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Effects of 2,4-Di-tert-butylphenol and Selected Herbicides which Induced Lipid Peroxidation on Quantum Yield and Membrane Integrity of Weedy Plants under Dark and Light Conditions

(Kesan 2,4-Di-tert-butilfenol dan Racun Herba Lipid Peroksida Terpilih pada Hasil Kuantum dan Integriti Membran Tumbuhan Rumpai di bawah Keadaan Gelap dan Terang)

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ABSTRACT

2,4-Di-tert-butylphenol (2,4-DTBP) has herbicidal properties that cause lipid peroxidation on plant tissues. The present study aimed at examining the phytotoxic effects of 2,4-DTBP compared to that of selected herbicides which induced lipid peroxidation based on quantum yield (Φ) and membrane integrity of two bioassay weed species namely Oldenlandia verticillata *and* Leptochloa chinensis *under light and dark conditions. Laboratory assays showed reduced Φ of 2,4-DTBP- and dinoterb-treated leaf discs within the first 3 h of the dark incubation period, with further decrease during the subsequent 15 h dark period and 6 h light period. Diuron drastically reduced the Φ of the bioassay species throughout the incubation period. The Φ of glufosinate-treated* O. verticillata *leaf discs was marginally reduced and decreased further upon light exposure; it had no effect on the Φ of* L. chinensis*. Fluridone, isoxaflutole, clomazone and oxyfluorfen also had negligible effect on Φ, whereas paraquat caused a rapid reduction in Φ upon light exposure for both bioassay species. 2,4-DTBP, paraquat and dinoterb induced electrolyte leakage during the dark incubation period; this was further increased in the presence of light for* O. verticillata *and* L. chinensis*. For both bioassay species, glufosinate caused a marked amount of electrolyte leakage, whereas diuron, fluridone, isoxaflutole, clomazone and oxyfluorfen had negligible effect on ion leakage. These results suggested that 2,4-DTBP has herbicidal activity comparable to that of dinoterb without dependence on light.*

Keywords: Electrolyte leakage; Leptochloa chinensis*;* Oldenlandia verticillata*; photosynthesis*

ABSTRAK

2,4-Di-tert-butilfenol (2,4-DTBP) mempunyai sifat racun herba yang menyebabkan peroksidaan lipid pada tisu tumbuhan. Kajian ini bertujuan untuk mengkaji kesan fitotoksik 2,4-DTBP berbanding racun herba lipid peroksida terpilih berdasarkan hasil kuantum (Φ) dan integriti membran melalui bioasai dua spesies rumpai iaitu Oldenlandia verticillata *dan* Leptochloa chinensis *di bawah keadaan terang dan gelap. Asai makmal mendedahkan penurunan Φ 2,4-DTBP- dan cakera daun dinoterb yang dirawat dalam tempoh 3 jam pertama dalam inkubasi yang gelap, dengan penurunan selanjutnya dalam tempoh gelap 15 jam dan tempoh cerah 6 jam yang seterusnya. Spesies bio-asai diuron berkurang secara drastik Φ sepanjang tempoh inkubasi. Φ daripada cakera daun* O. verticillata *yang dirawat glufosinat telah berkurang sedikit dan terus berkurang apabila terdedah pada cahaya; ia tidak mempunyai kesan pada Φ* L. chinensis*. Fluridone, isoxaflutole, clomazon dan oksifluorfen juga mempunyai kesan yang tidak dapat diabaikan pada Φ, sedangkan paraquat menyebabkan pengurangan pesat dalam Φ apabila pendedahan cahaya untuk kedua spesies bioassai. 2,4-DTBP, paraquat dan kebocoran elektrolit oleh dinoterb semasa tempoh inkubasi gelap; ini semakin meningkat di hadapan cahaya untuk* O. verticillata *dan* L. chinensis*. Bagi kedua-dua spesies bioassai, glufosinat menyebabkan kebocoran elektrolit yang ketara, sedangkan diuron, fluridone, isoxaflutole, clomazon dan oksifluorfen mempunyai kesan yang tidak dapat diabaikan pada kebocoran ion. Keputusan ini menunjukkan bahawa 2,4-DTBP mempunyai aktiviti racun herba yang setanding dengan dinoterb tanpa kebergantungan pada cahaya.*

Kata kunci: Fotosintesis; kebocoran elektrolit; Leptochloa chinensis*;* Oldenlandia verticillata

Introduction

Currently, there is increasing evidence that allelochemicals, which are natural plant products derived from higher plants, may be ideal sources of herbicides. For example, 2,4-Di-tert-butylphenol (2,4-DTBP), a phenolic compound isolated from plant tissues, has been shown to have herbicidal potential (Chuah et al. 2015). It is one of the natural compounds present in medicinal plants such as *Gynura cusimbua* (Rana & Blazquez 2007), *Pereskia bleo* (Malek et al. 2009), *Heliotropium indicum* (Oluwatoyin et al. 2011) and *Plumbago zeylanica* (Ajayi et al. 2011). Recently, Chuah et al. (2014) identified 2,4-DTBP in

the culm plus leaf extracts of Napier grass (*Pennisetum purpureum*). They found that 2,4-DTBP completely prevented root growth of *Leptochloa chinensis* in soil at an application rate of as low as 0.60 kg a.i. ha^{-1}.

A subsequent study by Chuah et al. (2015) showed that 2,4-DTBP induced oxidative stress by generating reactive oxygen species (ROS), thus leading to lipid peroxidation and membrane damage in root tissues and in the chloroplasts of leaf tissues in weedy plants. Halim et al. (2017) further demonstrated that 2,4-DTBP led to ultrastructural damage of chloroplasts, with a disorganised thylakoid system and undulating membranes, coupled with the absence of starch grains and an increased number of plastoglobuli. However, its phytotoxic activity was not compared to other herbicides known to cause lipid peroxidation due to the ROS effect. These herbicides include those which inhibit electron flow in photosystem II (PS II, diuron), capture electrons in PS I (paraquat) and inhibit carotenoid biosynthesis (fluridone, isoxaflutole, clomazone), protoporphyrinogen oxidase activity (oxyfluorfen), glutamine synthetase activity (glufosinate) (Monaco et al. 2002) and uncouple the process of oxidative phosphorylation (dinoterb) (Belbachir et al. 1980a, 1980b; Shao et al. 2007).

In general, lipid peroxidation is a process by which the ROS, including the superoxide radical, hydrogen peroxide, and singlet oxygen, attack lipids containing any number of carbon-carbon double bonds, particularly polyunsaturated fatty acids (PUFAs) (Ayala et al. 2014). In plants, lipid peroxidation is used as an indicator for oxidative stress, and as a mechanism of cellular injury (Hossam & Heba 2013). In plant cells, lipid peroxidation can occur both enzymatically and non-enzymatically, with lipid hydroperoxides formed as byproducts (Skorzynska-Polit 2007).

Some of the herbicides which cause lipid peroxidation have been proven to induce chlorophyll fluorescence (Dayan & Zaccaro 2012) and reduce the quantum yield of photosynthesis. In plants, chlorophyll absorbs light energy to undergo photosynthesis, while excess energy is released as heat and chlorophyll fluorescence (Maxwell & Johnson 2000). The ratio of variable chlorophyll fluorescence (Fv) to maximal chlorophyll fluorescence (Fm) provides the quantum yield (Fv/Fm) of photosynthesis and this measurement has become an indicator of photosynthetic efficiency (Emerson 1958). Notable electrolyte leakages have been detected in plants treated with lipid peroxidase herbicides (Dayan & Watson 2011). Electrolyte leakage is one of the stress responses due to loss of membrane integrity, particularly with regards to lipids (Harwood 1997) and may indirectly cause tissue damage (Dayan & Zaccaro 2012). Any process that causes destruction of cell membranes can occur with or without rupturing the membrane. Nevertheless, the effect is more noticeable when the plasma membrane is ruptured and the whole contents of the cell, including cytoplasmic and vacuolar compartments, are discharged (Duke & Kenyon 1993). In addition, it is believed that membrane integrity (Dayan & Watson 2011) and chlorophyll fluorescence (Dayan & Zaccaro 2012) are good indicators of the phytotoxic action of herbicides. To date, the effect of photoperiod on phytotoxic action of 2,4-DTBP remains unclear. Thus, the objective of the present study was to examine the phytotoxic effects of 2,4-DTBP on membrane integrity and the quantum yield of the weeds, *O. verticillata* and *L. chinensis*, compared to that of selected lipid peroxidase herbicides under both light and dark conditions.

Materials and Methods

Seeds of *O. verticillata* and *L. chinensis* were collected from an oil palm plantation in Gemuruh, Terengganu and rice fields of Pasir Puteh, Kelantan, respectively. Seeds were then sown and propagated at the Greenhouse of Universiti Malaysia, Terengganu. 2,4-DTBP (2,4-di-tert-butylphenol), paraquat (1,1'dimethyl-4,4'-bipyridinium), dinoterb (2-(1,1-dimethyl-ethyl)-4,6-dinitrophenol), fluridone (1-methyl-3-phenyl-5-[3-(trifluoromethyl) phenyl]-4(1*H*)-pyridinone), isoxaflutole (5-cyclopropyl-4-isoxazolyl)[2-(methylsulfonyl)-4-(trifluoromethyl) phenyl]-methanone, clomazone (2-[(2-chlorophenyl) methyl]-4,4-dimethyl-3-isoxazolidinone), and oxyfluorfen (2-chloro-1-(3-ethoxy-4-nitrophenoxy)-4-(trifluoromethyl)benzene) were all purchased from Sigma Chemical Co., Kuala Lumpur. For diuron (*N*'-(3,4-dichlorophenyl)-*N*,*N*-dimethyl-urea) and glufosinate (2-amino-4-(hydroxymethylphosphinyl)butanoic acid) chemical compounds of 98% purity were kindly supplied by Ancom Crop Care Sdn. Bhd.

Assessment of the effect of various chemical compounds on the quantum yield and membrane integrity of two bioassay weed species

Experiments were conducted in accordance to the methods described by Dayan and Zaccaro (2012) and Dayan and Watson (2011) with some modifications. Briefly, the lamina of the second fully expanded leaf of *O. verticillata* and *L. chinensis* was punched out with a cork borer in order to obtain discs of 5 mm diameter. Three leaf discs from each of the bioassay species namely *O. verticillata* and *L. chinensis* were placed in 5 mL of the test compound at a concentration of 75 mg/L in petri dishes (50 × 10 mm) (Naimah 2017). Distilled water was used as the control treatment and the control tissues were exposed to the same amount of acetone as the treated tissues, but without the test compounds. The final concentration of acetone in the dishes was 1% (v/v). Plates were incubated in darkness for 18 h, prior to exposure to light @ 100 µmol m^{-2} s^{-1} of photosynthetically active radiation (PAR) in a growth chamber at 25°C. The quantum yield was measured using a CI-340 hand-held photosynthesis system attached to a chlorophyll fluorescence modulator (CID Bio-Science, Inc., Camas, WA, USA); electrolyte leakage was measured using an electrical conductivity meter. A time-course experiment was performed by measuring quantum yield and electrolyte leakage of the leaf discs at predetermined time intervals (up to 24 h). Beginning with the first measurement, at 3 h after the

dark incubation period, a second measurement was made after 18 h (overnight), after which time the samples were placed in the light and a final measurement was made 6 h after light exposure. Each experiment was done twice and the design was a Complete Randomized Design (CRD) with three replicates. To evaluate the effects of the compounds on membrane integrity, the maximum conductivity was measured by boiling three leaf discs from each treatment for 20 min at 95°C. The quantum yield and electrolyte leakage values were expressed as a percentage of the quantum yield and electrolyte leakage observed in the control treatments.

STATISTICAL ANALYSIS

The data were checked for homogeneity of variance before being subjected to the one-way Analysis of Variance (ANOVA) using the software Statistical Package for Social Science (SPSS) version 20.0. The Tukey Test was used to compare all the possible pairs of means and identify pairs of means that were significantly different under the selected probability level ($p<0.05$). In certain cases, data of quantum yield and electrolyte leakage were subjected to log (x), and 1/(x) transformations before being subjected to one-way ANOVA.

RESULTS

Diuron treatment led to a rapid reduction in quantum yield, with *O. verticillata* being more sensitive to diuron than *L. chinensis* (Figure 1), but it did not induce electrolyte leakage within the 24 h incubation period for both weed species (Figure 2). Paraquat caused a slight reduction in quantum yield for *O. verticillata* but had no effect on *L. chinensis* during the dark incubation period. The reduction in quantum yield was most pronounced upon exposure of the leaf discs in paraquat to 6 h of the light period,

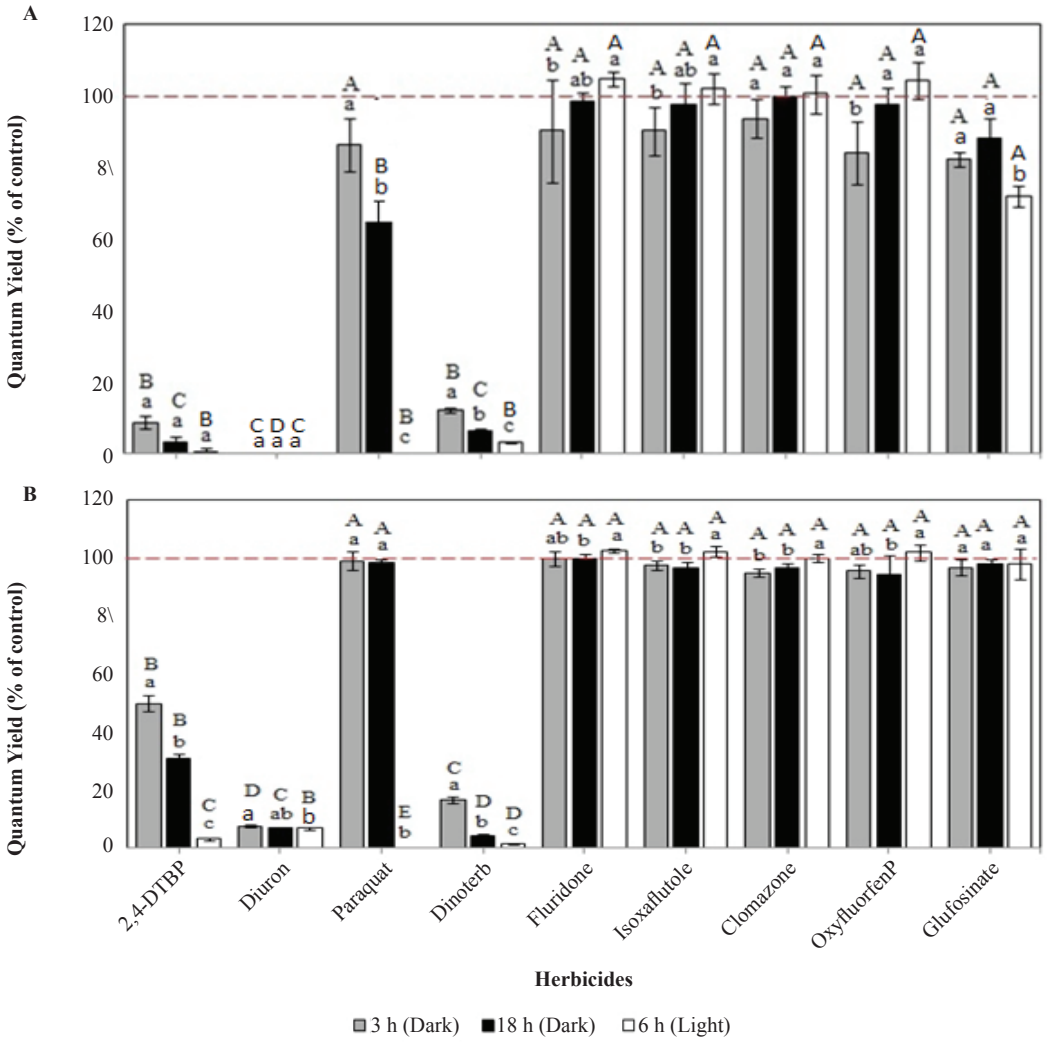

FIGURE 1. Quantum yields of *Oldenlandia verticillata* (A) and *Leptochloa chinensis* (B) leaf discs reduced by herbicides, which caused lipid peroxidation. Data represent the mean ± SD of six replications. The dotted line represents the quantum yield obtained without the addition of herbicide. Means followed by similar small letters have no significant differences among incubation periods within each herbicide while means followed by similar capital letters have no significant differences among herbicides tested within each incubation period after analysed by Tukey test at 5% of significance level

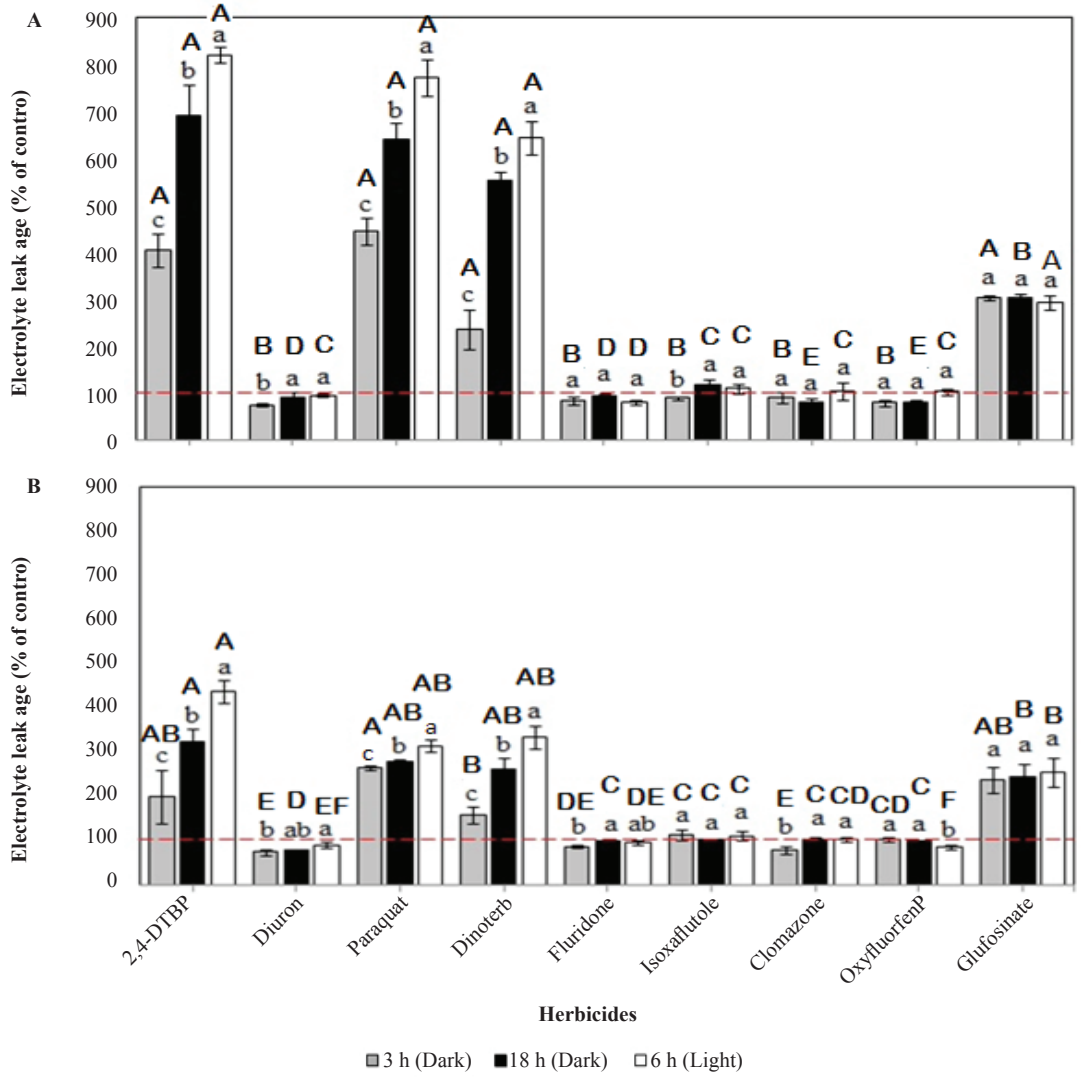

FIGURE 2. Electrolyte leakage of *Oldenlandia verticillata* (A) and *Leptochloa chinensis* (B) leaf discs induced by herbicides, which caused lipid peroxidation. Data represent the mean ± SD of six replications. The dotted line represents maximum leakage obtained by boiling the *O. verticillata* leaf discs without the addition of herbicide. Means followed by similar small letters have no significant differences among incubation periods within each herbicide while means followed by similar capital letters have no significant differences among herbicides tested within each incubation period after analysed by Tukey test at 5% of significance level

regardless of the bioassay species (Figure 1). In contrast, paraquat induced notable ion leakage during the dark period of incubation. This ion leakage was further enhanced when leaf discs were exposed to the 6 h light period, with *O. verticillata* being more sensitive to paraquat than *L. chinensis* (Figure 2).

For both bioassay species, fluridone did not exert any effects on the quantum yield or electrolyte conductivity within the 24 h incubation period. Isoxaflutole and clomazone followed a similar pattern (Figures 1 & 2). Similarly, oxyfluorfen had little to negligible effects on the quantum yield and ion leakage, respectively (Figures 1 & 2). Treatment with glufosinate reduced the quantum yield of photosynthetic electron transport in *O. verticillata* slightly during the dark incubation period; this effect was enhanced during the subsequent light incubation period, whereas the quantum yield of *L. chinensis* was unaffected (Figure 1). Glufosinate elicited dramatic electrolyte leakage within the first 3 h of the dark incubation period. This effect was sustained during the subsequent dark and light periods for both the bioassay species (Figure 2). Dinoterb and 2,4-DTBP caused a marked reduction in the quantum yield of photosynthetic electron transport and induced ion leakage after incubation of the leaf discs for 3 h in the dark. These effects were further enhanced over the following 15 h dark period and 6 h light period for both bioassay species. Statistical analysis revealed that there were no significant differences in quantum yield between 2,4-DTBP and dinoterb during the 24 h incubation period for *O. verticillata*. Similarly, there were no significant differences in electrolyte leakage between 2,4-DTBP and dinoterb during the 24 h incubation period for either *O. verticillata* or *L. chinensis*. In the present study, *O. verticillata* was considerably more sensitive

to dinoterb and 2,4-DTBP treatment than *L. chinensis* (Figures 1 & 2).

Discussion

Treatment with diuron led to a rapid reduction in quantum yield for both the bioassay species during the time span of the experiment (Figure 1). These results are consistent with the mechanism of action of the PS II inhibitors whereby the herbicide directly interferes with the photosynthetic electron flow (Dayan & Zaccaro 2012). These findings are in agreement with results reported by Dayan and Zaccaro (2012) where diuron-treated cucumber cotyledon discs were used. Herbicides that inhibit the PS II mechanism interrupt the electron flow by competing with plastoquinone at its Q_B binding site called D1 protein on PS II. Then, this competition blocks the electron flow through PS II because of the displacement of Q_B (Monaco et al. 2002) and effectively generates ROS (Merlin 1997). The ROS is produced when excess triplet chlorophyll is formed due to the singlet chlorophyll energy which cannot be transferred to the P_{680} reaction center. Consequently, disruption of plant cell membranes occurrs as a result of lipid peroxidation (Monaco et al. 2002).

Interestingly, in the present study diuron did not induce any electrolyte leakage for both the bioassay species during the 24 h of incubation (Figure 2). Similarly, Dayan and Watson (2011) demonstrated that no notable ion leakage on the cotyledon discs of cucumber occurred when they were treated with the PS II inhibitors such as atrazine, diuron and bentazon. In contrast, it has been reported that diuron and atrazine induce small levels of ion leakage in *Lemna* when exposed to continuous light at 275 µmol m^{-2} s^{-1} after incubation of tissues in 100 µM of herbicide solution for 20 h in the dark (Duke & Kenyon 1993). This may be due to the fact that *Lemna* is more sensitive to diuron than cucumber, *O. verticillata* and *L. chinensis*.

The PS I inhibitor, generates highly reactive free radicals after accepting electrons from PS II during the electron flow of photosynthesis. The herbicide acts as an electron acceptor itself by binding near the ferrodoxin binding site and accepts electrons, thereby becoming a free radical. Under normal circumstances, plastocyanin transfers its electrons through a series of steps to ferrodoxin and finally onto the NADP in PS I (Monaco et al. 2002). In the present study, a rapid reduction in the quantum yield of photosynthetic electron flow was evident when the paraquat-treated leaf discs were exposed to light, regardless of the bioassay species (Figure 1). The above results are similar to those reported by Dayan and Zaccaro (2012) for cotyledon discs of cucumber treated with paraquat at 100 µM. Interestingly, the onset of ion leakage occurred during the dark incubation period and the toxicity was further enhanced in the presence of light (Figure 2). Similar findings have been documented by Kim et al. (2001) who found that maize green tissues treated with paraquat at different concentrations (1, 10, and 100 µM) showed relatively low levels of electrolyte leakage within the 12 h dark period incubation, but a dramatic loss of membrane integrity during the following 'light' incubation period.

These results imply that the occurrence of ROS could be triggered by paraquat treatment not only upon exposure to light, but also in the dark. Hess (2000) stated that the toxicity of the PS I herbicides also occurs, albeit to a lesser extent, in the dark because the electron transport chain in mitochondria respiration possibly supplies the electrons for the paraquat-induced free radical formation. According to Asada (1999) and Edreva (2005), chloroplasts are a major source of ROS in green tissues under 'light' conditions. The superoxide radical is primarily produced as a result of oxidative stress in chloroplasts at PS I and singlet oxygen at PS II (Asada 1999). However, mitochondria are the main source of ROS production in non-green tissues or under dark conditions (Lascano et al. 2012; Rhoads et al. 2006), in which the superoxide radicals are generally produced at complexes I and III (Asada 1999).

Carotenoid biosynthesis inhibitors are known as 'bleaching herbicides' or 'bleachers' due to the bleaching of cells and the white foliage formed following treatment (Sandmann et al. 1991). Carotenoids such as phytofluene, tocopherols and isoprenoids play important roles in protection from the destruction of chlorophyll by light (photooxidation). The primary result of photooxidation can be observed by the loss of chlorophyll in plants treated with carotenoid biosynthesis inhibitors (Monaco et al. 2002). In these plants, some of the synthesized chlorophyll is transformed from the short-lived singlet form to the longer-lived triplet form after the chlorophyll has been electronically excited by absorbing light photons. Carotenoids are able to reduce the energy of triplet chlorophyll, including singlet oxygen produced from triplet chlorophyll, when generated under high light intensity. Singlet oxygen, and possibly triplet chlorophyll, initiate detrimental effects leading to membrane damage (Hess 2000).

The target sites for herbicides that inhibit carotenoid biosynthesis pathways are the enzymes phytoene desaturase (PDS), namely *p*-hydroxyphenylpyruvate dioxygenase (HPPD), and 1-Deoxy-$_D$-xylulose phosphate synthase (DXS) (Dayan & Zaccaro 2012). Fluridone is reported to be involved in the inhibition of the PDS enzyme, which leads to zero production of phytofluene (Monaco et al. 2002). Isoxaflutole is one herbicide known to inhibit the HPPD enzyme, which is involved in the biosynthesis of the prenylquinones namely plastoquinone and tocopherol (Grossmann & Ehrhardt 2007). Clomazone inhibits the DXS enzyme, which catalyzes the biosynthesis of the plastidic isoprenoids in the methylerythritol phosphate pathway (Lichtenthaler et al. 2000).

In the present study, fluridone, isoxaflutole, and clomazone had no effect on the quantum yields or electrical conductivity in the bioassay species tested (Figures 1 & 2). These results are in agreement with previous findings where cucumber cotyledon discs were used as the bioassay species (Dayan & Zaccaro 2012; Dayan & Watson 2011). These results were expected considering the relatively short assay duration period because the inhibition of the enzymes

PDS, HPPD, and DXS did not affect the levels of preexisting carotenoids. As a result, reduction in quantum yield and ion leakage were not detected. Nevertheless, it is suggested that prolonged exposure to carotenoid biosynthesis inhibitors may affect the quantum yield and lead to ion leakage due to tissue bleaching (Dayan & Zaccaro 2012).

Enzyme protoporphyrinogen oxidase (PROTOX) is the primary herbicide target in chlorophyll biosynthesis and is responsible for converting protoporphyrinogen IX to protoporphyrin IX. This enzyme is involved in chlorophyll and heme synthesis, whereas in mitochondria it is involved in non-plastidic heme synthesis. Accumulation of protoporphyrinogen IX occurs when protoporphyrin IX cannot be formed because PROTOX is inhibited by oxyfluorfen. Once protoporphyrinogen IX leaks out of the chloroplast into the cytoplasm, it is oxidized to protoporphyrin IX and it then reacts with oxygen and light to form singlet oxygen in the presence of light. Lipid peroxidation and membrane disruption occur when singlet oxygen rapidly reacts with the lipids in cell membranes, which then leads to plant death (Monaco et al. 2002). Surprisingly, oxyfluorfen had no effect on ion leakage, irrespective of the bioassay species tested in the present study (Figure 2). On the contrary, PROTOX inhibitors such as acifluorfen and sulfentrazone caused rapid electrolyte leakage in cucumber in the presence of light at 1000 µmol m^{-2} s^{1} (Dayan & Watson 2011).

Most likely the light intensity of 100 µmol m^{-2} s^{-1} used in the present study was too low to induce detectable electrolyte leakage in the leaf discs of the bioassay species treated with oxyfluorfen (Figure 2). A study by Renner and Fausey (2001) demonstrated that *Chenopodium album* and *Amaranthus retroflexus* could be controlled effectively at a higher light intensity of 1000 µmol m^{-2} s^{-1}, rather than the low light intensity of 4 µmol m^{-2} s^{-1} when treated with the PROTOX inhibitors, flumiclorac and fluthiacet. The high light intensity showed better efficacy of PROTOX inhibitors because of the high amount of free radicals produced (singlet oxygen) that promoted lipid peroxidation and destruction of cell membranes. When plants are exposed to low light intensity, the harmful effects of PROTOX inhibitors decrease as the amount of free radicals produced is reduced (Krämer & Schirmer 2007). However, oxyfluorfen had negligible effects on the quantum yields of *O. verticillla* and *L. chinensis* (Figure 1), although the PROTOX inhibitors, acifluorfen and sulfentrazone have been reported to reduce the photosynthetic electron flow in cucumber upon exposure to a light intensity of 100 µmol m^{-2} s^{-1} (Dayan & Zaccaro 2012), which is similar to the light intensity used in the present study. This may be due to the fact that both bioassay species tested are less sensitive to oxyfluorfen compared to cucumber.

Glufosinate is the only commercial inhibitor of glutamine synthetase in the nitrogen assimilation pathway of plants, a key enzyme in glutamine biosynthesis. Formation of glutamine and glutamate is inhibited by glufosinate, which disrupts many important nitrogen metaboliic and synthetic reactions in plants. Electron flow in photosynthesis is indirectly inhibited through the decrease in amino donors (from glutamate) for glyoxylate. In the light, inhibition of electron flow in photosynthesis causes induction of lipid peroxidation from a build-up of triplet chlorophyll (Monaco et al. 2002). In the present study, glufosinate caused a greater reduction in the quantum yield of *O. verticillata* during the light than during the dark period compared to that of *L. chinensis* (Figure 1). A similar trend was observed in glufosinate-treated cucumber leaf discs (Dayan & Zaccaro 2012). Since light is necessary for the two main ammonia-producing reactions in plants (nitrite reduction and the photorespiratory conversion of glycine to serine), greater reduction in quantum yield during the dark period may be due to less ammonia accumulation during the dark after glufosinate treatment. Additionally, photosynthesis is indirectly inhibited by an accumulation of glyoxylate in photorespiration, even though the inhibition of the light reaction in photosynthesis may already be directly caused by the accumulation of ammonia. Thus, lipid peroxidation in membranes occurs due to the inhibition of the photosynthetic light reaction (Hess 2000).

In contrast, there was a marked amount of ion leakage following treatment with glufosinate for both the bioassay species (Figure 2). However, Dayan and Watson (2011) reported that glufosinate caused only a small amount of ion leakage on cucumber leaf discs during the 24 h incubation period. Dayan and Watson (2011) stated that prolonged exposure of leaf discs to glufosinate is likely to have greater effects due to the toxicity of ammonia. The results from the present study differ from those of Dayan and Watson (2011) perhaps due to the different levels of sensitivity of the bioassay species to glufosinate.

Dinoterb, a phenol herbicide, is a weak acid known to induce the uncoupling of photooxidative phosphorylation and oxidative phosphorylation (Terada 1990). These uncoupling reactions can occur in both mitochondria and chloroplasts (Belbachir et al. 1980a, 1980b). At higher concentrations, dinoterb may induce a similar response in mitochondria because dinoterb inhibits the electron transport chain, most likely before cytochrome c. However, at low concentrations, dinoterb is reported to uncouple oxidative phosphorylation in mitochondria and interfere with respiratory oxygen consumption (Belbachir et al. 1980a, 1980b), thus leading to the failure of ATP synthesis (Merlin 1997). Dinoterb acts as an inhibitor of light-dependent reactions in chloroplasts, with the site of inhibition located before plastoquinone but near PS II (Belbachir et al. 1980a, 1980b). In short, dinoterb has a multifaceted mechanism of action involving the inhibition of photosynthesis and ATP synthesis. In this study, dinoterb led to a marked reduction in the photosynthetic electron flow rate and induced notable ion leakage during the dark incubation period. The effects were further enhanced during the subsequent light period (Figures 1 & 2). In another study, dinoterb caused a reduction in electron flow in cotyledon discs of cucumber during the dark incubation period and this effect was sustained upon exposure to light (Dayan & Zaccaro 2012). Meanwhile, Dayan and Watson (2011) reported that dinoterb caused electrolyte leakage on

the cotyledon discs of cucumber during the dark incubation period and this effect was most pronounced after light exposure at 1000 µmol m^{-2} s^{1}.

It has been proven that 2,4-DTBP induces oxidative stress through the generation of ROS in weedy plants (Chuah et al. 2015). A previous study showed that the quantum yields of *L. chinensis* and *O. verticillata* were reduced by 82% and 83%, after treatment with 2,4-DTBP at the concentration of 50 and 200 mg/L, respectively, within 24 h dark incubation period (Chuah et al. 2015). These observations are consistent with those of Yu et al. (2006), where it was reported that treatment of 2,4-DTBP on eggplant also caused a reduction in quantum yield. In the present study, one would expect that 2,4-DTBP could cause ion leakage during a 24 h incubation period (Figure 2). Even though 2,4-DTBP and paraquat showed similar trend in eliciting electrolyte leakage during the 24 h incubation period (Figure 2), paraquat had negligible effects on quantum yield during the dark incubation period (Figure 1). 2,4-DTBP followed a similar pattern to that of dinoterb, causing a reduction in quantum yield and inducing electrolyte leakage during the assay (Figures 1 & 2). These results suggested that both 2,4-DTBP and dinoterb are not dependent on light to reduce the efficiency of photosynthesis and destroy membrane integrity, as the effects can be observed irrespective of dark or light incubation periods.

Conclusion

It can be concluded that the quantum yields of 2,4-DTBP, diuron, and dinoterb-treated leaf discs were rapidly reduced during the dark incubation period and the subsequent light period, whereas 2,4-DTBP, paraquat, and dinoterb caused dramatic ion leakage during the 24 h incubation period. The results of the study suggested that the reduction in quantum yield precedes the electrolyte leakage for both 2,4-DTBP- and dinoterb-treated leaf discs. 2,4-DTBP has potent light-independent herbicidal activity comparable to that of dinoterb, affecting photosynthetic efficiency and causing the loss of membrane integrity. Further studies are necessary to confirm whether 2,4-DTBP inhibits photosynthetic electron transport by uncoupling the process of oxidative phosphorylation.

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