Furthermore, the crop *Z. mays*, which is also a monocot, was initially affected by derivative **3**, but probably metabolized the derivative, because the plant recovered.

Derivatives **1**, **2**, **4**, **5**, and **6** are probably metabolized to inactive molecules by the plants. Otherwise, these molecules may be sequestered by a cell component(s) that does not allow them to reach the photosynthetic machinery. In this regard, the

dicot species studied (*P. vulgaris* and *P. ixocarpa*) were not affected by any of the 2,5-diamino-*p*-benzoquinones tested. The differential sensitivity to derivative **3** found on monocot plants may reflect different rates of absorption, translocation, interaction, or detoxification.

Farange and Long (*17*) reported that, in *Triticum aestivum* L., exposure to O_3 results in a reduction in A_{sat} by 35%, and this can be accounted for by the significant reduction in $V_{c,max}$. The authors suggested that the effect could be attributed to a decrease in the concentration of active Rubisco enzyme. In this study, we found a similar result when *E. crusgalli* leaves were sprayed with **3**, suggesting that in this case the quantity of active Rubisco also decreases.

At variance with paraquat, derivative **3** did not affect plasma membrane integrity measured as electrolyte leakage or loss of total chlorophyll contents. This may be explained when it is considered that paraquat generates ROSs in photochemically active tissues, and ROSs produce lipoperoxidation (*4*). In contrast, the semiquinone form of **3** produced by PSI-mediated reduction has a tendency to react with itself, producing a quinone and a quinol (*4*). This would result in less ROS production and lipid peroxidation than with paraquat (*2*−*4*). Thus, even though the dry weight data demonstrate that in *E. crusgalli* derivative **3** is a herbicide, the action mechanism of derivative **3** seems to involve mainly an inhibition of photosynthesis which occurs at least at two different levels: (i) as a PSI electron acceptor and (ii) at the level of carboxylation of CO_2, in the dark reactions.

ABBREVIATIONS USED

A, rate of CO_2 assimilation; A_{sat}, CO_2 assimilation at saturation; C_i, intercellular CO_2 concentration; Chl, chloropyll; DCMU (diuron), 3-(3,4-dichlorophenyl)-1,1-dimethylurea; F_v/F_m, maximal apparent efficiency of PSII in dark-adapted leaves; G_s, stomatal conductance; PSI, photosystem I; PSII, photosystem II; $V_{c,max}$, maximum rate of carboxylation estimated in vivo from the response of *A* to C_i.

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