applied sciences

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Three-Dimensional Analysis of the Impact of Different Concentrations of Glyphosate on the Growth of Cocoa (*Theobroma cacao*)

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Abstract: Ecuadorian cocoa possesses important organoleptic characteristics such as aroma and flavor, called fine and aromatic cocoa. The objective of this study was to evaluate the initial growth responses of young cocoa seedlings to glyphosate in a dose progression in 45 cocoa plants (5 months old), which were transplanted into pots with substrate adjusted to pH 6.0–6.5. Glyphosate doses (0 to 904 g e.e. ha^{-1}) were applied every two weeks, evaluating the impact at 30 and 60 days post-application. Data on shikimate accumulation parameters, chlorophyll content and PSII quantum efficiency were subjected to multivariate analysis using a three-dimensional scatter plot. The results indicated that high concentrations of glyphosate contributed to higher shikimate concentration and lower PSII quantum efficiency. The findings for the variables crop damage, stem height and stem diameter were evaluated by ANOVA. Similarities were reported between the results of the variables height and diameter, and significant differences ($p < 0.05$) in the variable crop damage for all treatments were also reported. In terms of phytotoxic reaction and growth parameters, the most efficient treatment was DO4, since the seedlings with this dosage showed a low percentage of damage (10%) and the best indices in terms of height and diameter. The least efficient treatment was D15. The control plants (DO1) showed a crop damage of >50% because the absence of control favored weed proliferation. These indications highlight the need to adjust glyphosate doses according to the specific needs of each crop and the development stage of the plant in order to reduce negative effects and maximize potential benefits.

Keywords: *Theobroma cocoa*; seedlings; dose-response; glyphosate; phytotoxicity

Citation: Valenzuela-Cobos, J.D.; Guevara-Viejó, F.; Galindo-Villardón, P.; Vicente-Galindo, P. Three-Dimensional Analysis of the Impact of Different Concentrations of Glyphosate on the Growth of Cocoa (*Theobroma cacao*). *Appl. Sci.* **2024**, *14*, 8180. https://doi.org/10.3390/app14188180

Academic Editor: Daniela Gwiazdowska

Received: 21 May 2024
Revised: 5 July 2024
Accepted: 22 July 2024
Published: 11 September 2024

1. Introduction

Cocoa (*Theobroma cacao*) is a tree native to countries in America, Africa, Asia and Oceania located in equatorial and tropical latitudes which have a tropical-humid environment [1]. The fruit of the tree consists of a cob containing between 20 and 50 seeds with highly valued organoleptic characteristics, such as aroma and flavor. These kernels are the main ingredient in the chocolate industry and are used in the production of a wide range of manufactured products, including cosmetics, beverages and pharmaceuticals [2]. In economic terms, cocoa is an agricultural product of vital relevance for the world market. According to ICCO [3], during the period 2019–2020 world production totaled 4.7 million tons. Of this annual production, 18.4% (0.9 million tons) was generated in Latin America, with the Ivory Coast (2.1 million tons) and Ghana (0.8 million tons) leading production, followed by Ecuador (0.32 million tons). In Ecuador, cocoa cultivation is the economic mainstay of many rural families that are dedicated solely to this activity, especially in the foothills of the Andes Mountains [4,5]. According to a report issued by the National

Finance Corporation (CFN) [6], the harvested area increased by 13% between 2013 and 2016, contributing 12% to the national agricultural Gross Domestic Product (GDP) and 1.50% to the total GDP in 2019.

A limiting factor in agricultural production is the presence of so-called "weeds". These weeds act at the same tropical level as crops, competing for soil nutrients, water and solar energy, and serve as a refuge for thriving pests. In plantations of Theobroma cacao, allelopathy secreted by weeds has the potential to decrease total bean yield and quality by up to 30%. To control the growth of these unwanted hosts, cultural techniques, such as tillage, and chemical methods, such as glyphosate, have been implemented, of which the use of herbicides has shown greater efficacy [7,8].

Glyphosate, a broad-spectrum herbicide, acts as the predominant chemical component in numerous systemic herbicides intended for the management of a wide range of annual and perennial plant species [9]. This compound regulates weed growth by interfering with the production of aromatic amino acids, which are essential for the synthesis of essential proteins in the primary and secondary metabolic processes of the plants affected by its action [9,10]. Glyphosate is currently the most widely used herbicide worldwide and is considered the safest for human health under the technical conditions indicated: direct spraying on weeds, use in concentrations between 1 and 3% and low doses and low exposure time for the person handling the herbicide [11]. Scientists and regulatory authorities globally, including the US EPA, have evaluated the carcinogenic potential of glyphosate since 1985 in animals and concluded that it does not exhibit genotoxicity within the suggested dose range. For this reason, the use of the pesticide continues to demonstrate safety for the health of farmers, while increasing crop yield and quality, generating higher profits for farmers and the agricultural industry [12,13].

Although pesticides help protect the cocoa plant, their indiscriminate use can cause damage such as stunting or even death. Adu-Yeboah et al. (2023) demonstrated that high doses of glyphosate result in significant accumulation of shikimate and decreased photosynthesis, which affects the health and growth of cocoa [14]. On the other hand, Kayode Olufemi et al. (2020) observed that moderate doses may be less harmful, suggesting that glyphosate, applied with caution, could be part of a weed management regime without significant detrimental effects [15]. In Ecuador, there is little scientific research on the collateral impact of glyphosate application for weed control on cocoa plantations of commercial interest. In this context, it is important to examine the developmental responses of Ecuadorian cocoa seedlings in the presence of different doses of glyphosate, using multivariate statistical analysis tools to reveal complex relationships between the parameters under study, which cannot be visualized with the naked eye.

The multivariate data analysis revolution began to spread in the 1990s. Data mining techniques, such as three-dimensional scattering algorithms, involve the synthesis of large volumes of information in a lower-dimensional space. The graphical representation is carried out in a two-dimensional environment expressing points for observations and vectors for variables. The main advantage of this type of approach, in contrast to traditional statistical methods of classification/sorting, is the localization and understanding of patterns, trends and sequences hidden in the data. The application of three-dimensional scatter plots in agronomic data promises to positively transform decision making in agriculture, leading to significant improvements in the efficiency of weed control treatments and, consequently, in productivity [16].

The main objective of this paper was to implement a three-dimensional dispersion analysis as a statistical visualization tool to evaluate the influence of different glyphosate-based treatments on 45 cocoa plants (five months old).

2. Materials and Methods

2.1. Seedling Selection and Sample Preparation

This experimental study was conducted at the "Esmeraldas" farm, located in the city of Montalvo, province of Los Ríos, Ecuador. Five-month-old Theobroma cacao plants of the

CCN-51 variety grown under in situ conditions on this property were used for this purpose. Forty-five seedlings were selected and distributed in a sampling area of approximately 400 m^2, all free of visible diseases, nutritional deficiencies or any other physical anomaly. The characteristics considered for selection were similarity in size, height, structure and general physical condition. The selected seedlings were labeled and registered for follow-up and subsequent analysis.

Seedlings were transplanted in 7 L pots with a substrate composed of a mixture of 40% compost, 30% coconut fiber, 20% coarse sand and 10% vermiculite. This substrate was adjusted to a pH of 6.0–6.5 by adding agricultural lime. Samples of the substrate (500 g) were collected for the determination of physicochemical parameters under standardized methodology. The quantification of organic matter was performed following the Walkley–Black method, whose approach is based on oxidation (potassium dichromate) and acidity (sulfuric acid), followed by titration with ferrous sulfate. Phosphorus availability was extracted by Olsen's method, using sodium bicarbonate, molybdate reagents and ascorbic acid, with absorbance at 800 nm. The number of exchangeable cations (K, Ca, Mg) was measured by the ammonium acetate method, while the exchangeable acidity (H+Al) was calculated by titration after extraction with 1 N KCl. The sum of exchangeable bases (SB) resulted from the total amount of exchangeable basic cations K, Ca and Mg. The effective cation exchange capacity (T) resulted from the sum of SB and H+Al. Finally, base saturation (V) was calculated as the ratio of the sum of exchangeable bases to the effective cation exchange capacity. The report revealed the following contents: organic matter (M.O.) = 25.0 g.·dm^{-3}, P (resin) = 30.0 mg·dm^{-3}, K = 2.0 mmolc·dm^{-3}, Ca = 25.0 mmolc·dm^{-3}, Mg = 15.0 mmolc·dm^{-3}, H+Al = 20.0 mmolc·dm^{-3}, SB = 42.0 mmolc·dm^{-3} and T = 62.0 mmolc·dm^{-3}, with a base saturation (V) of 68% [17].

2.2. Treatments and Experimental Design

The experiment consisted of the direct spraying of different doses of glyphosate (Vargas brand, Quito, Ecuador) on transplanted cocoa seedlings. A total of 15 treatments were used, and the doses of each were assigned randomly, combining amounts implemented in previous studies using glyphosate, ranging from 0 to 904 g e.e. ha^{-1}. They were applied by foliar spraying, simulating an accidental supply and ensuring foliar coverage. The process was repeated with a frequency of 15 days for 2 months. Plant evaluations were carried out in two instances: on Days 30 and 60 after the first herbicide application [18].

2.3. Measurement of Variables

At the end of the experiment (60 days), the following variables were measured: phytotoxic crop damage reactions (%) and plant biochemical reactions such as shikimate accumulation (μg/g). To assess plant photosynthetic health, chlorophyll content (mg/m^2) and PSII quantum efficiency (Fv/Fm) were calculated. Subsequently, growth parameters such as plant height (m) and stem diameter (cm) were measured. These variables allowed a comprehensive evaluation of the effect of glyphosate on various physiological and growth aspects of cocoa seedlings. The data were used to confirm the homogeneity of the seedlings collected and to correlate the results of the analyses with the physical characteristics of the plants.

2.3.1. Measurement of Crop Damage

In young cocoa seedlings, visual evaluations of phytotoxicity caused by glyphosate were carried out 60 days after application. A herbicide tolerance scale from 1 to 9 developed by the European Weed Research Council (EWRC) was used for this purpose. The scale ranked plant response as follows: (1) no visible effects; (2) very mild effects, such as stunting or dwarfing and chlorosis; (3) mild effects with stunting and chlorosis, but these effects can be reversed; (4) moderate effects of chlorosis and/or stunting, with most being possibly reversible; (5) severe chlorosis and/or stunting; (6) more severe damage; (7) increased severity of damage; (8) even greater severity of damage and (9) plant death [19].

2.3.2. Photochemical Activity of the PSII

To evaluate quantum efficiency, PSII chlorophyll fluorescence was measured using a portable fluorometer (PAM 2100, WALZ, Effeltrich, Germany). Measurements were performed on fully expanded leaves of the fifteen cocoa seedlings selected for each dose. The slides were adapted to a photon flux density (FFD) of 1000 μmol·m^{-2} s^{-1}, and instantaneous maximum (F'm) and stable (F) fluorescence measurements were recorded, following the methodology of Genty et al. [20].

2.3.3. Shikimate Accumulation

To evaluate the accumulation of Shikimate, plant material was crushed in a mortar with liquid nitrogen to a fine powder, then weighed and stored at −80 °C. To determine the amount of shikimic acid by HPLC, 900 μL of 0.25 N HCl was placed in a microcentrifuge tube with 100 mg of crushed plant tissue and allowed to stand for 5 min. Subsequently, the extracts were vortexed for 5 min and ultrasonication was performed for a period of 8 min at 25 °C (2210 Branson Ultrasonicator, Markham, OH, Canada). The cellular components were then separated by centrifuging at maximum speed in a benchtop microcentrifuge for 10 min. The sediment was discarded and the clear liquid was passed through a 0.22 μm syringe filter (MilliporeSigma™ Millex®, San Jose, CA, USA) for direct HPLC analysis of shikimate according to the methodology of Kretzmert et al. [21].

2.3.4. Chlorophyll Content

Leaf tissue samples were collected in the morning between 8:00 and 10:00 am to ensure consistency in moisture content and metabolic activity. The collected leaves were immediately placed in sealed plastic bags and stored on ice for transport to the laboratory. Fifty grams of sample were used. They were crushed with 80% acetone in a biker until a homogeneous mixture was obtained. The mixture was centrifuged to separate the liquid extract (containing chlorophyll) from the solid residues. The extract was analyzed with a spectrophotometer. The absorbance was measured at different specific wavelengths of 666 nm, 649 nm and 470 nm using 95% ethanol as a target.

Chlorophyll content in cocoa seedlings was determined following Arnon's equations by measuring the absorbance of the sample at different wavelengths [22]. The total chlorophyll content, as seen in Equation (3), is the sum of chlorophyll a (Chl a) content (Equation (1)) and chlorophyll b (Chl b) content (Equation (2)). In the formulas, A666 refers to the absorbance measured at 666 nm and A649 to the absorbance measured at 649 nm:

$$c_a = 13.77 * A_{666} - 4.63 * A_{649} \quad (1)$$

Equation (1). Chlorophyll a (Chl a)

$$\boldsymbol{c_b} = 24.63 * \boldsymbol{A}_{649} - 6.66 * \boldsymbol{A}_{666} \quad (2)$$

Equation (2). Chlorophyll b (Chl b)

$$\mathbf{c_t} = 7.11 * \mathbf{A}_{649} + 20 * \mathbf{A}_{666} \quad (3)$$

Equation (3). Total Chlorophyll Content

2.4. Statistical Analysis

A three-dimensional scattering analysis was performed to track the patterns between biochemical reactions (shikimate accumulation) and photosynthetic health parameters (chlorophyll content, PSII quantum efficiency) of cocoa plants after application of different doses of glyphosate. Multivariate analysis was performed with R software, version 4.1.1 (R Core Team, Vienna, Austria). In addition, the results of phytotoxic reaction (crop damage) and growth parameters (height, diameter) were studied by analysis of variance (ANOVA) to evaluate the significance of differences at the $p < 0.05$ level. Subsequently, Duncan's test was applied ($p < 0.05$). This process was carried out in the software (SPSS ver. 26.0.0.0.0).

2.5. Three-Dimensional Scatter Plot

Three-dimensional scatter plots are an extension of two-dimensional scatter plots and are used to identify possible correlations between observations and variables in a data set on a single plot of three mutually perpendicular axes, where each variable is interpreted as a Cartesian coordinate vector. Cartesian coordinate vectors are defined such that each element of the matrix is the inner product of the vectors corresponding to its rows and columns [23].

The analysis of a three-dimensional scatter plot goes beyond data visualization: it also involves the understanding of different statistical tools involved, such as three-dimensional Euclidean space and measures of central tendency. The exploration begins with the definition of a three-dimensional Euclidean space, with a set of the tern (x, y, z) of real numbers, formally defined as $\mathbb{R}^3$. The observations represent the relationship between the three variables, where f is a function of two variables, $z = f(x,y)$.

The Euclidean distance between two observations (Equation (4)) indicates the distance between the influence of two different treatments $P(p_1, q_1, r_1)$ and $Q(p_1, q_1, r_1)$ [24].

$$d(P,Q) = \sqrt{(x_2 - x_1)^2 + (y_2 - y_1)^2 + (z_2 - z_1)^2} \quad (4)$$

Equation (4). Euclidean distance fitted to a three-dimensional plane.

The calculation of the mean of each variable (Equation (5)) allows us to understand the concentration of the data in each dimension and to detect possible biases in the data [25].

Mean for variable "*xa*"

$$\overline{a} = \frac{1}{n} \sum_{i=1}^{n} a_i \quad (5)$$

Equation (5). Average of the variables in a three-dimensional plane.

Where:

- $\overline{a}$ represents the mean of the variable.
- a_i represents each individual observation of the variable.
- n is the total number of observations.
- a can be x, y, z depending on the variable of interest.

Covariance (Equation (6)) measures the linear relationship between two variables, indicating the degree to which these two change together. Given a positive covariance, the variables tend to increase; given a negative covariance, one variable tends to increase while the other variable tends to decrease and given a covariance close to zero, a weak linear relationship between the variables is inferred.

Covariance between variable "*a*" and variable "*b*".

$$Cov(a,b) = \frac{1}{n-1} \sum_{i=1}^{n} (a_i - \overline{a})\left(b_i - \overline{b}\right) \quad (6)$$

Equation (6). Calculation of covariance in a three-dimensional plane.

Where:

- $\overline{a}$ and $\overline{b}$ represents the mean of the variable.
- a_i and b_i are the individual observations of the variables a and b, respectively.
- n is the total number of observations.
- a and b can be any pair of the variables x, y, or z to compute $Cov(x,y)$, $Cov(x,z)$ or $Cov(y,z)$.

Pearson's correlation (Equation (7)) is another measure of association, which allows us to denote how related two variables are to each other [26]. For a three-dimensional plane, the Pearson correlation is calculated in pairs.

Correlation between variable "a" and variable "b".

$$r_{a,b} = \frac{Cov\ (a, b)}{\sigma_a \sigma_b} \tag{7}$$

Equation (7). Calculation of Pearson's correlation in a three-dimensional plane. Where:

- $\boldsymbol{r_{a,b}}$ represents the correlation coefficient between the variables $\boldsymbol{a}$ and $\boldsymbol{b}$.
- $\boldsymbol{Cov(a, b)}$ is the covariance between the variables $\boldsymbol{a}$ and $\boldsymbol{b}$.
- $\boldsymbol{n}$ are the standard deviations of the variables $\boldsymbol{a}$ and $\boldsymbol{b}$, respectively.
- $\boldsymbol{a}$ and $\boldsymbol{b}$ can be any pair of the variables x, y, or z to compute $\boldsymbol{r_{x,y}}$, $\boldsymbol{r_{x,z}}$ or $\boldsymbol{r_{y,z}}$.

3. Results and Discussion

The purpose of this study was to expose, by means of a three-dimensional scatter plot and ANOVA, the complex effects of different doses of glyphosate on various parameters of cocoa seedling development. The treatments used in the research, with their respective doses, were the following: DO1 (0 g e.e. ha^{-1}); DO2 (25 g e.e. ha^{-1}); DO3 (50 g a.e. ha^{-1}); DO4 (80 g e.e. ha^{-1}); DO5 (144 g e.e. ha^{-1}); DO6 (288 g e.e. ha^{-1}); DO7 (325 g e.e. ha^{-1}); DO8 (432 g e.e. ha^{-1}); DO9 (535 g e.e. ha^{-1}); DO10 (625 g e.e. ha^{-1}); DO11 (700 g e.e. ha^{-1}); DO12 (768 g e.e. ha^{-1}); DO13 (803 g e.e. ha^{-1}); DO14 (864 g e.e. ha^{-1}) and DO15 (904 g e.e. ha^{-1}).

Three plants were treated for each treatment and the characteristics of each group were evaluated after the application of the doses.

3.1. Multivariate Statistics for the Analysis of Photosynthetic Health and Biochemical Reaction of Seedlings

Figure 1 expresses the three-dimensional scatter plot that relates to the patrons existing between the parameters of photosynthetic health and biochemical reaction. Plane 1, corresponding to the horizontal variable, represents the accumulation of shikimate; Plane 2, belonging to the vertical variable, denotes the quantum efficiency of PSII and Plane 3, corresponding to the depth variable, collects information about chlorophyll content. The Euclidean distance allowed us to quantify how far apart the different treatments are according to the combination of their effects on the plants. The graph reduced the complexity of the multivariate information and reflects the normal distribution of 15 points, concerning the number of treatments, around the three Cartesian coordinates (x, y and z).

In this analysis, we observed that high concentrations of glyphosate, particularly from DO5 treatment, showed higher shikimate accumulation and lower chlorophyll content, contributing to lower quantum efficiency. On the other hand, lower concentrations of glyphosate, from DO1 to DO4, exhibited the highest ranges of PSII quantum efficiency and chlorophyll content. Plant performance responses were not perfectly positively correlated with applied doses, which may be attributed to the influence of other factors, such as genetic variability and the interaction of allelopathy-producing microorganisms, among others. Deterioration in quantum efficiency was found to be associated with a reduction in chlorophyll content, which implies a decrease in the plant's ability to capture and use light energy effectively. As a cluster analysis, it was determined that the DO4 (80 g e.e. ha^{-1}) treatment was the one that showed the highest balance among the evaluated parameters, emphasizing the higher PSII quantum efficiency and the higher chlorophyll content.

Similar studies showed that glyphosate can interfere with the enzyme EPSPS, which is essential for amino acid synthesis in plants, which could be a contributing factor to the observed effects on cocoa growth [27]. In another study, De Maria [28] indicated that the phytotoxic effect on tissues affects young leaves more due to the degree of increased shikimate in the tissues. The herbicidal activity of glyphosate lies in its ability to inhibit the EPSPS enzyme, blocking the shikimate pathway [29]. This pathway is essential for the biosynthesis of aromatic compounds in plants. In the study of Mueller et al. [30], shikimate was found to accumulate in glyphosate-resistant and glyphosate-sensitive Conyza canadensis plants. This phenomenon suggests that shikimate accumulation may not be directly related to glyphosate resistance, indicating that other mechanisms may be involved in the resistance of some plants to this herbicide [31].

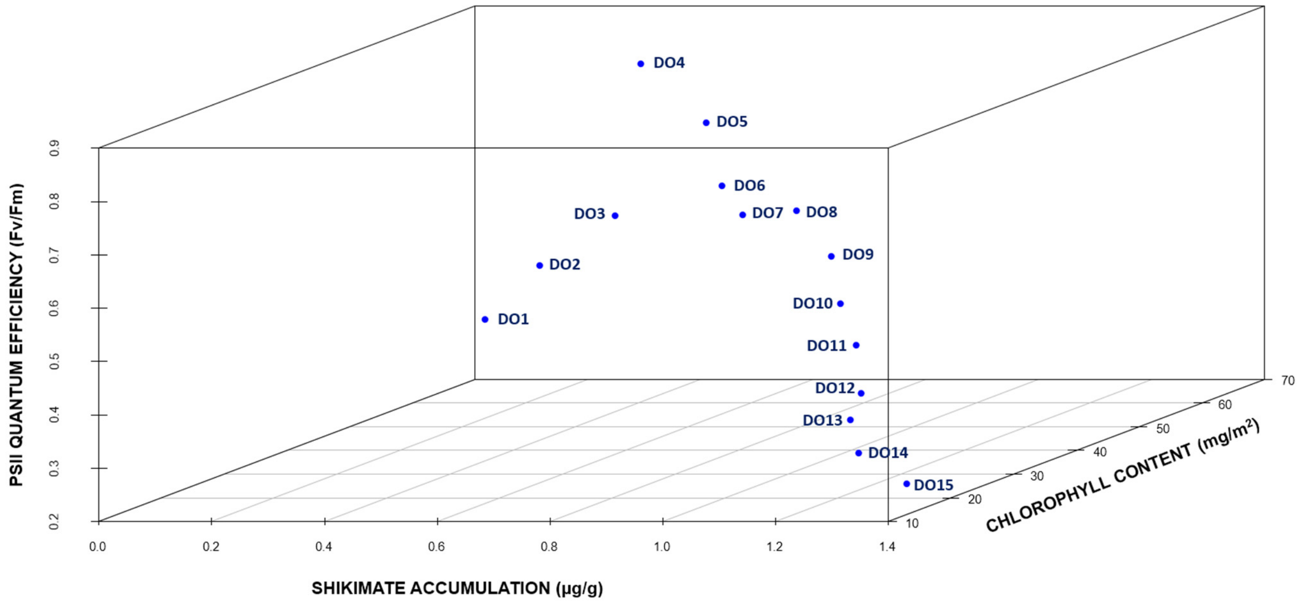

Figure 1. Three-dimensional scatter plot of biochemical reactions and photosynthetic health of cocoa seedlings as a function of different doses of glyphosate. Euclidean distance was calculated considering the following variables: shikimate accumulation, chlorophyll content and PSII quantum efficiency.

For Maxwell and Johnson [32], the quantum efficiency of photosynthesis, especially the PSII parameter (Φ), is crucial to evaluate the response of plants to abiotic stresses such as drought, salinity and temperature extremes. These environmental conditions are important to determine and control for effective plant development. Quantum efficiency is fundamental for the design of lighting systems in controlled agriculture, such as greenhouses or vertical farming systems, where artificial light is used to maximize photosynthesis. In another study, Mateos-Naranjo et al. [33] indicated that exposure to glyphosate can cause alterations in the ultrastructure of chloroplasts in eucalyptus plants, which could negatively influence quantum efficiency. Glyphosate can inhibit chlorophyll synthesis by interfering with the production of key precursors, leading to a decrease in the ability of plants to capture light energy and effectively photosynthesize. Other studies showed that herbicide application to soybean significantly reduced chlorophyll content, which is related to a lower rate of photosynthesis and compromised plant growth [34]. Chlorophyll is not only essential for light absorption during photosynthesis but also plays a key role in protecting plant tissues against photooxidative damage. As chlorophyll concentration decreases, plants suffer a reduction in biomass and their overall capacity for growth and development.

A similar study by Zobiole et al. [35] observed that soybean plants treated with glyphosate showed a significant reduction in PSII quantum efficiency and a decrease in chlorophyll concentration. On the other hand, the study of Adu-Yeboah et al. [14] indicated that a glyphosate dosage of 360 g a.e. ha^{-1} or more resulted in >60% leaf damage and a more than 10-fold increase in shikimate accumulation. On the other hand, in the study of Adu-Yeboah et al. [14], whose experimentation was carried out under field conditions in Ghana, the sensitivity of cocoa plantations to spraying with different doses of glyphosate was also indicated. The authors found that glyphosate doses ≥360 g a.e. ha^{-1} caused leaf damage to more than 60% of plant structures, a greater than 10-fold increase in shikimate accumulation and a significant decrease in chlorophyll content. These findings highlight the need to develop and promote the use of herbicides with more specific active ingredients in order to minimize the collateral impact on non-target plants.

3.2. Analysis of Variance (ANOVA) for the Evaluation of Phytotoxic Reaction and Growth Parameters (Height, Diameter)

Table 1 shows the results of the investigation with respect to phytotoxic reaction and growth parameters. The findings indicated that there were similarities between treatments in terms of crop damage, height and stem diameter. Higher concentrations of the herbicide inhibited plant development, while lower concentrations showed fewer adverse effects. The means obtained allowed establishing that the most efficient treatment was DO4, using a dose of 80 g e.a. ha^{-1} since the seedlings treated with this dosage showed a low percentage of damage and the best indices in terms of height and diameter. The least efficient treatment was D15 because the seedlings with this dosage showed a lower development and the second-highest percentage of damage to the crop.

Table 1. Effect of glyphosate application on growth parameters of cocoa seedlings.

Treatment	Crop Damage (%)	Height (m)	Diameter (cm)
DO1	$23.00^{b} \pm 2.64$	$0.66^{ce} \pm 0.04$	$1.10^{d} \pm 0.11$
DO2	$15.33^{a} \pm 0.28$	$0.70^{d} \pm 0.02$	$1.13^{de} \pm 0.12$
DO3	$12.00^{a} \pm 2.64$	$0.75^{f} \pm 0.02$	$1.18^{fg} \pm 0.15$
DO4	$10.00^{a} \pm 1.13$	$0.83^{h} \pm 0.05$	$1.23^{g} \pm 0.03$
DO5	$25.00^{b} \pm 1.56$	$0.77^{fg} \pm 0.02$	$1.16^{fg} \pm 0.03$
DO6	$33.00^{c} \pm 1.73$	$0.75^{eg} \pm 0.03$	$1.10^{de} \pm 0.04$
DO7	$50.16^{d} \pm 0.28$	$0.78^{e} \pm 0.11$	$1.15^{ef} \pm 0.04$
DO8	$53.41^{de} \pm 2.96$	$0.66^{de} \pm 0.03$	$1.05^{cd} \pm 0.06$
DO9	$56.33^{e} \pm 1.15$	$0.64^{cd} \pm 0.03$	$1.00^{cd} \pm 0.13$
DO10	$53.50^{de} \pm 1.56$	$0.65^{cd} \pm 0.04$	$1.03^{cd} \pm 0.09$
DO11	$55.00^{de} \pm 1.29$	$0.62^{ac} \pm 0.06$	$0.95^{bc} \pm 0.04$
DO12	$72.33^{f} \pm 1.51$	$0.60^{bc} \pm 0.07$	$0.93^{b} \pm 0.05$
DO13	$75.66^{f} \pm 1.08$	$0.57^{bc} \pm 0.05$	$0.90^{b} \pm 0.09$
DO14	$71.66^{f} \pm 1.88$	$0.53^{ab} \pm 0.11$	$0.91^{b} \pm 0.07$
DO15	$75.00^{f} \pm 1.21$	$0.50^{a} \pm 0.07$	$0.85^{a} \pm 0.09$

Note: The results of crop damage, stem height and diameter data are presented according to each treatment applied; Different letters within a column indicate significant differences ($p < 0.05$).

Leaf damage was measured in percentages, showing necrosis, chlorosis and wilting of the plant. Cocoa plants belonging to the control treatment (DO1) showed a crop damage of >50% because the absence of control favored the proliferation of weeds. A similar result occurred with the study of Ríos [31]: the control proved to be lower due to the high density of weeds competing for light, nutrients and space. The doses of 803 and 904 g e.e. ha^{-1} caused leaf scorch in young leaves, as well as changes in the shape of leaf margins and depigmentation along the leaflets. After application of doses of 144 and 288 g a.e. ha^{-1}, cocoa seedlings exhibited depigmentation on older leaves, abnormal coloration, wavy blade-shaped edges and blighted leaves but produced healthy new leaves 60 days after application. Although the symptoms were worrisome, the plants recovered.

The study by Martínez Ramírez et al. [32] indicated that the highest dose of 0.200 $kg \cdot ha^{-1}$ caused significant phytotoxic effects. Initially, phytotoxicity symptoms, such as chlorosis, were severe, reaching a grade 4 but then the symptoms decreased to a grade 3 after 45 days of application and were completely eliminated after 60 days, leaving no residual effects on the treated plant population. This observation is important because it shows how the dose of a chemical can affect plant health in the short term, but also indicates long-term recovery if conditions permit [33].

A similar study was conducted by Konrad et al. [36], in which they observed that low doses of glyphosate did not interfere with plant growth for the BRS-Maués cultivar. In addition, the morphology of plants exposed to high doses of this herbicide was also affected, with a reduction in stem height and diameter. Helander, et al. [37] observed that in trees and shrubs the herbicide can significantly reduce both height and stem diameter, especially in young plants. This is due to inhibition of essential amino acid synthesis and general damage to photosynthetic capacity.

In contrast, Magalhães Farias et al. [38] found that insufficient g a.e. ha^{-1} of glyphosate on H. brasiliensis caused damage by reducing plant development in height and diameter. However, they recovered the growth of these plants within 180 days after application. In the statistical analysis of Neves et al. [39], they observed a 15% increase in the height variable at doses of 18–33.5 g a.e. ha^{-1} and maximum responses of 16% at doses of 27 g a.e. ha^{-1}. Glyphosate is a broad-spectrum herbicide that can function as a plant growth regulator, provided that the correct doses are applied with precision. Similarly, Silva et al. [40] reported that, at low doses, the herbicide can stimulate an increase in plant height, coinciding with the results found in this study.

Barbosa et al. [41] found that this herbicide on coffee seedlings can reduce their growth, with more pronounced effects when the herbicide is absorbed by the leaves. These effects include reductions in height, root length and total dry mass. In the study by Gonçalves et al. [42], high doses of glyphosate were found to cause severe intoxication in coffee seedlings, resulting in significant reductions in height, leaf area, and shoot and root biomass. However, the use of foliar fertilizers can mitigate the negative effects of these doses. It is important to know the dose-response effect of the plant to glyphosate because extremely high doses affect foliar and root uptake. This study could be relevant to improve management and dosage practices in agricultural applications to minimize plant damage and control pests or diseases.

4. Conclusions

In terms of photosynthetic health, high rates of shikimate accumulation, specifically from DO5 treatment, contributed to a detrimental effect on seedlings, contributing to lower quantum efficiency. Meanwhile, lower concentrations of glyphosate, from DO1 to D04, exhibited higher PSII quantum efficiency and higher chlorophyll content.

In terms of phytotoxic reaction and growth parameters, the most efficient treatment was DO4 (80 g e.e. ha^{-1}), since the seedlings with this dosage showed a low percentage of damage (10%) and the best indices in terms of height and diameter. The least efficient treatment was D15.

The cocoa plants belonging to the control treatment (DO1) showed a crop damage of >50% due to the fact that the absence of control favored the proliferation of weeds; this allows us to deduce that the application of glyphosate is necessary.

Author Contributions: Conceptualization, J.D.V.-C. and F.G.-V.; formal analysis, J.D.V.-C. and F.G.-V.; investigation, F.G.-V.; methodology, J.D.V.-C. and F.G.-V.; supervision, P.G.-V. and P.V.-G.; writing—original draft, J.D.V.-C., F.G.-V., P.G.-V. and P.V.-G.; writing—review and editing, P.G.-V. and P.V.-G. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the Universidad Estatal de Milagro (UNEMI) Scholarship.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The original contributions presented in the study are included in the article, further inquiries can be directed to the corresponding author.

Acknowledgments: The authors are grateful to the Universidad Estatal de Milagro (UNEMI).

Conflicts of Interest: The authors declare no conflicts of interest.

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