Journal of High School Science

The effect of herbicides on the rates of photosynthesis in spinach leaves.

D.Zemedkun, A.Alaparmak, S.Apte[1]
Harmony Science Academy, Euless, TX 76040

Abstract

The ratio of the rate of photosynthesis to the rate of respiration (P/R) was measured in spinach leaves using the leaf-disc experiment. The effect of herbicides and an insecticide on this ratio was measured. The herbicides and the insecticide significantly altered the P/R ratio of the spinach leaves. A comparison of the magnitude of the P/R ratio of spinach leaves exposed to herbicides or the insecticide with that of the control enabled the deduction of the general site of action of the compounds in the photosynthesis – respiration pathway.

Introduction

Photosynthesis is an anabolic endothermic redox reaction by which photoautotrophs reduce CO_2 to produce carbohydrates using photons from the sun as an energy source. Photosynthesis occurs in two stages. In the light-dependent reactions, photons react with antenna pigments in the light harvesting complex (photosystems II and I) to set up an electron transport chain reducing $NADP^+$ to NADPH. The enzymatic photolysis of water compensates for the loss of electrons by the antenna pigments leading simultaneously to the generation of O_2 gas as a byproduct and a proton gradient across the chloroplast membrane. ATP synthase uses this chemiosmotic proton gradient for the conversion of ADP to ATP. In the light independent or "dark" reactions, the enzyme Ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO) captures CO_2 from the atmosphere and reduces it to 3-carbon sugars *via* the Calvin cycle, using the NADPH and ATP manufactured from the light-dependent reactions.

Cellular respiration is a catabolic exothermic redox reaction by which photoautotrophs oxidize carbohydrates to produce smaller molecules (*via* the pentose phosphate and phenol pyruvic acid pathways), ATP (by utilizing the chemiosmotic proton gradient in the mitochondrial electron transport chain) and the reducing equivalents NADH and $FADH_2$ produced in the tricarboxylic acid (TCA) cycle, CO_2 and H_2O using the TCA and O_2 as the terminal electron acceptor.

[1]Corresponding author, Harmony Science Academy, Euless, TX 76040, sapte@harmonytx.org

Herbicides are chemical compounds that interfere with photosynthesis and cellular respiration processes to kill unproductive photoautotrophs. Their structure is not a good predictor of the site of action of these compounds because of the ubiquity of redox reactions throughout the photosynthesis/respiration regime. In addition, a combination of these compounds is usually used in commercially available herbicides to maximize their lethality which makes for even poorer prediction for a specific site of action that is predominantly toxic toward different photoautotrophs. Different herbicides have differing effects on the photosynthesis and cellular respiration processes.

Materials

Spinach leaves were obtained from several grocery stores. A Pioneer model balance, Item PA153, d=0.001 g, Ohaus Corporation, NJ, USA was used for weighing samples. Other chemicals were, Simple Truth™ liquid dish soap, lot # S152501201307569, Kroger Co. OH, USA, Citric acid solution 0.1 M lot # 177951 Flinn Scientific, IL, USA, sodium phosphate heptahydrate, dibasic lot # AD-13164-1 Carolina Biological supply Co., NC, USA and sodium bicarbonate Arm and Hammer lot # WW 7268, Church and Dwight Co. NJ, USA. An incandescent bulb of 40 W was used to illuminate the leaf discs in the culture tube, a ¼ inch single hole puncher was used to make the spinach leaf discs. The culture tube containing the leaf discs suspended in 3 mL of the either the citrate-phosphate buffer solution or 3 mL of the herbicide or insecticide solution along with one drop of dish soap was subjected to vacuum (Mityvac™ model MV8010, Lincoln Industrial Corp, MO, USA) to ensure maximum liquid penetration into intercellular spaces. A ceramic mortar and pestle was used to crush the leaf punches in alcohol, the fine colloidal suspension subsequently being filtered through a 11 cm qualitative filter paper, Ahlstrom filtration LLC, PA, USA. The absorbance was measured using a spectrophotometer catalog no. AP7038, Flinn Scientific, IL, USA. The intensity of light, pH and temperature were measured with appropriate probes connected to a Vernier LabQuest™, Vernier Software and Technology, OR, USA. The herbicides/insecticides used were: Triazicide™ insect killer containing gamma-cyhalothrin 0.08% lot No. U020318T2203, Spectracide™ containing Diquat dibromide 0.12%, Fluazifup-p-butyl 0.06%, Dicamba, dimethylamine salt 0.04% lot No. U071218BM11:17:37 United Industries Corp. MD, USA and Knockout™ weed and grass killer containing Glyphosate isopropylamine salt 1.92% lot No. A1805403A GroTec Inc. GA, USA, A micrometer screw gauge d = 0.01 mm, was used to measure the thickness of the leaf discs.

Methods

Non-wilted, green spinach leaves were stored in closed polyethylene bags in the refrigerator between 4ºC and 8ºC. They were used as-is within 2 weeks of purchase. Leaves were

punched at the edges taking care to avoid including large veins into the disc. Preliminary experiments demonstrated that leaf discs obtained from leaves with axial lengths ranging from 5 cm to 9 cm and equatorial lengths ranging from 3 cm to 5 cm had similar average weights invariant of batches sourced at different times from different grocery stores. The weight of one leaf disc was obtained by dividing the weight of 10 leaf discs weighed on the scale precise to 0.001 g. The experiment was repeated 10 times. The leaf thickness was measured and recorded as the mean of 10 trials. The density of all solutions was assumed to be equal to that of a 0.22% sodium bicarbonate solution.

Using standard mensuration formulae and assuming that the leaf disc would sink when its density became greater than that of the surrounding bicarbonate solution at that temperature, the percent of air space in the leaf punch could be calculated (Table 2, row 6). Using the ideal gas equation, this enabled the calculation of the moles of O_2 gas in the leaf punch, and consequently, the calculation of the rate of oxygen released or consumed as $\mu m\ m^{-2}\ s^{-1}$ during photosynthesis or respiration respectively (Table 3).

10 discs were obtained from a leaf using the hole punch. They were transferred to a borosilicate glass culture tube and mixed gently with 3 mL of the either the citrate-phosphate buffer solution or 3 mL of the herbicide or insecticide solution. One drop of the liquid dish soap was added. The herbicide/insecticide solutions were obtained from the primary packaging plastic container as is, except for the Spectracide™; which was diluted 1100 times with water before mixing with the leaf discs. The contents of the tube were subjected to three cycles of vacuum (< 200 mm Hg) using the hand held vacuum pump, each cycle lasting for 4 minutes. To the tube was then added 15 mL of a 0.22% (26 mM) sodium bicarbonate solution. The bicarbonate solution was prepared immediately before use in a 100 mL volumetric flask. The tube was shaken gently until the leaf discs settled to the bottom. The tube was illuminated with a 40 W incandescent bulb kept at a distance of < 2 cm from the side of the tube. A light sensor placed with its sensor end close to the tube bottom was used to ensure that the light intensity was the same for all experiments. During the ongoing experiment, the tube was shaken gently (swirling motion 6 times) at 1 minute intervals. The time for any leaf disc to float to the top of the solution was recorded until such time as all the leaf discs floated to the top of the solution; i.e. became floaters from sinkers. At this time, the lamp was turned off and the tube was transferred to a closed unlighted room. During this dark exposure, the tube was gently shaken every 15 minutes and the time for any leaf disc to sink to the bottom of the tube was recorded (the door of the room was left ajar to enable observation) until such time as all the leaf discs sank to the bottom of the solution; i.e. became sinkers from floaters.

The rise in temperature of the solution in the test tube over a period of 60 minutes was measured with a Go™Temp temperature probe attached to a Vernier LabQuest™ . All the solutions were exposed to the same temperature change throughout exposure. The light spectrum emitted by the lamp was viewed through a spectroscope and photographed using

a smartphone. All the solutions were exposed to the same light intensity.

The pH of the solutions was measured using a Vernier pH probe attached to the Vernier LabQuest^{TM}. The probe was calibrated with pH 4.0 and pH 7.0 solutions prior to use. The light intensity was measured using a Vernier T1 light probe attached to the Vernier LabQuest^{TM}. Since the light intensity within 2 cm of the lamp was off-scale, the intensity in mW cm^{-2} was measured at various distances from the lamp and then extrapolated to the distance of 2 cm from the lamp using a fitted power function.

The results were graphed with time in minutes as the independent variable and the number of floaters or sinkers as the dependent variable. The points were joined by a freehand curve and the effective time for 50% of the leaf discs to float, designated as ET_{50L}, or to sink, designated as ET_{50D}, was calculated. A representative curve is shown in figure 4. The rate of photosynthesis was calculated using equation 1 below (1),

Rate of photosynthesis = $[1/ET_{50L}] + [1/ET_{50D}]$ equation 1

The rate of respiration was calculated using equation 2 below,

Rate of respiration = $[1/ET_{50D}]$ equation 2

The ratio of the rate of photosynthesis to the rate of respiration could hence be calculated.

Chlorophyll absorbance was measured using a spectrophotometer. 10 leaf discs were completely ground in 10 ml of absolute alcohol for < 1 minute. The suspension was then filtered through the filter paper and its absorbance was measured in a 1 cm ID test tube from 400 to 500 and 600 to 700 nm at intervals of 20 nm, blanking the instrument at each wavelength with absolute alcohol (see figure 6). For generating a standard curve, the absorbance of 3, 6, 10, 15 and 20 leaf punches, ground and filtered as above was measured at two wavelengths, 440 and 660 nm. The standard curves were generated in anticipation of chlorophyll degradation in the presence of herbicide/insecticide.

Independent two-tailed unpaired Student's *t* tests were used to assess the statistical difference between the P/R ratios for the leaf discs exposed to different herbicides/insecticide from control or from one another. Differences between means were considered significant at a p-value < 0.05.

Results and discussion

The average weight of a leaf disc was 0.061 g ± 0.86% (average mass ± % relative standard deviation) (n=10, leaf discs weighed=10). The thickness of the leaf was found to be 0.053 cm ± 0.11%, consistent with that reported in the literature (2). The ET_{50L} and ET_{50D} values

obtained with spinach leaf disks at day 0 from purchase and at day 14 from purchase were similar (data not shown), hence leaves were stored and used within 2 weeks of purchase.

Figures 1 and 2 illustrate the experimental set up. The lamp, the culture tube and the light intensity detector are visible, as are the leaf discs with some in the process of floating up.

Figure 1: The lamp. Light intensity measurement Tube and the culture tube with solution and leaf Discs during the experiment.

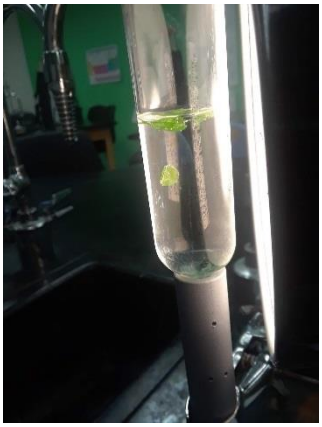


Figure 2: Similar to figure 1 with lighter surroundings.

Figure 3 shows that the lamp emitted the full spectrum of visible light conducive to maximum photosynthesis.

Figure 3: Light spectrum emitted by lamp

Figure 4 shows a representative graph of the number of floaters and sinkers with time. It can be seen that both the rates of photosynthesis and respiration can be described by smooth functions which validates using the parameters of ET_{50L} and ET_{50D} to calculate the P/R ratio.

Figure 4: Representative graph of measurement of floaters and sinkers

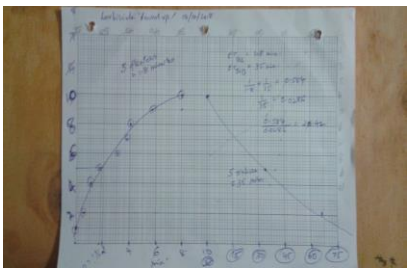


Figure 5 shows the photon flux intensity as a function of distance from the lamp. At a distance of 2 cm, the photon flux intensity is calculated as 153.0 $mW.cm^{-2}$ or 0.153 $J.cm^{-2}.s^{-1}$. Using the wavelength of maximum absorption of chlorophyll as 440 nm (figure 6) and the equation $E= hc/\lambda$, where h is Planck's constant in J.s, c is the speed of light in $m.s^{-1}$ and λ is the wavelength in m, yields an energy of 4.5 $x10^{-19}$ J/photon. There are hence 3.38 x 10^{17} photons impinging on the tube per cm^2.s. Since it requires 8 photons to release an O_2 molecule during photosynthesis (3), it is evident that the light intensity is not a limiting factor for photosynthesis in the experiment. The temperature of the solution in the culture tube increased by < 2° C after an illumination period of 60 minutes.

Figure 5: Photon flux as a function of distance from the lamp

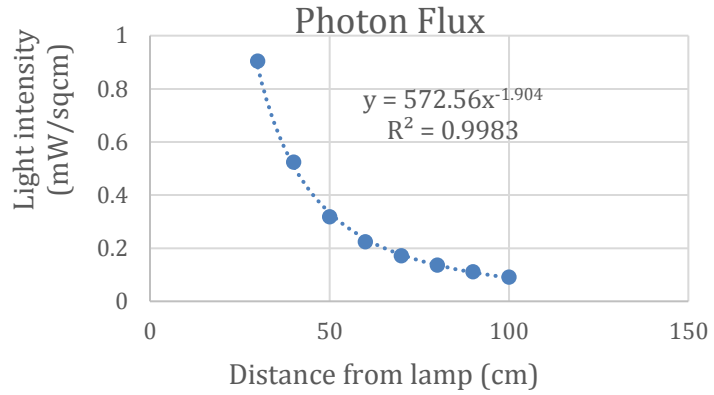


Figure 6 shows a representative visible absorption spectrum for chlorophyll. Absorption maxima were found at 440 and 660 nm. The spectra of chlorophyll obtained from leaf discs in the citrate-phosphate buffer and in the various herbicide/insecticide solutions were superimposable, as was their absorbance (data not shown), suggesting that no degradation of chlorophyll occurred when exposed to these herbicides/insecticide for the duration of the experiment.

Figure 6: Representative VIS absorption curve for chlorophyll

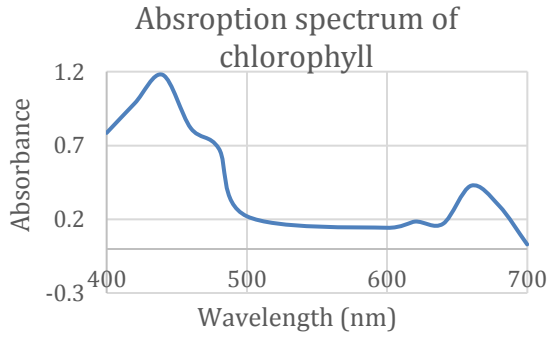
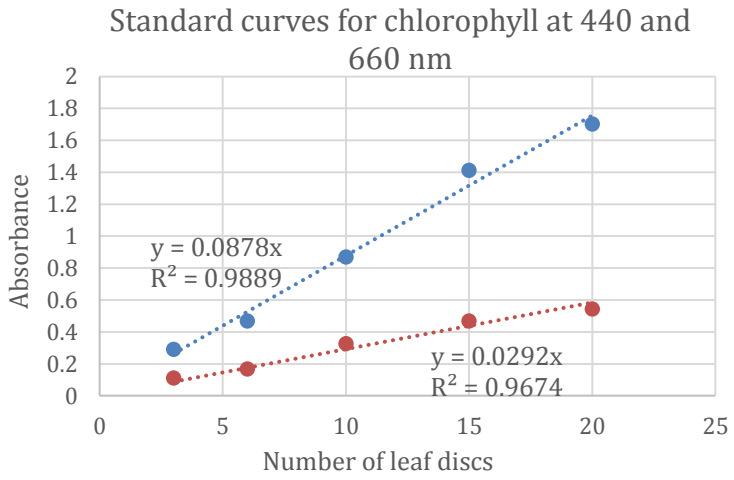


Figure 7 shows the standard curves of chlorophyll at 440 nm (the line with the greater slope) and 660 nm. The standard curves at both wavelengths were linear till a concentration of 14.3 uM (at 660 nm) with R^2 >0.96. Using an extinction coefficient value of 45700 M^{-1} cm^{-1} (4) in absolute alcohol at 666 nm, the concentration of chlorophyll in 10 leaf discs was calculated to be 7.16 uM or 0.64 mg mL^{-1} using a molar mass of chlorophyll of 893.51 g mol^{-1}.

Figure 7: Standard curve for chlorophyll at 440 and 660 nm



Since the concentrations of the herbicides used differed by orders of magnitude, concentration effects could potentially contribute to the difference in the rates of photosynthesis and/or the rates of respiration observed. As a first approximation for comparison of the relative 'lethality' of the herbicides on spinach leaves, the ratio of their concentration used to the LD_{50} (acute, oral administration in rats) was used. Since the ratios for glyphosate and gamma-cyhalothrin were of the same order of magnitude (equi-lethal), any differences in photosynthetic or respiration rates could reasonably be assumed not to result from differences in concentration. On the other hand, the herbicide (Spectracide) containing diaquat and fluazifop-p-butyl needed to be diluted 1100X in order to decrease lethality to an extent to obtain floatation.

The pH of the herbicide/insecticide and bicarbonate medium in which the spinach leaves were submerged ranged from 6.5 to 7.7. Using the Henderson-Hasselbach equation, and a pK_1 of 6.1 for H_2CO_3, the ratio of [HCO_3^{-1}] to [CO_2] was calculated as 2.5 and 39.8 at pH values of 6.5 and 7.7 respectively. The HCO_3^{-1} concentration in the glyphosate containing solution was hence 7.4 mM while it was 0.65 mM in the others. Even though the bicarbonate concentration used in the study was limiting for photosynthesis ($1/ET_{50L}$ plateau at 0.3% from reference 2), the rate of photosynthesis of the leaf discs exposed to the Knockout™ was not significantly greater than those of the control (p =0.32) or the leaf discs exposed to Triazide™

(p =0.19) but significantly greater than the those exposed to Spectracide™ (p = 0.013). However, since the P/R ratios for leaf discs exposed to Triazide™ and Knockout™ were not significantly different, the difference in pH was not a confounding factor in the experiment.

Table 1: Lethality comparison for concentrations of different herbicides

Compound	LD_{50} (acute, rat, oral) mg Kg^{-1}	Concentration used in this study [Molar]	Ratio of concentration to LD_{50}
Glyphosate	5600	8.4×10^{-2}	1.5×10^{-5}
Diquat	120	3.17×10^{-6}	2.6×10^{-8}
fluazifop-p-butyl	3600	1.42×10^{-6}	3.9×10^{-10}
Gamma-cyhalothrin	79	1.78×10^{-3}	2.3×10^{-5}

There were no floaters under illumination when water was substituted for the 0.22% $NaHCO_3$ solution. The absence of CO_2 results in accumulation of NADPH - and a depletion of $NADP^+$ - immediately upstream of the Calvin cycle. The absence of the terminal electron acceptor; $NADP^+$; blocks electron transfer downstream of photosystem I and prevents the generation of O_2 that otherwise occurs as a byproduct of reaction 1. The removal of CO_2 has also been shown to inhibit the Hill reaction (5) by suppressing electron flow on the donor side of PSII (6) as well as to inhibit the oxidation of water due to it being required in the water oxidizing complex of photosystem II (7). The transformation of incident light into reducing power (NADPH) and its utilization in carbon fixation differ by 15 orders of magnitude (8). The photochemical reactions of photosystem II (PSII) and PSI occur at an - orders of magnitude - faster time scale than electron transport and metabolism. A low CO_2 concentration causes a metabolic imbalance between the formation and utilization of reducing equivalents leading to a high ratio of 'closed' to 'open' PSII reaction centers via metabolic feedback loops. Such photo inhibitory conditions result in a reduced rate of water photolysis and the formation of damaging reactive oxygen species (ROS) (8). All these factors result in an attenuated rate of accumulation of oxygen.

$$2\ NADP^+ + 2\ H_2O + light \rightarrow 2\ NADPH + O_2 + 2\ H^+ \qquad \text{reaction 1}$$

There is hence not enough oxygen gas to displace the liquid in the leaf discs to decrease density and enable flotation. A similar result was obtained when the leaf discs were exposed to undiluted Spectracide™. The diquat and fluazifop-butyl herbicide constituents of Spectracide™ are classified as photosystem II and photosystem I disruptors respectively (9). No leaf discs floated under illumination when exposed to undiluted Spectracide™. It required a 1100 X dilution of Spectracide™ with water to enable flotation to occur.

Glyphosate inhibits the enzyme 5-enolpyruvylshikimate 3-phosphate (EPSP)

synthase, that is responsible for the synthesis of aromatic amino acids phenylalanine, tyrosine and tryptophan (10). It consequently causes an accumulation of shikimic acid and some hydroxybenzoic acids in leaves. Downstream byproducts of the shikimate pathway, Chorismate and Arogenate negatively regulate the shikimate pathway by inhibiting the enzyme 3-deoxy-D-arabinoheptulosonic acid-7-phosphate (DAHP) synthase, the first enzyme involved in the shikimic pathway. Glyphosate abrogation of the synthesis of Chorismate and Arogenate therefore causes an upregulation of carbon-flux into the shikimate pathway, siphoning carbon flow both from the pentose phosphate cycle and the tricarboxylic acid cycle into the shikimic acid pathway (11, 12). This leads to an accumulation of shikimic acid and shikimate-3-phosphate in the chloroplasts, which become a C-sink (13). Furthermore, glyphosate also reduced ribulose 1,5-biphosphate carboxylase oxygenase activity (Rubisco) as well as phosphoenolpyruvate carboxylase activity in nodulated lupine plants (14). Although glyphosate has been shown to inhibit photosynthesis by decreasing the biosynthesis of chlorophyll (15), photosynthetic electron transport and light harvesting are not affected (16). Under our experimental conditions, there was no noticeable change in either the visible absorption spectrum, or the absorbance of chlorophyll after exposure to the herbicides/insecticide. The spectra were superimposable (data not shown). The net effect was to cause a rapid cessation of carbon fixation and consequently a significant increase in the P/R ratio within the experimental regime.

Since the leaf disc sinks once all the O_2 in the air space has been utilized and replaced with the immersion liquid, the percent air space in the leaf punch needs to be at least 63.4% for the liquid filled leaf disc density to be 0.9976 g mL^{-1}. The air space volume in C_3 dicotyledonous species such as *Nicotiana tabacum* (tobacco) and *Vicia faba* (broad bean) has been reported to be 55% (17). The value of 63.4% hence does not seem unreasonable and presents a new method by which the intercellular air space can be calculated using immersion, the leaf disc dimensions and mensuration formulae.

Table 2: Mensuration results from punched leaf

Row #	Attribute	Expression used to calculate value
1	Average weight of 10 punched leaves (g)	0.061
2	Average weight of 1 punched leaf (g)	[(row1)/10] = 0.0061
3	Quarter inch punch radius (cm)	[0.25 X 2.54 /2] = 0.3175
4	Leaf disc thickness (cm)	0.053
5	Leaf disc volume (cm^3)	[π x (row3)^2 x (row4)] = 0.0168
6	Air space in leaf (%)	63.4
7	Leaf volume without air (cm^3)	[(100-(row6)) x 0.01 x (row5)] = 0.00614
8	Leaf surface area (m^2)	[2 x π x ((row3)/100)^2] = 6.3 x 10^{-5}
9	Volume of O_2 in leaf disc (cm^3)	[(row5) - (row7)] = 0.0106
10	Moles of O_2 in leaf disc at 20^{o}C	[((row9) x 0.001) / (0.08206 x 293)] = 4.42 x 10^{-7}
11	Density of leaf disc with air (g/mL)	[(row2)/(row5)] = 0.3636
12	Density of leaf disc with liquid (g/mL)	[((row2) + (row9))/ (row5)] = 0.9976

Table 3: Ratio of the rate of photosynthesis to the rate of respiration

	Herbicide concentration [Molar]	pH	ET_{50L} (min) ± standard deviation	ET_{50D} (min) ± standard deviation	P, rate of photosynthesis O_2 released	R, rate of respiration O_2 consumed	P/R ratio ± standard deviation
					µmol $O_2/m^2.s$		
Control	NA	7.41	9.97 ± 6.12	26.83 ± 2.84	11.68	4.34	2.69 ± 1.34
KnockOut™ Glyphosate isopropylamine	8.4×10^{-2}	6.54	5.21 ± 3.76	121.67 ± 87.51	22.35	0.957	23.35 ± 3.83
Spectracide™ Diquat dibromide Fluazifop-p-butyl Dicamba dimethylamine	3.17×10^{-6} 1.42×10^{-6} 1.36×10^{-6}	7.72	14.67 ± 0.73	6.5 ± 3.54	7.94	17.92	0.44 ± 0.18
Triazide™ Gamma cyhalothrin	1.78×10^{-3}	7.72	8.8 ± 1.0	252 ± 50	13.23	0.462	28.63 ± 2.46

As can be seen from table 3, the R/P ratio for the control is 0.37. This is consistent with the literature reported carbon balance of vegetation wherein the respiration to photosynthesis ratio is constrained to a narrow range from 0.4 to 0.5 (18). The rate of photosynthesis expressed in µmoles m^{-2} s^{-1} is of the same order of magnitude and consistent with literature reported values (19, 20). The P/R ratio for the leaf discs in the control experiment was significantly different from that of the leaf discs exposed to Spectracide™ ($p = 0.024$), Knockout™ ($p = 0.001$) and Triazide™ ($p = 0.0001$). The P/R ratios of leaf discs exposed to Knockout™ and Triazide™ were not significantly different ($p = 0.076$).

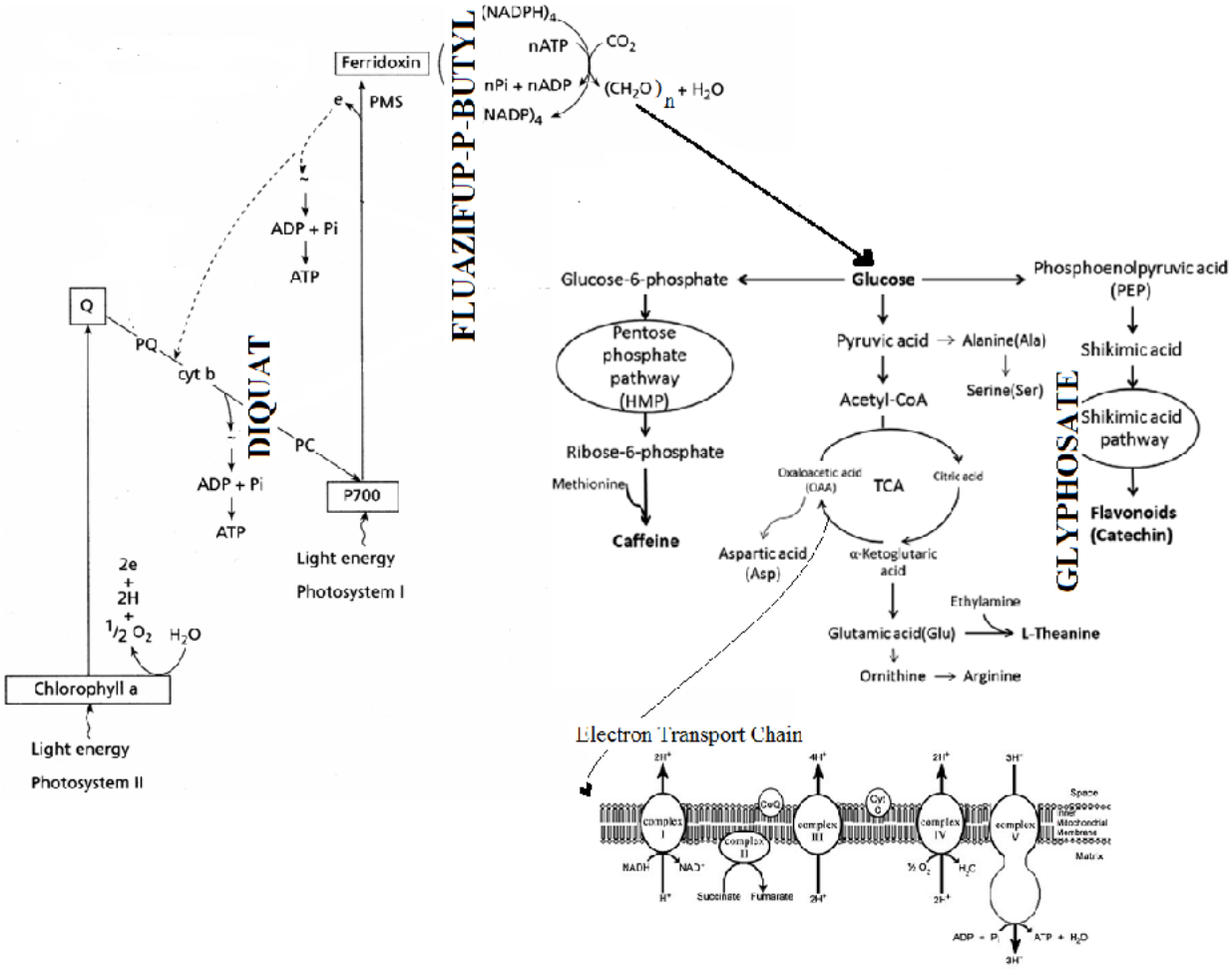
It can be inferred from Table 3 that when the P/R ratio is lesser than that of the control, electron transfer disruptions are caused before CO_2 fixation (light reactions), while when the ratio is greater than that of the control, CO_2 fixation pathways (dark reactions) are disrupted. Since the number of compounds subjected to this relationship is small, more comprehensive studies are needed to confirm this effect. If valid, a mere comparison of the P/R ratio becomes indicative of the general site of action of the compound under investigation. Furthermore, the generalized site of action of electron or pathway disruptions due to environmental changes can be determined by comparing the P/R ratio due to the environmental change to that of the control.

Table 3 also shows that it is possible to predict the site of action of any particular herbicide or chemical by determining the P/R ratio for that particular substance. Diquat is known to be electron diverter/disruptor in the light reaction of photosynthesis (21), hence the P/R ratio for a herbicide containing this compound is less than

that of the control when applied at sub-lethal levels (dilution > 1100 times). At lethal levels (dilution < 1000 times), diquat diverts photosynthetic electrons to oxygen, producing superoxide (O_2^-) radicals, which are disproportionated to H_2O_2 by superoxide dismutase. The peroxide produced readily accepts electrons from the reduced herbicide to form hydroxyl (OH^-) radicals (22). The net effect is to prevent floatation by the consumption of oxygen derived from photolyzed water (23). It was indeed found that the spinach leaf discs did not photosynthesize (float) when they were exposed to any dilutions of Spectracide™ < 1000 times with water. At dilutions > 1100X, the rate of accumulation of photolyzed water derived O_2 is greater than its consumption by diaquat to form O_2^-, H_2O_2, and OH^- so that floatation can still occur.

It can be predicted from table 3 that gamma cyhalothrin (Triazide™) modulates CO_2 fixation pathways downstream of the Calvin cycle because its P/R ratio is similar to that of glyphosate; a compound that is known to block the shikimic acid pathway. Indeed, although there have been no studies performed on its role in photosynthesis, cyhalothrin is known to be a potent inhibitor of complex I of the mitochondrial electron transport chain (ETC) (24).

Figure 8: Site of action of herbicides (adapted from reference 26)



Conclusions

The herbicide Knockout™ and the insecticide Triazide™ both significantly increased the P/R ratio of spinach leaves from that of the control. The herbicide Spectracide™, on the other hand, significantly decreased the P/R ratio.

There is a general appreciation that the equilibrium P/R ratio ranges from 2.0 to 2.5 (18), shows a decrease in stressed systems and varies with temperature (25). It does not seem to be recognized however, that the magnitude of this ratio may actually be a predictor of the specific stressed site(s) in the photosynthesis or respiratory pathways. Also unrecognized is the fact that not only is a decrease in this ratio a qualitative indicator of stress, but an increase in the ratio also signifies a disruption in the electron transport pathways that modulate both photosynthesis and respiration.

An examination of the Photosynthesis to Respiration (P/R) ratio in spinach leaves using the classic and simple leaf-disk experiment enabled the deduction of the general site of

action of herbicides/insecticides in the photosynthesis-respiration cycle. A P/R ratio of < control may imply that electron transfer is disrupted in Photosystems I and/or II; upstream of the Calvin cycle, while a P/R ratio of > control may implicate disruptions in the tricarboxylic acid cycle (TCA), mitochondrial ETC or shikimic acid pathways downstream of the Calvin cycle. It may be possible to predict the site of action with even more specificity using empirically generated relationships between the P/R ratio and the specific site of action. However, more compounds with known mechanisms of action need to be studied to validate this result.

References:

1. Steucek GL, Hill RJ, Photosynthesis: I: An assay utilizing leaf disks, Am. Biol. Teacher, 47(2), 96-99, 1985.
2. Boese SR, Huner NPA, Effect of growth temperature and temperature shifts on spinach leaf morphology and photosynthesis, Plant Physiol., 94, 1830-1836, 1990.
3. Hill JF, Govindjee, The controversy over the minimum quantum requirement for oxygen evolution, Photosynth. Res., 122(1), 97-112, 2014.
4. Ishida A, O'Donnell D, Quantification of Chlorophyll A using UV-Vis Absorbance Spectroscopy, poster date unknown, website address: https://www.hamline.edu/WorkArea/DownloadAsset.aspx?id=4295023241
5. Stern BK, Vennesland B, The effect of carbon dioxide on the Hill reaction, J. Biol. Chem., 237(2), 596-602, 1962.
6. Klimov VV, Allakhverdiev SI, Feyziev YaM, Baranov SV, Bicarbonate requirement for the donor side of photosystem II, FEBS lett., 363(3), 251-255, 1995.
7. Klimov VV, Baranov SV, Bicarbonate requirement for the water-oxidizing complex of photosystem II, Biochim. Biophys. Acta., 1503(1-2), 187-196, 2001.
8. Huner NPA, Oquist G, Sarhan F, Energy balance and acclimation to light and cold, Trends in plant sci., 3(6), 224-230, 1998.
9. Fuerst EP, Norman MA, Interaction of herbicides with photosynthetic electron transport, Weed Sci., 39(3), 458-464, 1991.
10. Duke SO, Powles SB, Glyphosate: a once-in-a-century herbicide, Pest manage. Sci., 64, 319-325, 2008.
11. Servaites JC, Tucci MA, Geiger DR, Glyphosate effects on carbon assimilation, ribulose biphosphate carboxylase activity, and metabolite levels in sugar beet leaves, Plant Physiol., 85, 370-374, 1987.
12. Liu DF, Ai GM, Zheng QX, Liu C, Jiang CY, et.al., Metabolic flux responses to genetic modification for shikimic acid production by Bacillus subtilis strains, Microbial Cell Factories, 13, 40-50 , 2014.

13. Siehl D, Inhibitors of EPSPS synthase, glutamine synthetase and histidine synthesis. In Roe R, Burton J, Kuhr R, eds. Herbicide activity: toxicology, biochemistry and molecular biology . Amsterdam: IOS Press, 37–67.
14. De María N, Becerril JM, García-Plazaola JI, Hernandez A, De Felipe MR, Fernandez-Pascual M, New insights on glyphosate mode of action in nodular metabolism: Role of shikimate accumulation. Journal of Agricultural and Food Chemistry 54, 2621–2628, 2006.
15. Vivancos PD, Driscoll SP, Bulman CA, Ying L, Emami K, Treumann A, et.al., Perturbations of Amino Acid Metabolism Associated with Glyphosate-Dependent Inhibition of Shikimic Acid Metabolism Affect Cellular Redox Homeostasis and Alter the Abundance of Proteins Involved in Photosynthesis and Photorespiration, Plant Physiol., 157, 256-268, 2011.
16. Ireland CR, Percival MP, Baker NR, Modification of the induction of photosynthesis in wheat by glyphosate, an inhibitor of amino acid metabolism. J Exp Bot., 37, 299–308, 1986.
17. Byott GS, Leaf air space systems in C3 and C4 species, New Phytol., 76, 295-299, 1976.
18. Oijen MV, Schapendonk A, Hoglind M, On the relative magnitudes of photosynthesis, respiration, growth and carbon storage in vegetation, Ann. Bot., 105, 793-797, 2010.
19. Yamori W, Noguchi K, Terashima I, Temperature acclimation of photosynthesis in spinach leaves: analyses of photosynthetic components and temperature dependencies of photosynthetic partial reactions, Plant, Cell and Environment, 28, 536-547, 2005.
20. Downton WJS, Grant WJR, Robinson SP, Photosynthetic and stomatal responses of spinach leaves to salt stress, Plant Physiol., 77, 85-88, 1985.
21. Farrington JA, Ebert M, Land EJ, Fletcher K, Bipyridylium quaternary salts and related compounds. V. Pulse radiolysis studies of the reaction of paraquat radical with oxygen. Implications for the mode of action of bipyridyl herbicides, Biochim. Biophys. Acta, 14(3), 372-381, 1973.
22. Upham BL, Hatzios KK, Counteraction of paraquat toxicity at the chloroplast level, Z. Naturforsch, 42c, 824-828, 1987.
23. Boger P, Kunert KJ, Phytotoxic action of Paraquat on the photosynthetic apparatus, Z. Naturforsch, 33c, 688-694, 1978.
24. Gassner B, Wuthrich A, Scholtysik G, Solioz M, The pyrethroids Permethrin and cyhalothrin are potent inhibitors of the mitochondrial complex I, J. Pharmacol. Exp. Ther., 281(2), 855-860, 1997.
25. Ikkonen EN, Shibaeva TG, Titov AF, Influence of daily short-term temperature drops on respiration to photosynthesis ratio in Chilling sensitive plants, Russian J. Plant Physiol., 65(1), 78-83, 2018.
26. Ashton FM, Crafts AS, 1981, Mode of action of Herbicides, 2nd ed., New York, John Wiley and Sons.