Pesticide Biochemistry and Physiology 102 (2012) 189–197

Contents lists available at SciVerse ScienceDirect

Pesticide Biochemistry and Physiology

journal homepage: www.elsevier.com/locate/pest

Chlorophyll fluorescence as a marker for herbicide mechanisms of action

Franck E. Dayan [a,*], Maria Leticia de M. Zaccaro [b,1]

[a] USDA-ARS, Natural Products Utilization Research Unit, P.O. Box 8048, University, MS 38677, USA
[b] Faculdade de Ciências Agrárias e Veterinárias, Universidade Estadual Paulista 14884-900, Jaboticabal, SP, Brazil

ARTICLE INFO

Article history:
Received 23 November 2011
Accepted 16 January 2012
Available online 30 January 2012

Keywords:
Photosynthesis
Induced chlorophyll fluorescence
Photosynthetic electron rate
Mode of action
Herbicide
Mechanism of action
Light-dependent
Light-independent

ABSTRACT

Photosynthesis is the single most important source of O_2 and organic chemical energy necessary to support all non-autotrophic life forms. Plants compartmentalize this elaborate biochemical process within chloroplasts in order to safely harness the power of solar energy and convert it into usable chemical units. Stresses (biotic or abiotic) that challenge the integrity of the plant cell are likely to affect photosynthesis and alter chlorophyll fluorescence. A simple three-step assay was developed to test selected herbicides representative of the known herbicide mechanisms of action and a number of natural phytotoxins to determine their effect on photosynthesis as measured by chlorophyll fluorescence. The most active compounds were those interacting directly with photosynthesis (inhibitors of photosystem I and II), those inhibiting carotenoid synthesis, and those with mechanisms of action generating reactive oxygen species and lipid peroxidation (uncouplers and inhibitors of protoporphyrinogen oxidase). Other active compounds targeted lipids (very-long-chain fatty acid synthase and removal of cuticular waxes). Therefore, induced chlorophyll fluorescence is a good biomarker to help identify certain herbicide modes of action and their dependence on light for bioactivity.

Published by Elsevier Inc.

1. Introduction

The appearance of photosynthesis marked a critical turning point in the evolution of life on earth. Prior to that point, life was confined to prokaryotic organisms growing in extreme environments (e.g., deep-sea hydrothermal vents) where energy-generating chemosynthetic oxidation–reductions occurred in the absence of oxygen and light [1].

Photosynthesis, which consists primarily in converting light energy into chemical energy while using CO_2 and releasing O_2, is an energy resource 3–4 orders of magnitude larger than previous processes [2]. This process originally evolved in bacteria and is believed to have been introduced into eukaryotic organisms through endosymbiosis to form what eventually stabilized as chloroplasts [3]. The efficiency of photosynthesis ultimately enabled the development of more complex oxygenic organisms and the slow transition from aqueous to terrestrial environments. As the atmosphere enriched in O_2, other life forms relying on this molecule for aerobic respiration evolved. Today, all eukaryotic life depends on photosynthesis, as it is the only significant solar energy storage process on earth [4].

Plants have evolved an elaborate physiological and biochemical framework to support the proper function of photosynthesis. Higher plants developed sophisticated architectures (i.e., from subcellular compartmentalization to specialized tissues and organs) to maximize their ability to harness the power of solar energy. Photosynthesis is separated in two broad functions, the light reaction (or Hill reaction) and the dark reaction (or Calvin cycle). The light reaction involves the splitting of water into oxygen, protons and electrons. The electrons are energized by the light energy trapped by the reactions centers and channeled through the photosynthetic electron transport system to generate NADPH. This process concentrates protons inside the lumen of the thylakoids, which leads to ATP synthesis as the protons are released back in the cytosol through a coupling factor/ATPase complex. These two primary forms of chemical energy are then utilized to produce a more mobile and stable form of energy, sugar, through the dark reaction.

Photosynthesis requires a complex environment of subcellular membranes within the chloroplast, the synthesis of the most abundant pigments in the world (chlorophylls and carotenoids) [5,6], fairly elaborated antioxidative mechanisms to quench the excess energy generated under high light intensities [7], many enzymes and proteins involved in carbon fixation, including the most abundant protein in the world (Rubisco) [8,9], and numerous processes needed to provide water and shuttle photosynthates to other parts of the cell and to the rest of the plant [10,11]. All of these processes are interdependent, and stresses (biotic or abiotic) challenging the integrity of the plant cell are likely to affect photosynthesis.

The paradigm underlying chlorophyll fluorescence analysis is that the light energy absorbed by chlorophylls is used to drive

* Corresponding author.
E-mail addresses: fdayan@olemiss.edu, franck.dayan@ars.usda.gov (F.E. Dayan).
[1] Current address: Sitio Manga Rosa, Cx Postal 001, Ribeirão Preto 14110-970, SP, Brazil.

0048-3575/$ - see front matter Published by Elsevier Inc.
doi:10.1016/j.pestbp.2012.01.005

photosynthesis (photochemical energy), and excess energy is released as non-photochemical energy, such as heat and chlorophyll fluorescence. While chlorophyll fluorescence accounts for 1–2% of the total light absorbed, it is easily measured and has been a powerful tool to investigate plant physiological processes. Carefully designed experiments can provide detailed information into the various steps of the Hill reaction, but fluorescence is a reflection of carbon fixation as well as the overall stress status of a plant [12,13]. Therefore, such analysis has yielded important information on herbicides that directly interfere with photosynthetic electron transport (e.g., photosystem I and II inhibitors). Newer techniques such as phytofluorography can visualize real-time movement of certain herbicides in plants and calculate their respective ability to translocate (systemicity index) [14]. Chlorophyll fluorescence is affected by other light-dependent herbicides, such as the inhibitors of glutamine synthetase, protoporphyrinogen oxidase, and carotenoid biosynthesis [15]. Additionally, herbicides causing peroxidation of membrane lipid bilayers affect the stability of the photosynthetic apparatus and may indirectly induce chlorophyll fluorescence.

In light of the long history of using herbicides to probe plant biochemical process [16,17], a simple three-step assay was developed to test selected herbicides representative of all the known herbicide mechanisms of action and determine whether induced chlorophyll fluorescence a suitable biomarker to help identify certain herbicide modes of action. A number of natural phytotoxins are also included in this survey. Any activity detected as changes in chlorophyll fluorescence is discussed in the context of the compounds respective mechanisms of action and their potential requirement for light.

2. Materials and methods

2.1. Chemicals

Diclofop-methyl, 2-[4-(2,4-dichlorophenoxy)phenoxy]-propanoic acid methyl ester; alachlor, 2-chloro-*N*-(2,6-diethylphenyl)-*N*-(meth oxymethyl)-acetamide; sulfentrazone, *N*-[2,4-dichloro-5-[4-(difluoromethyl)-4,5-dihydro-3-methyl-5-oxo-1*H*-1,2,4-triazol-1-yl]-phenyl]-methanesulfonamide; clomazone, 2-[(2-chlorophenyl) methyl]-4,4-dimethyl-3-isoxazolidinone; atrazine, 6-chloro-*N*2-ethyl-*N*4-(1-methylethyl)-1,3,5-triazine-2,4-diamine; bentazon, 1*H*-2,1,3-benzothiadiazin-4(3*H*)-one, 3-(1-methylethyl)-2,2-dioxide; paraquat, 1,1′-dimethyl-4,4′-bipyridinium; dinoterb, 2-(1,1-dimethylethyl)-4,6-dinitro-phenol; imazapyr, 2-[4,5-dihydro-4-methyl-4-(1-methylethyl)-5-oxo-1*H*-imidazol-2-yl]-3-pyridinecarboxylic acid; metsulfuron, 2-[[[[(4-methoxy-6-methyl-1,3,5-triazin-2-yl)amino] carbonyl]ami no]sulfonyl]-benzoic acid; glyphosate, *N*-(phosphonomethyl)glycine; asulam, *N*-[(4-aminophenyl)sulfonyl]-carbamic acid methyl ester; endothall, 7-oxabicyclo[2.2.1]heptane-2,3-dicarboxylic acid; 2,4-D, 2-(2,4-dichlorophenoxy)-acetic acid; quinclorac, 3,7-dichloro-8-quinolinecarboxylic acid were purchased from ChemService (West Chester, PA 19381).

Carbetamide, (2*R*)-*N*-ethyl-2-[[(phenylamino)carbonyl]oxyl]propanamide; EPTC, *N*,*N*-dipropyl-carbamothioic acid *S*-ethyl ester; isoxaflutole, (5-cyclopropyl-4-isoxazolyl)[2-(methylsulfonyl)-4-(tri fluoromethyl) phenyl]-methanone; triclosan, 5-chloro-2-(2,4-dichlorophenoxy)-phenol; pelargonic acid, nonanoic acid; diuron, *N*′-(3,4-dichlorophenyl)-*N*,*N*-dimethyl-urea; pendimethalin, *N*-(1-ethylpropyl)-3,4-dimethyl-2,6-dinitrobenzenamine; oryzalin, 4-(dipropylamino)-3,5-dinitro-benzenesul fonamide; dichlobenil, 2,6-dichlorobenzonitrile; isoxaben, *N*-[3-(1-ethyl-1-methylpropyl)-5-isoxazolyl]-2,6-dimethoxy-benzamide; MSMA, monosodium methylarsonate; cercosporin, (13b*R*)-5,12-dihydroxy-8,9-bis[(2*R*)-2-hydroxypropyl]-7,10-dimethoxy-perylo[1,12-def]-1,3-dioxepin-6,11-dione; acifluorfen-methyl, 5-[2-chloro-4-(trifluoromethyl)phenoxy]-2-nitro-benzoic acid methyl ester; norflurazon, 4-chloro-5-(methyla mino)-2-[3-(trifluoromethyl)phenyl]-3(2*H*)-pyridazinone; glufosinate, 2-amino-4-(hydroxymethylphosphinyl)butanoic acid were purchased from Sigma–Aldrich (St. Louis, MO 63103).

Fluridone, 1-methyl-3-phenyl-5-[3-(trifluoromethyl)phenyl]-4(1*H*)-pyridinone was a gift from SePRO Inc. (Carmel, IN 46032). Cinmethylin, (1*R*,2*S*,4*S*)-*rel*-1-methyl-4-(1-methylethyl)-2-[(2-methylphenyl)methoxy]-7-oxabicyclo[2.2.1]heptanes was a gift from Dupont de Nemours (Newark, DE 19711). Dehydrozaluzanin C, (3a*S*,6a*R*,9a*R*,9b*S*)-octahydro-3,6,9-tris(methylene)-azuleno[4,5-b]furan-2,8(3*H*,4*H*)-dione was kindly provided by Dr. J.C.G. Galindo (University of Cadiz, Spain).

2.2. Assessing the effect of compounds on photosynthetic efficiency

Cucumber seedlings were grown in a growth chamber with a continuous light for 7 days. Three 12-mm cotyledon discs were placed on a 2% sucrose/1 mM 2-(*N*-morpholino)ethanesulfonic acid buffer (MES, pH 6.5) containing 100 μM of each of the compound tested [18] in 60 × 15 mm Petri plates. Each plate contained 5 mL of buffer. Control tissues were exposed to the same amount of acetone as 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} photosynthetically active radiation) in an incubator (Model CU-36L5, Percival Scientific, Boone, IA 50036). Photosynthetic quantum yield (Y) and electron transport rate (ETR) were measured using a pulse-modulated fluorometer (Opti-Science, Model OS5-FL, Tyngsboro, MA 01879). The instrument was set on Kinetic Mode and adjusted so that the initial Ft (instantaneous fluorescence signal) value in the control samples was approximately 210. The instrument detector gain was set between 75 and 85. Quantum yield was determined by the following light treatment: each cycle consisted of a 0.8 s pulse of saturating light generated with a laser diode actinic source to saturate PSII, followed by a 4 s far-red light pulse used to re-oxidize PSII, and a 10 s delay to allow PSII to regain steady-state conditions. A total of seven cycles were performed for each sample. ETR values were expressed as percents of the ETR average values observed in control treatments.

A time-course experiment was performed by measuring induced fluorescence on cotyledon discs at predetermined time intervals (up to 24 h). Beginning 3 h after a dark incubation period, another measurement was made after 18 h (overnight), at which time the samples were placed in the light and a final measurement was made after 6 h on light exposure. Each experiment consisted of three replicates.

3. Results and discussion

3.1. Herbicides targeting electron transports

Photosynthesis and cellular respiration are two of the most important physiological processes involving elaborate electron transport chains. Since reactions involving electron transfer are accompanied by potentially reactive and harmful intermediates, these processes are compartmentalized in lipid bilayers to avoid direct contact with water or high concentrations of oxygen, and are surrounded by numerous antioxidative protective mechanisms (both chemical and enzymatic) to quench excess reactive oxygen species [19]. The electron transport-dependent light reaction of photosynthesis is compartmentalized within the thylakoid membranes of the chloroplasts, whereas cellular electron transport is localized within the inner mitochondrial membrane. Photosynthetic electron