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# Photosynthetic Inhibitors

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## 1. Introduction

Life on Earth is dependent on sunlight. In the process known as photosynthesis, plants, algae and certain bacterias are capable of using this source of energy to drive the synthesis of organic compounds. The oxygenic photosynthesis results in the release of molecular oxygen and the removal of carbon dioxide from the atmosphere that is used to synthesize carbohydrates. This process, which involves a complex series of electron transfer reactions, provides the energy and reduced carbon required for the survival of virtually all life on our planet, as well as the molecular oxygen necessary for the survival of oxygen consuming organisms (Nelson, 2011; Nelson & Yocum, 2006; Rutherford & Faller, 2001).

In modern agriculture, farmers continuously face a battle to achieve products in high yields and better quality to feed an ever increasing world population (Stetter & Lieb, 2000). The optimization of agriculture techniques demands, along with other requirements, the application of crop protection agents to control a variety of diseases and pests, among which are weeds. Weeds compete with crops for nutrients, water, and physical space, may harbor insect and disease pests, and are thus capable of greatly undermining both crop quality and yield. In view of the problems caused by weed species, their control is highly desirable.

Among the methods to control weeds, the use of herbicides or weed killers has become the most reliable and least expensive tool for weed control in places where highly mechanized agriculture is practiced. Since the introduction of 2,4-dichlorophenoxyacetic acid (2,4-D) in 1946, several classes of herbicides were developed that are effective for broad-spectrum of weed control (Böger et al, 2002; Cobb, 1992; Ware, 2000).

It is well know that various compounds can interfere with photosynthetic electron transport. This fact has been explored by agrochemical companies to develop an assortment of herbicides to control weeds. Some representative members of commercial photosynthetic inhibitors are diuron (1), atrazine (2), paraquat (3) e diquat (4) (Figure 1).

The photosynthetic inhibitors can be divided into two distinct groups, the inhibitors of photosystem II exemplified by diuron (1) and atrazine (2) and the inhibitors of photosystem I such as paraquat (3) and diquat (4). It is worth to mention that compounds that inhibit photosystem II account for 30% of the sales in the herbicide market (Draber, 1992).

Although there are a variety of herbicides that can control a broad spectrum of weeds, there is still a necessity for the development of new active ingredients. Several reasons can be mentioned to support this statement. Herbicides should have a favorable combination of properties, such as high specific activity, low application rates, crop tolerance, and low mammalian toxicity. Increasing public concern for environmental pollution derived from

agricultural practices also requires that herbicides be rapidly degraded by soilborne microorganisms. Moreover, during the past years, intensive and repeated applications of the same active ingredients cause the selection for and development of herbicide resistance (Devine & Shukla, 2000; Beckie, 2006; Gressel, 2009; Preston, 2004). Starting from 1960s, hundreds of weed biotypes have been reported as surviving herbicide application (Heap, 2011).

Intensive efforts have thus been undertaken to discover new compounds with favorable environmental and safety features to selectively control weeds. In this regard, the photosynthetic system has been target aiming to find new weed killers. In this book chapter, it will be covered the developments concerning the search for new photosynthetic inhibitors during the last ten years.

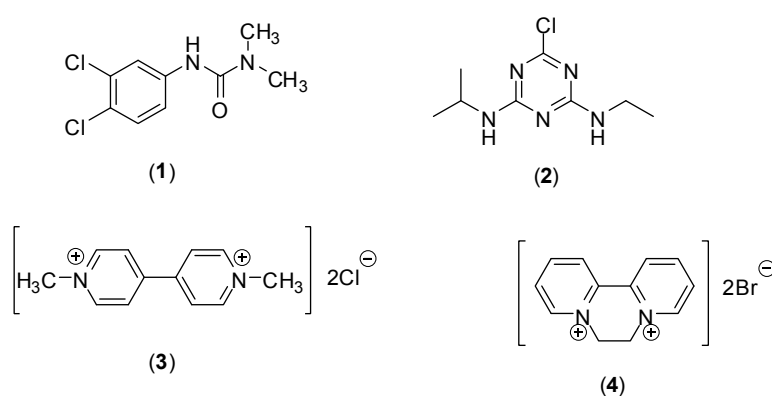


Fig. 1. Examples of commercial photosynthetic inhibitors.

## 2. Natural products as source of photosynthetic inhibitors

The strategies used to identify new chemical agents to control weeds can no longer be distinguished from pharmaceutical research and development (Short, 2005). Three major different approaches have been employed. The first one refers to the systematic screening of large numbers of synthetic compounds. Subsequently, lead compounds are optimized. This has been the most widely used strategy by agrochemical companies (Böger et al, 2002; Cobb, 1992; Rüegg et al, 2006; Ware, 2000). The second one is the rational design of specific inhibitors of key metabolic processes (Lein et al, 2004). However, to date such an approach has not been fully successful to produce commercial herbicides.

A third strategy is related to the exploitation of natural products either directly as herbicides (Copping & Duke, 2007) or as leads for the development of new herbicides (Barbosa et al, 2008; Dayan et al, 1999; Dayan et al, 2009; Duke et al, 2000a, 2002; Hütter, 2011; Macías et al, 2007; Pillmoor et al, 1993; Scharader et al, 2010; Strange, 2007; Vyvyan, 2002). This strategy can be considered attractive for several reasons. A vast array of compounds has been isolated from nature and most of them have not been evaluated for agrochemical purposes. Different from what happens to the majority of synthetic agrochemicals, most natural products are water soluble. Moreover, due to natural selection these compounds can present bioactivity in very low concentration. The great variety of chemical structures found in nature can afford chemical agents for weed control that are toxicologically and environmentally benign. Furthermore, the molecular sites where natural products exert their action can be quite different from known molecular targets (Duke et al, 2005; Duke,

Duke et al, 2000b). This is particularly important to overcome the resistance problem. It is worth to mention that the few natural products based herbicides mesotrione, sulcotrione, cinmethylin, bialaphos and glyphosate (vide infra) act on molecular sites that were not known before they were introduced.

Even though the great variety of natural products has been relatively little explored, several active principles were discovered in this way (Figure 2).

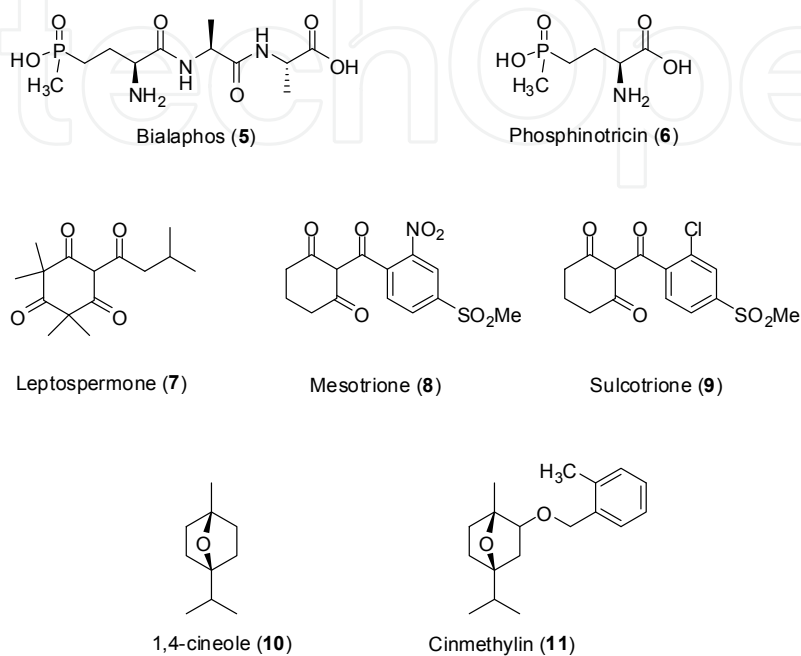


Fig. 2. Structures of compounds 5-11 mentioned in the text.

Bialaphos (5) and phosphinotricin (6) were originally isolated from various strains of the bacteria *Streptomyces* spp. (Saxena & Pandey, 2001). Bialaphos is a proherbicide that is metabolized into the active ingredient phosphinotricin in the treated plant. Currently, bialaphos is commercialized in Japan with the name of Herbiace®. It should be mentioned that phosphinotricin (6) is also produced synthetically as a racemic mixture and commercialized as glyphosate. Leptospermone (7), a major component of the essential oil of *Leptospermum scoparium* (Myrtaceae) (van Klink et al, 1999), was chemically modified to make mesotrione (Mitchell et al, 2001). Mesotrione (8) is the active principle of the commercial herbicide Callisto®, which is commercialized by Syngenta and suitable for use in corn fields. Another example is sulcotrione (Chaabane et al, 2005), an herbicide marketed in Europe by Bayer Crop Science under the trade name Mikado®. Sulcotrione (9) is used to control a broad range of annual and perennial broadleaf weeds in maize and sugar cane crops. The commercial herbicide cinmethylin (11) (Figure 2) is a 2-benzyl ether derivative of the natural product 1,4-cineole (10) that was developed to control annual grasses (Romagni et al, 2000a,b; Duke & Oliva, 2004). Moreover, an increasing number of other natural products have been described in the literature as potential leads for the development of chemical agents for weed control among which are coumarins, benzoquinones, flavonoids, terpenoids and lactones (Barbosa et al, 2008). The natural product pool has been explored in the search of new photosynthetic inhibitors and the advances in this regard will be described below.

The diterpene labdane-8 $\alpha$ ,15-diol (**12**) and its acetyl derivative (**13**) (Figure 3) were isolated from the hexan extract of the stems of *Croton clatoglanduliferus*. Biological assays carried out with intact spinach chloroplasts showed that these compounds are capable of interfere with ATP synthesis (Morales-Flores et al., 2007).

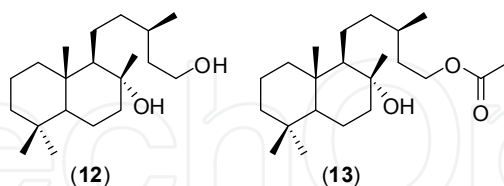
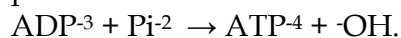


Fig. 3. Structures of labdane-8 $\alpha$ ,15-diol and its acetyl derivative.

It is well established that in the oxygenic photosynthesis there is a transfer of electron from water to NADP<sup>+</sup> affording the reduced form NADPH. In this biochemical process, two different reaction centers, known as photosystem II and photosystem I, work concurrently but in series. In the presence of light photosystem II feeds electrons to photosystem I. The electrons are transferred from photosystem II to the photosystem I by intermediate carriers as described by the well known Z scheme of photosynthesis (Figure 4). The net reaction is the transfer of electrons from water to NADP<sup>+</sup>.

It has been proposed that electron transport is indirectly coupled to phosphorylation (ATP synthesis) through an electrochemical potential of hydrogen ions (protons) build up across the biological membranes involved in the electron transport (Mitchel, 1961). The electrochemical gradient, in turn, is consumed in the formation of ATP from ADP and inorganic phosphate. There is proportionality between changes in pH and changes in hydrogen ion concentrations. The electron transport gives two protons translocated for each electron transferred from photosystem II to photosystem I (Allen, 2003). At pH 8.0 one proton ion is consumed irreversibly in the synthesis of ATP:



As can be noticed in the previous equation, ATP formation can be measured in vitro, using intact spinach chloroplast, by determining the basicity of the medium. In the presence of an artificial electron receptor such as methylviologen and under continuous actinic illumination, the pH rise continues to increase linearly with time corresponding to the steady state rate of ATP formation. Under appropriate conditions (Morales-Flores et al., 2007), the rate of ATP formation can be determined by back titration with hydrochloric acid using a microelectrode attached to a potentiometer.

Diterpenes (**12**) and (**13**) (Figure 3) inhibited ATP synthesis coupled to electron transport from water to methylviologen in freshly lysed intact spinach chloroplasts in a concentration dependent manner. The IC<sub>50</sub> (the concentrations causing 50% inhibition in vitro) were determined being equal to 72  $\mu\text{mol L}^{-1}$  for compound (**12**) and 10  $\mu\text{mol L}^{-1}$  for compound (**13**) (Morales-Flores et al., 2007). As mentioned above, the light-dependent phosphorylation is coupled to electron transport flow. Thus, ATP formation can be inhibited by (a) blockage of the electron transport within thylakoid chain, (b) by dissipation of the H<sup>+</sup> gradient, that is, uncoupling of the ATP synthesis process from the electron transport, and (c) by direct inhibition of the H<sup>+</sup>-ATPase complex. Reagents that block electron transport avoid ATP synthesis because the generation of the transmembrane electrochemical gradient is not formed; as previously stated, the driving force for ATP synthesis is dependent upon electron flow. Chemicals that increase proton permeability of thylakoid membranes uncouple

phosphorylation from electron flow. Uncoupling agents inhibit ATP synthesis by decreasing the proton gradient but allow electron transport to occur at high rates. In contrast, direct inhibitors of photophosphorylation block both phosphorylation and that portion of electron transport that is a consequence of proton efflux linked to phosphorylation (Veiga et al, 2007a).

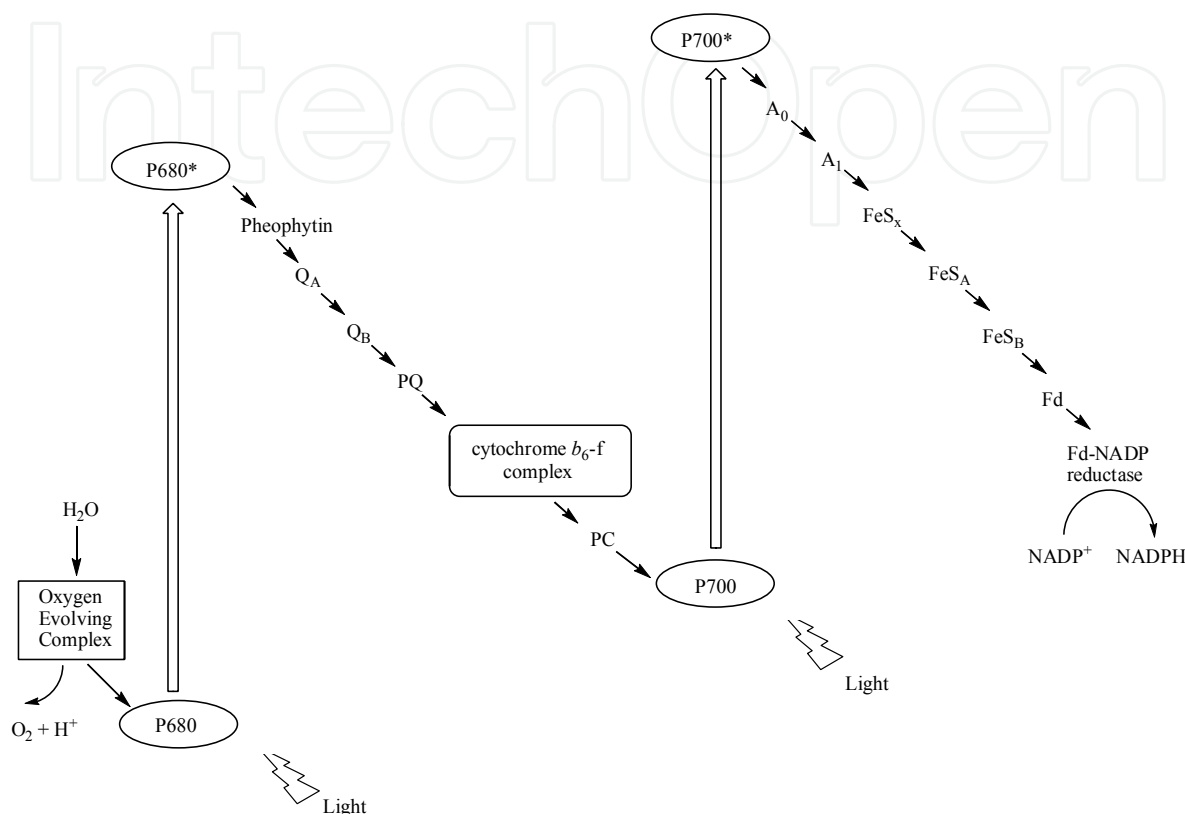


Fig. 4. The Z scheme and the electron transfer processes involved in oxygenic photosynthesis.

In order to elucidate the mechanism of action of **(12)** and **(13)** on photosynthesis, their effect on non-cyclic electron transport from water to methylviologen (basal, phosphorylating, and uncoupled) was investigated. It was found that as concentration of these compounds increased up to 300  $\mu\text{mol L}^{-1}$ , the rates of basal, phosphorylation, and uncoupled electron transport were inhibited being basal and uncoupled electron rates the more affected. The  $\text{IC}_{50}$  found for compound **(12)** were 200  $\mu\text{mol L}^{-1}$  (basal) and 76  $\mu\text{mol L}^{-1}$  (uncoupled) while for **(13)**  $\text{IC}_{50}$  for these rates were 31 and 71.5  $\mu\text{mol L}^{-1}$  for basal and uncoupled, respectively. The partial reactions involved in the electron transport flow can be characterized in great detail by using highly specific artificial donors and acceptors (Trebst, 2007; Morales-Florest et al, 2007; as cited in Allen & Holmes, 1986). The effect of the natural products **(12)** and **(13)** (Figure 3) on photosystem I and II and their partial reactions were evaluated. It was determined that terpene **(12)** inhibited the partial reaction from water to DCPIP (2,6-dichlorophenol indophenol). Inhibition of this partial reaction indicates that the compounds inhibits the range of reactions of photosystem II electron transport chain from water to Q<sub>B</sub> (Figure 4), because oxidized DCPIP accepts electrons at this level. It was also found that **(12)** inhibits the photosystem II partial reaction from water to sodium silicomolybdate (SiMo).



Inhibition of this partial reaction means that the (12) affects the partial reaction from water to quinone  $Q_A$  since SiMo is used as electron acceptor at or before  $Q_A$  site (Morales-Flores et al, 2007, as cited in Giaquinta & Dilley, 1975). The effect of compound (12) on the electron flow from diphenylcarbazine (DPC) to DCPIP was also evaluated. In this case, DCPD donates electrons at  $P_{680}$  level and the evaluation is carried out with Tris treated chloroplast. Such a treatment inhibits the oxygen evolving complex activity. Compound (12) did not inhibit this last partial reaction (electron flow from DPC to DCPIP). The results found for the activities of compound (12) on the partial reactions of photosystem II led to the indication that for this natural product the first site of interaction and inhibition is at the oxygen evolving complex. Further investigations carried out with terpene (12) showed that it is able to inhibit the partial reaction from reduced phenylmetasulfate (PMS) and methylviologen. While PMS donates electrons at the  $P_{680}$  level, methylviologen accepts electrons at  $F_x$  level in photosystem I. Therefore, it was established that the second site of interaction of compound (12) is located between  $P_{700}$  and  $F_x$  of photosystem I electron transport chain (Morales-Flores et al, 2007).

The effect of (13) on the partial reactions mentioned above was also evaluated being found the following results. It inhibited the partial reactions from water to DCPIP, from water to SiMo, and from DPC to DCPIP. These results indicate that the compound inhibits the electron flow in photosystem II in the evolving complex system as well as in the path between  $P_{680}$  and  $Q_A$  or at least at  $P_{680}$  site. The labdane 13 also inhibited the partial reaction from reduced PMS to oxidized methylviologen (Morales-Flores et al, 2007).

The survey of literature revealed that various other terpenes (Figure 5) have been evaluated as potential inhibitors of photosynthesis in a similar manner described for compounds (12) and (13).

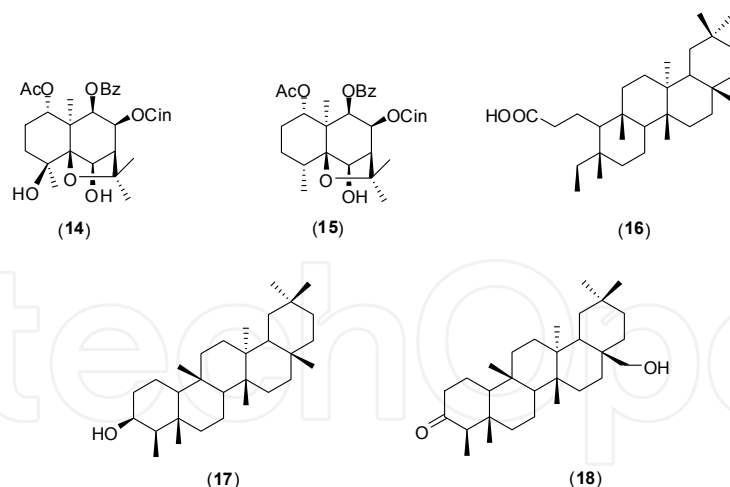


Fig. 5. Structures of photosynthetic inhibitor terpenes 14-18.

The sesquiterpenoids (14) and (15) were isolated from the dichloromethane extract of the leaves of *Celastrus vulcanicola* (Torres-Romero et al, 2008). Similar to what happens to other terpenes, both compounds are capable of inhibiting the photosynthetic photophosphorylation in isolated spinach chloroplasts from water to methylviologen in a concentration-dependent manner ( $IC_{50} = 25 \mu\text{mol L}^{-1}$  for 14 and  $IC_{50} = 44 \mu\text{mol L}^{-1}$  for 15). At lower concentrations (up to  $50 \mu\text{mol L}^{-1}$ ) compound (14) inhibited basal and phosphorylating electrons rates (approximately 83% and 79%, respectively); however, at

higher concentrations this terpenes reversed both electron transport rates (at 300  $\mu\text{mol L}^{-1}$ , the electron flow rates were 32% and 68% for basal and phosphorylating, respectively). In addition, this compound also inhibited the uncoupled electron transport rate. Further investigations showed that (14) has two targets of interaction: one is located at the oxygen-evolving complex, and the other located at the  $\text{H}^+$ -ATPase complex. Compound (15) behaves likewise to (14) albeit being less active (Torres-Romero et al, 2008).

The fractioning of the hexane extract from air-dried leaves of *Maytenus imbricata* led to the isolation of seco-3,4-triterpenoid (16). It inhibits the formation of ATP coupled to electron transport from water to methylviologen and its  $\text{IC}_{50}$  was 148  $\mu\text{mol L}^{-1}$ . Evaluation of the effect of increasing concentration of (16) on basal, phosphorylating, and uncoupled electron flow rates showed that the phosphorylating and uncoupled electron flow are inhibited up to 300  $\mu\text{mol L}^{-1}$ ; after this concentration both activities are enhanced. Experimental results support the fact that this triterpenoid acts on the oxygen evolving complex since it inhibits the electro flow from water to sodium silicomolybdate (SiMo). Moreover, it behaves as uncoupler activating the  $\text{Mg}^{+2}$ -ATPase complex (Souza & Silva et al, 2007).

Epifriedelinol (17) and canophyllol (18) (Figure 5) are friedelane triterpenoids isolated from the stems of *Celastrus vulcanicola*. Their activities on photosynthesis as determined in vitro can be summarized as follows. Epifriedelinol (17) and canophyllol (18) inhibits the ATP synthesis coupled to electron transport from water to methylviologen as concentration increases presenting  $\text{IC}_{50} = 82 \mu\text{mol L}^{-1}$  for compound (17) and 124  $\mu\text{mol L}^{-1}$  for compound (18). The evaluation of their effects on noncyclic electron transport from water to methyl viologen under basal, phosphorylating, and uncoupled conditions showed that epifriedelinol (17) did not affect these processes. On the other hand, compound 18 partially inhibited the electron transport rates at different degrees. Both compounds moderately enhanced the light-activated  $\text{Mg}^{+2}$ -ATPase activity indicating that they affect the variation of pH and thus avoiding the ATP formation. Thus, the friedelane triterpenoids (17) and (18) has two targest of interaction: one is a decrease of pH variation blocking the ATP formation and the second by interacting and inhibiting the  $\text{Mg}^{+2}$ -ATPase activity in thylakoids. It was also found that compound (18) is capable of inhibiting the electron flow from water to the electron acceptor 2,5-dichloro-1,4-benzoquinone (DCBQ) acting, therefore, as Hill reaction inhibitor (Torres-Romero et al, 2010).

Besides terpenes, the effects of several other natural products (Figure 6) as photosynthetic inhibitors have been investigated.

Demuner and co-workers reported the isolation of 4-methoxy-5-methyl-6-(3-methylbut-2-enyloxy)isobenzofuran-1(3H)-one (19) from the phytopathogenic fungus *Nybmia alternantherae* (Demuner et al, 2006). This isobenzofuranone acts as Hill reaction inhibitor and uncoupler of photosynthesis, displaying inhibitory activity on ATP synthesis ( $\text{IC}_{50} = 66 \mu\text{mol L}^{-1}$ ).

Siderin (20) is a secondary metabolite produce by *Toona ciliate* (Meliaceae). Veiga and co-workers showed that this coumarin inhibited ATP synthesis presenting  $\text{IC}_{50} = 27.0 \mu\text{mol L}^{-1}$  and did not inhibit photosystem I electron transport. Siderin does inhibit partial reactions of photosystem electron flow frow water to DCPIP, from water do sodium silicomolybdate (SiMo), and partially inhibits electron flow from DPC to DCPIP. All these results support the fact that the site of inhibition of (20) is the donor and acceptor sites of photosystem II, between  $\text{P}_{680}$  and  $\text{Q}_A$  (Veiga et al, 2007b).

The lactone lasiodiplodin (21) was isolated from the ethanolic extract from the fungus *Botryosphaeria rhodina*. As the concentration of (21) is raised, ATP synthesis is inhibited



( $IC_{50} = 35.6 \mu\text{mol L}^{-1}$ ). The investigation of the phytotoxic effect of this compound on photosynthesis resulted in the identification of three new different sites of interaction and inhibition: one at  $CF_1$  ATPase complex, the second in the oxygen evolving complex, and the third at the electron transfer path between  $P_{680}$  and  $Q_A$ . These targets are different from that of displayed by synthetic herbicides. This finding corroborates the fact that the exploitation of the natural product pool can afford compounds presenting different molecular targets compared to well known herbicides (Veiga et al, 2007a).

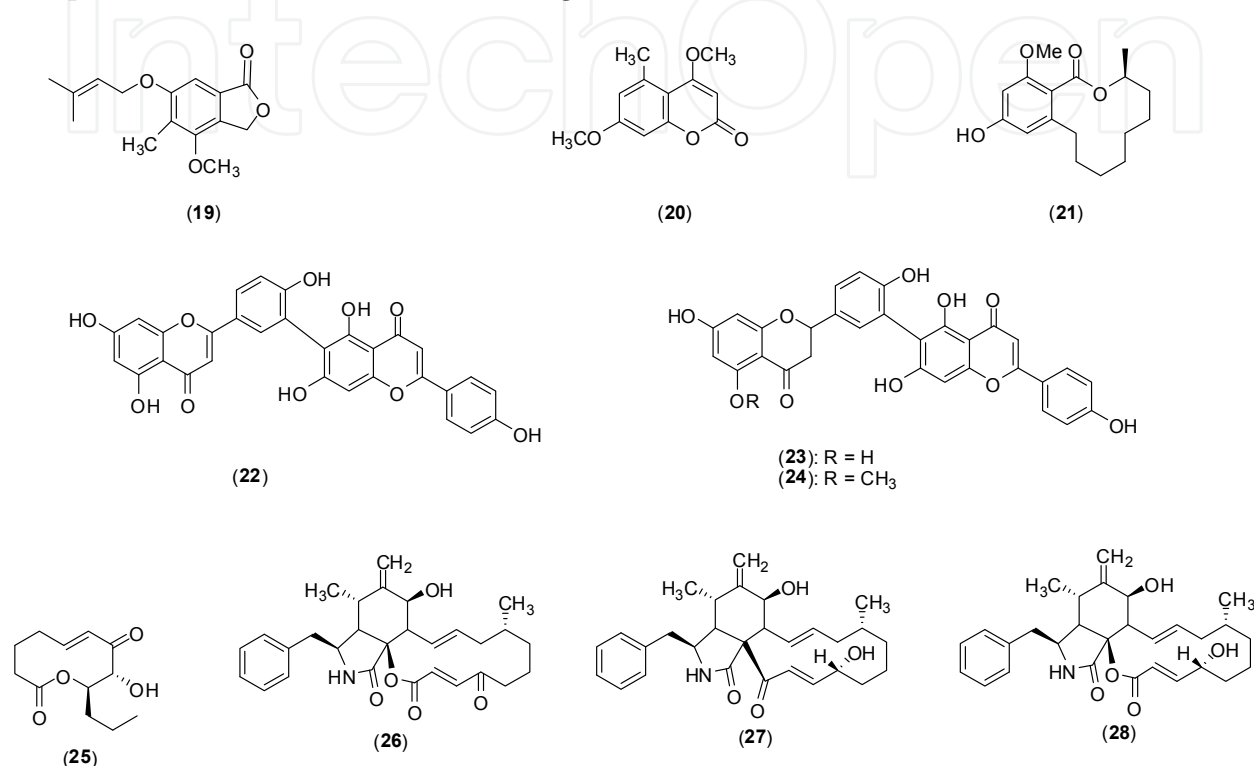


Fig. 6. Structures of compounds **19-28** mentioned in the text.

The bioactivity-guided chemical analysis of *Selaginella lepidophylla* resulted in the isolation of the biflavonoids robustaflavone (**22**), 2,3-dihydrorobustaflavone (**23**), and 2,3-dihydrorobustaflavone-5-methyl ether (**24**) (Figure 6). The *in vitro* assays revealed that all the isolated flavonoids inhibited the ATP synthesis coupled to electron flow from water to methylviologen in isolated freshly lysed intact spinach chloroplasts. The  $IC_{50}$  values for this activity were 44, 39, and 79  $\mu\text{mol L}^{-1}$  for compounds (**22**), (**23**), and (**24**), respectively. Considering the three modes of electron transport (basal, uncoupling and phosphorylating) from water to methylviologen, all of them are partially inhibited by the three flavonoids being compound (**22**) the most active. The mode of action of these compounds were further investigated. Compound (**22**) did not affect the photosystem I. However, it interacts with photosystem II in the span of oxygen evolving complex to  $P_{680}$ . The interaction and inhibition target of (**23**) was located at  $Cytb_6f$  complex to plastocyanin (PC) (Figure 4). Flavonoid (**24**) had no effect on photosystem I or II. However, it acts as energy transfer inhibitors since increasing concentrations of (**22**), (**23**), and (**24**) inhibited the  $Mg^{+2}$ -ATPase activity (Aguilar et al, 2008).

A set of fifteen substances belonging to two groups of organic compounds, nonenolides and cytochalasins, had their phytotoxic effects evaluated against the perennial weed species

*Cirsium arvense* and *Sonchus arvensis* (Berestetskiy et al, 2008). Among the nonelides evaluate by leaf disc-puncture bioassay, stagonolide A (**25**) displayed the highest phytotoxic effect (necrosis diameter > 6 mm) on leaves of *C. arvense*. Considering the cytochalasins, only cytochalasyn A (**26**) showed high phytotoxic effect on this weed species (necrosis diameter ~3 mm) (Berestetskiy et al, 2008).

The most phytotoxic compound against *S. arvensis* was the cytochalasyn deoxaphomin (**27**) (necrosis diameter ~7 mm). High phytotoxic effects were also observed for stagonolide A (**25**), cytochalasin A (**26**) and cytochalasin B (**28**) (necrosis diameter ~ 4.5, 5.5 and 4 mm, respectively) (Berestetskiy et al, 2008).

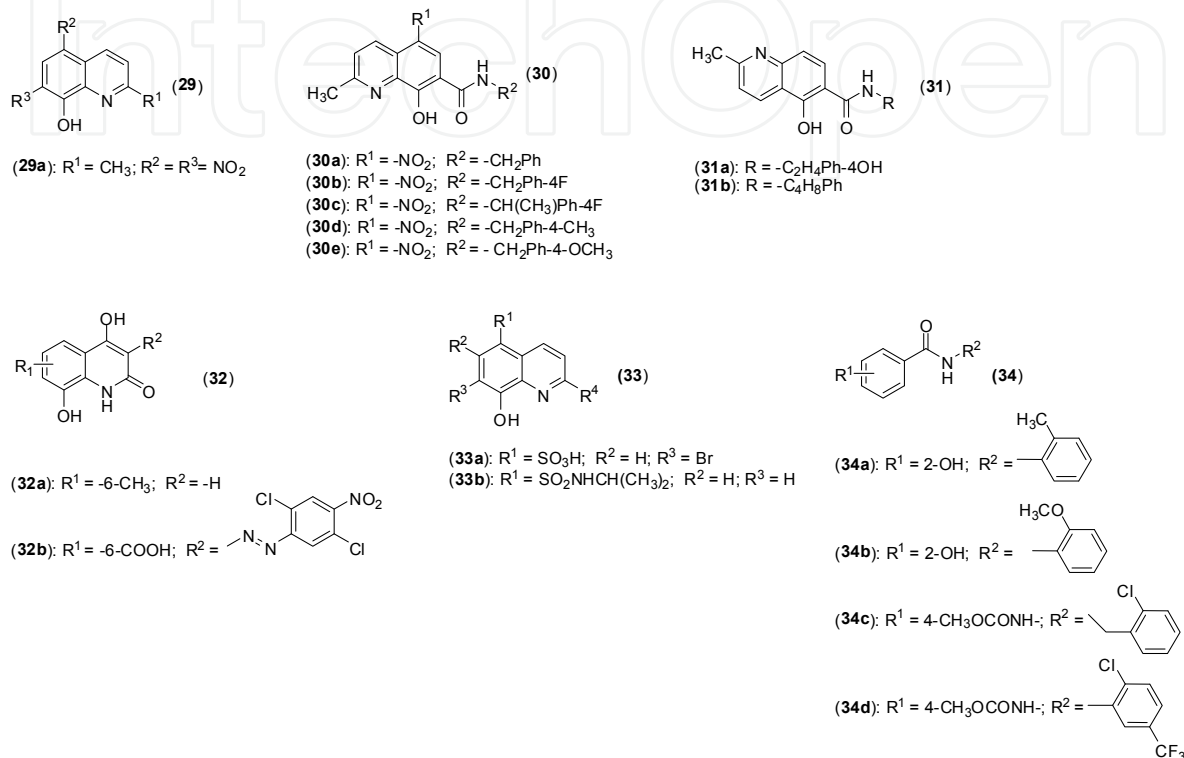
Photometric assays in the range of 450-950 nm showed that stagonolide A (**25**) and cytochalasin B (**28**) presented effects on photosynthesis. Twenty four hours after the observation of the first necrosis on leaf discs, both compounds caused significant decrease of the light absorption at the wave length 450 nm by *C. arvense*. This experimental observation was associated with the reduction of  $\beta$ -carotene or/and chlorophyll *b* content in leaf tissue of *C. arvense* since these pigments have a peak of resonant absorption near the 450 nm. At the wavelength of 530 nm and 550 nm, it was noticed increase in the light absorption for compounds (**25**) and (**28**). However, stagonolide A (**25**) had significantly stronger effect at 550 nm compared to cytochalasin B (**28**). Since cytochromes have a peak absorption in the range of 530-550 nm the substances (**25**) and (**28**) probably increased the concentration of these proteins, and did not affect electron transport. Treatment of leaf discs by stagonolide (**25**) also led to reduction of light absorption in the wavelength region between 630-690 nm by *C. arvense* leaves (Berestetskiy et al, 2008). The peaks of light absorption in this region are characteristic of chlorophyll intermediates, phytychlorophyllide and chlorophyllide (Berestetskiy et al, 2008, as cited by Duke et al, 1991).

### 3. Synthetic studies towards the discovery of new photosynthetic inhibitors

In the research and development of new agrochemicals, organic synthesis plays an important role. After the discovery of a lead structure, an optimization process of it is carried out in order to improve the biological activity as well physico-chemical properties of the lead. Within this context, organic chemists utilize a vast array of chemical reactions to prepare derivatives of a lead compound. In this section it will be discussed recent advances concerning synthetic studies aimed to discover new photosynthetic inhibitors. Details about the preparation of the mentioned compounds can be found in the cited references and will not be covered herein since this is not the focus of this book chapter.

Several papers has been published in the last few years dealing with the synthesis of various aromatic nitrogenated compounds and their biological evaluation as photosynthetic inhibitors (Jampilek et al, 2009a,b; Musiol et al, 2007; Musiol et al, 2008; Musiol et al, 2010; Otevrel et al, 2010). The general structures and their associated most active compounds are presented in Figure 7 along with the IC<sub>50</sub> data. For all of these substances, the in vitro evaluation of their inhibitory activity on the electron transport in isolated intact spinach chloroplasts was determined spectrophotometrically using the artificial electron acceptor 2,6-dichlorophenol indophenol (DCPIP). The rate of photosynthetic electron transport was monitored as photoreduction of DCPIP. The biological assays utilized diuron (**1**) (Figure 1) as positive control which the IC<sub>50</sub> determined under the conditions used in the experiments was 1.9  $\mu\text{mol L}^{-1}$ .

Quinoline is a structural motif found in various classes of bioactive compound. Musiol and co-workers prepared seventeen quinoline derivatives presenting the general structure (29). Some of the synthesized compound could not be biologically evaluated due to poor water solubility. The ten studied quinolines presented low inhibitory activity on photosynthetic electron transport with  $IC_{50}$  values ranging from 26 to 487  $\mu\text{mol L}^{-1}$ . Compound (29a) was the most active presenting  $IC_{50} = 26 \mu\text{mol L}^{-1}$  (Musiol et al, 2007).



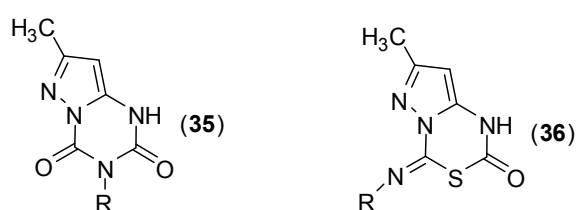
Compound	$IC_{50}$ ( $\mu\text{mol L}^{-1}$ )
29a	26.0
30a	32.0
30b	95.0
30c	8.7
30d	107.0
30e	137.0
31a	16.0
31b	7.2
32a	157
32b	126
33a	33.5
33b	54.4
34a	2.7
34b	1.6
34c	1.6
34d	1.0
Diuron	1,9

Fig. 7. Structures of aromatic nitrogenated compounds 29-34.

For the two groups of amides (structures **30** and **31**) based on the quinoline scaffold system, it was found that for the seventeen compounds of general structure (**30**) the most active ones were those possessing the nitro group ( $-\text{NO}_2$ ) at the  $\text{R}^1$  position (**30a-30e**). Compounds lacking this functionality at the specified position were completely inactive. Considering the quinolines (**31**), no simple structure-activity relationship explaining the observed activity was found being the two most active derivatives (**31a**) and (**31b**) (Musiol et al, 2008).

In a series of twelve ring-substituted 4-hydroxy-1*H*-quinolin-2-one derivatives (general structure **32**), all of the compounds displayed very low inhibitory activity ( $\text{IC}_{50}$  ranging from 126 to 925  $\mu\text{mol L}^{-1}$ ) on electron transport in photosynthesis from water to DCPIP (Jampilek et al, 2009a). The best activities were observed with derivatives (**32a**) and (**32b**).

In another series of hydroxylated derivatives, among the fourteen ring-substituted 8-hydroxyquinoline derivatives (**33**), two compounds (**33a**, **33b**) showed moderate inhibitory activity (Musiol et al, 2010).



- (**35a**): R = phenyl  
 (**35b**): R = cyclohexyl  
 (**35c**): R = benzyl  
 (**35d**): R = ethyl  
 (**35e**): R = butyl  
 (**35f**): R = sec-butyl
- (**36a**): R = cyclohexyl  
 (**36b**): R = benzyl  
 (**36c**): R = ethyl  
 (**36d**): R = butyl  
 (**36e**): R = butyl

Compound	$\text{IC}_{50}$ ( $\mu\text{mol L}^{-1}$ )
<b>35a</b>	169±86
<b>35b</b>	0.32±0.19
<b>35c</b>	42±8
<b>35d</b>	32±8
<b>35e</b>	6.6±0.8
<b>35f</b>	2.6±0.6
<b>36a</b>	0.21±0.03
<b>36b</b>	18±4
<b>36c</b>	2.9±0.6
<b>36d</b>	0.57±0.11
<b>Diuron</b>	0.27±0.02
<b>Hexazinone</b>	0.11±0.01
<b>Lenacil</b>	0.08±0.02

Fig. 8. Structures of pyrazoles **35** and **36** and the corresponding  $\text{IC}_{50}$  data.

The investigation of the influence of twelve ring substituted salicylanilides and carbamoylpehnylcarbammates, represented by the general structure (**34**), on photosynthetic apparatus afforded compounds (**34a-34d**) (Figure 7) with biological activities compared do diuron. The remaining evaluated compounds displayed low to moderate activity. In addition, it was also suggested based on experimental results that the site of action of the

compounds could be in  $Q_B$ , which is the second quinone acceptor on the oxidizing site of photosystem II.

The ability of ten pyrazole derivatives presenting the general structures (35) and (36) to act as photosystem II inhibitors was evaluated (Vicentini et al, 2004). In the presence of increasing concentrations of the compounds, it was observed that all of them are capable of interfering with the light-driven reduction of ferricyanide by isolated spinach chloroplasts. As can be seen in Figure 8, with the only exception of compound (35a), the  $IC_{50}$  ranged from 0.2 to 42  $\mu\text{mol L}^{-1}$ . It is important to mention that the effectiveness of the most active compounds was comparable to three commercial herbicides (diuron, hexazinone and lenacil) used as positive controls in the experiments.

The pyrazoles (35) and (36) were also evaluated in vivo against the blue-green alga *Spirulina platensis* as well as the eukaryotic alga belonging to the genus *Chlorella*. Once again, for some derivatives the observed activities are remarkable and comparable to commercial herbicides used as positive controls (Vicentini et al, 2004).

Even more remarkable biological activity was displayed by another series of pyrazoles presenting the general structures (37) and (38) (Figura 9). All of these compounds inhibited the Hill reaction from water to ferricyanide in the presence of illuminated chloroplasts, with  $IC_{50}$  ranging from  $10^{-6}$  mol  $L^{-1}$  to  $10^{-4}$  mol  $L^{-1}$ . The efficacy of (37j) ( $IC_{50} = 0.64 \mu\text{mol L}^{-1}$ ) is comparable to commercial herbicides such as diuron, lenacil and hexazinone ( $IC_{50} = 0.27$ , 0.08 and 0.11  $\mu\text{mol L}^{-1}$ ) (Vicentini et al, 2005).

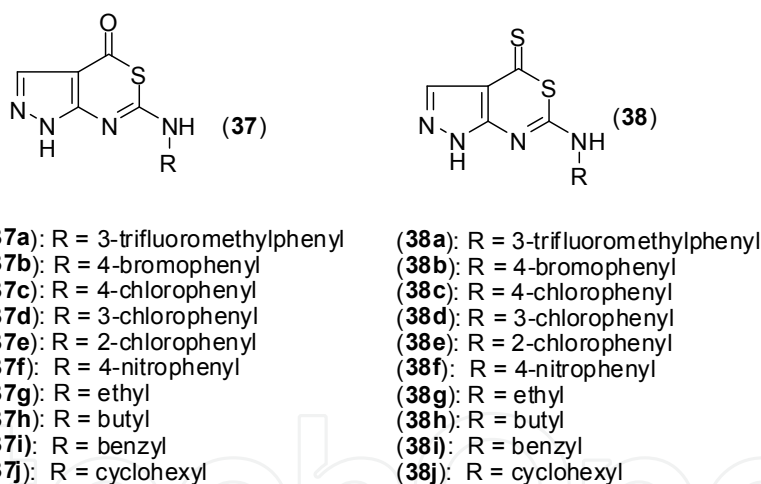


Fig. 9. Structures of pyrazoles 37 and 38 mentioned in the text.

One strategy that could be used to discover novel herbicides is to synthesize analogues of a natural products which is known to present phytotoxic activity. It is also possible to take a structure of a phytotoxic natural product itself and carry out structural modifications on it. Such an approach can result in compounds with improved biological activity as well better physico-chemical properties. The natural product (39) (Figure 10) was isolated from *Pterodon polygalaeflorus*. Biological evaluation of (39) as potential inhibitor of ATP synthesis in isolated chloroplasts from spinach revealed that this substance is completely inactive. However, its  $\beta$ -lactone derivative (40) inhibited this process as concentration increases. For this compound, the  $IC_{50}$  was 90  $\mu\text{mol L}^{-1}$ . Similar to other terpenes previously described in this chapter, lactone (40) did not affect photosystem I but it did inhibit electron transport through photosystem II by targeting the oxygen evolving complex as well as the redox



enzymes of the electron transport chain in the span between  $P_{680}$  and  $Q_A$  (King-Díaz et al 2006).

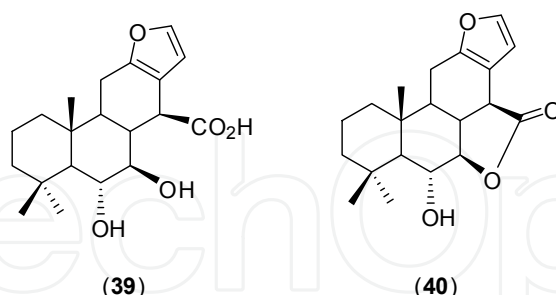
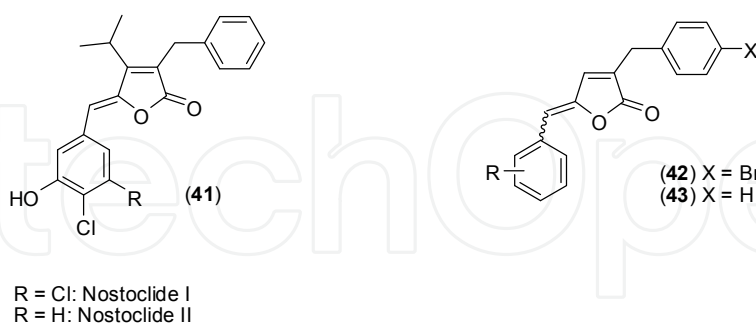


Fig. 10. Structure of diterpene (39) and its synthetic derivative (40).

Other derivatives of compound (39) have been recently evaluated and present phytotoxic properties on photosynthesis (King-Díaz et al, 2010).

The nostoclides (41) correspond to a pair of naturally-occurring lactones produced by a cyanobacterium (*Nostoc* sp.) symbiont of *Peltigera canina* (L.). It has been suggested that these chlorinated compounds may be allelopathic agents since *P. canina* cultures are usually not contaminated with microorganisms (Yang et al, 1993). In view of that, it was decided to investigate the potential phytotoxicity of nostoclides analogues (Barbosa et al. 2006). In this context, several derivatives (general structures 42 and 43, Figure 11) were prepared, and their ability to interfere with the light-driven reduction of ferricyanide by isolated spinach chloroplasts thylakoid membranes (Hill reaction) was subsequently evaluated (Barbosa et al., 2007; Teixeira et al, 2008).

A number of nostoclides derivatives, at various degrees, exhibited inhibitory properties in the micromolar range against the basal electron flow from water to ferricyanide. As a general trend, the non-brominated derivatives (43) presented higher effectiveness than their brominated counterparts. The most active compounds (43a-43e) derivatives along with their  $IC_{50}$  values are presented in Figure 11.



Compound	Benzylidene group	$IC_{50}$ ( $\mu\text{mol L}^{-1}$ )
43a	Z-4-nitrobenzylidene	1.7±0.7
43b	Z-2-fluorobenzylidene	10.1±3.2
43c	Z-4-trifluoromethylbenzylidene	8.3±2.3
43d	Z-2-trifluoromethylbenzylidene	11.8±4.5
43e	Z-4-ethylbenzylidene	9.1±3.2

Fig. 11. Structure of natural product nostoclides and some synthetic analogues.

More recently, a QSAR (Quantitative Structure Activity Relationship) investigation was carried out on a series of nostoclide analogues presenting the general structure (43) to correlate molecular descriptions with their in vitro biological activity (the ability to interfere with light-driven reduction of ferricyanide by isolated spinach chloroplasts thylakoid membranes). The results of this investigation suggested that the degree of inhibition efficiency of this class of compounds is intimately associated with their polarity (Teixeira et al, 2009). Thus, it is likely that new nostoclide analogues with higher polarity could display improved biological activity. At the moment that this book chapter is written, there is no report on the literature concerning the synthesis of nostoclide analogues with higher polarity than the previous ones already prepared.

#### 4. Conclusions

The ongoing need for new agents to control weeds has stimulated the search for new photosynthetic inhibitors. We described in this chapter a variety of compounds presenting this type of activity. The natural products have been explored toward this end resulting in the identification of compounds with various structural motifs. Such an approach has resulted in the discovery of photosynthetic inhibitors with new modes of action. This, in turn, can be helpful in dealing with resistance a problem to be faced in weed management. It is possible to anticipate that promising inhibitors of photosynthesis will certainly be found by exploring the natural product pool. From nature, it is also possible that more active compounds with low toxicity and improved selectivity will be found. Promising photosynthetic inhibitors has also been revealed by the synthetic studies. One important challenge in the field of weed management is related to selectivity. In other words, chemicals should exert their action only on weeds. As can be noticed in the discussion of the studies published in the literature during the last years, this issue has not been addressed. Future works should also be concerned with this important matter.

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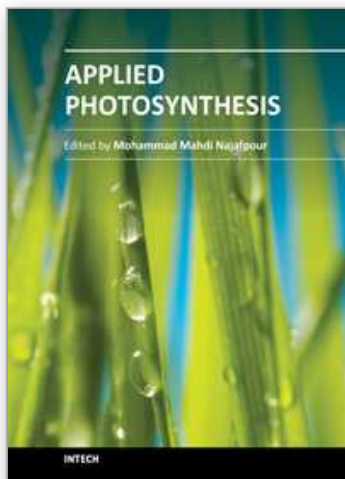
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Photosynthesis is one of the most important reactions on Earth, and it is a scientific field that is intrinsically interdisciplinary, with many research groups examining it. This book is aimed at providing applied aspects of photosynthesis. Different research groups have collected their valuable results from the study of this interesting process. In this book, there are two sections: Fundamental and Applied aspects. All sections have been written by experts in their fields. The book chapters present different and new subjects, from photosynthetic inhibitors, to interaction between flowering initiation and photosynthesis.

#### **How to reference**

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