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Do sub-doses of glyphosate and 2,4-D cause hormesis in the pineapple plants? Insights into the response of the pineapple plants to this phenomenon

Glauber Ferreira Barreto[1]*, José Maria Arcanjo Alves[1], Paulo Roberto Ribeiro Rocha[1], Thaís Santiago Castro[1], Deyse Cristina Oliveira da Silva[1], Sonicley da Silva Maia[1], José de Anchieta Alves de Albuquerque[1], Leandro Torres de Souza[1]

[1]Universidade Federal de Roraima, Boa Vista - RR, Brazil
*Corresponding author, e-mail: glauberfbarreto@gmail.com

Abstract

The slow growth of the pineapple (*Ananas comosus (L.) Merr*.) during the vegetative phase prolongs the crop cycle, resulting in higher production costs. When used in low doses, some herbicides can stimulate plant growth, a phenomenon known as hormesis. The aim of this study was to determine the effects of sub-doses of glyphosate and 2,4-dichlorophenoxyacetic acid (2,4-D) on growth in the Pérola cultivar of the pineapple. The treatments under study consisted of glyphosate applications of 0, 1.8, 3.6, 7.2, 18, 36, 72, 180, 360 and 720 g a.e. ha-1, and 2,4-D applications of 0, 1.68, 3.35, 6.70, 16.75, 33.5, 67, 167.5, 335 and 670 g a.e. ha-1. The experiments were completely randomised with four replications. The plants were grown in the greenhouse in pots with a capacity of 8 dm3 substrate. Growth variables were evaluated in the plants, together with the total and partitioned accumulated biomass (leaf, stem and root). The data were submitted to analysis of variance and regression. The pineapple showed greater phytotoxicity from the glyphosate herbicide at applications ranging from 18 to 720 g a.e. ha-1 and from 2,4-D in the range of 167.5 to 670 g a.e. ha-1. The hormesis effect was evidenced by the 32.25% increase in root fresh weight at the dose of 1.8 g a.e. ha-1 glyphosate. However, the slow growth of the pineapple was not overcome by the hormesis effect of the herbicides.

Keywords: *Ananas comosus,* growth stimulus, glyphosate, sub-dose of herbicide, 2,4-D.

Introduction

The pineapple is a perennial herbaceous plant belonging to family Bromeliaceae, which comprises around 56 genera (Wali, 2019). Of these, the only species of genus Ananas that produces an edible fruit, and is marketed and grown in 85 countries of the tropical and subtropical regions is *A. comosus* var. *comosus* (Zhang et al., 2016; Chen et al., 2019). However, the varieties of *A. comosus* grown in these regions show slow growth during the vegetative growth phase (VGP), thereby prolonging the crop cycle, which can vary from 12 to 30 months until the first fruit is produced. The prolonged crop cycle affects production costs due to the need to control weeds, insect pests and diseases (PY et al., 1984; Catunda et al., 2005; Kist et al., 2011).

The VGP is considered one of the longest, ranging from 8 to 18 months. It is also considered the most important phase in the production cycle of the pineapple, as it is during this period that the plants acquire sufficient size for floral induction (natural or artificial), and the maximum nutritional reserve necessary for the development and quality of the fruit (Kist et al., 2011; Caetano et al., 2013; Franco et al., 2014). However, even when all the optimal growing conditions are provided, the pineapple shows slow growth during the VGP (Cuzzuol et al., 2005).

The slow growth of the pineapple during the VGP may be associated with intrinsic factors, such as its crassulacean acid metabolism (CAM), which allows it to tolerate long periods of drought, albeit resulting in slow growth, as it depends on an environment with conditions that are favourable to its metabolism (CAM) for CO_2 assimilation and fixation to organic acids, which are used by the Calvin cycle to maintain growth and development in the plant (Dodd et al., 2003; Yang et al., 2015; Rainha et al. , 2016).

In view of this, it is presumed that the slow growth

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of the pineapple plant can be overcome by stimulating plant growth through the application of sub-doses of a toxic product (herbicide), a phenomenon known as hormesis. The hormesis effect occurs when a plant is exposed to low doses of a herbicide for example, which results in various stimuli, such as the growth stimulus (Calabrese & Blain, 2011).

The hormesis effect caused by herbicides has been demonstrated in maize, eucalyptus, pine (Velini et al., 2008), cotton (Américo et al., 2016) and soya (Pinheiro et al., 2021). However, there appear to be no studies of hormesis for stimulating vegetative growth in the pineapple. The aim of this study, therefore, was to determine the hormesis effect of sub-doses of glyphosate and 2,4-D herbicides on vegetative growth in the pineapple.

Material and Methods

Experimental site

Two parallel experiments were conducted in a greenhouse at the Centre for Agrarian Sciences of the Federal University of Roraima (2°52'20.7" N, 60°42'44.2" W, altitude 90 m).

The temperature and humidity inside the greenhouse were monitored to maintain a temperature variation of 32 ± 8°C, with the relative humidity controlled at 70%, using a forced ventilation system. An automated micro-sprinkler irrigation system was used, with an irrigated water volume of 2.5 mm day^{-1}.

Experimental design and treatments

The experimental design for both herbicides was completely randomised, with four replications. For the experiment with glyphosate (Glyphosate Nortox SL, 360 g a.e. L^{-1}), ten treatments were used, corresponding to doses of 0, 1.8, 3.6, 7.2, 18, 36, 72, 180, 360 and 720 g a.e. ha^{-1} and for the experiment with 2,4-D (2,4-D Nortox, 670 g a.e. L^{-1}), the treatments corresponded to doses of 0, 1.68, 3.35, 6.70, 16.75, 33.5, 67, 167.5, 335 and 670 g a.e. ha^{-1}. The herbicides and their respective doses were applied thirty days after transplanting the plants of the 'Pérola' pineapple. This cultivar was chosen as it is one of the most cultivated in Brazil (Silva et al., 2020).

Each experimental unit comprised one polyethylene pot with a capacity of 8 dm^3, containing a substrate consisting of a 2:1 mixture of an organic compound based on carbonised rice husks and a soil classified as a typical dystrophic Yellow Oxisol with a sandy clay loam texture, collected from an arable layer at a depth of 0.00 to 0.20 m and incubated for thirty days with dolomitic limestone. The analysis of the chemical attributes of the substrate is shown in (**Table 1**).

Setting up the experiments and applying the treatments

Before setting up the experiments, the pup pineapple plants were standardised to an average height of 25 ± 2 cm and fresh weight of 300 g ± 1 g; they were pre-rooted in plastic trays (length 50 cm x width 30 cm x height 10 cm) for 30 days and then transferred to the pots. During the pre-rooting period, the plants received three applications of a nutrient solution based on urea (10 g L^{-1}) and potassium chloride (5 g L^{-1}). The nutrient solution was applied via the leaves using a manual sprayer at a volume of 10 mL plant^{-1}, which was applied on the first day of pre-rooting (1st application); 15 mL plant^{-1}, applied ten days after the start of pre-rooting (2nd application); and 20 mL plant^{-1}, applied twenty-five days after the start of pre-rooting (3rd application). After transplanting the pineapple plants to the pots, base and topdressing fertiliser was applied based on the chemical analysis of the substrate and following the recommendations of Santos et al. (2020) for the pineapple.

The treatments were applied in a single step 30 days after transferring the plants to the pots. The application was carried out in the morning, between 08:00 and 09:00, under an average temperature of 29°C, relative humidity of 65% and no wind. At the time of application, the plants were placed outside the greenhouse, and after three days of herbicide application, the pots were again returned to the greenhouse. The plants were irrigated before the treatments were applied; after application, the soil moisture was maintained by manual irrigation using a watering can.

The herbicide doses were applied using a backpack sprayer at a constant pressure of 2 kgf cm^{-2} maintained by compressed CO_2, and equipped with a bar with two nozzles, 0.50 m from the centre of the bar, and one TeeJet EZ 8002 fan-spray nozzle. The sprayer was set to a constant rate to distribute a spray volume of 180 L ha^{-1} over the plants, keeping to a distance of 0.50 m above the target.

Variables under evaluation

A visual assessment of phytotoxicity was made 7, 42, 77, 147 and 217 days after applying the herbicides (DAA), using a scale from 0% (no damage) to 100% (complete death of the plant) (FRANS, 1972). Chlorosis, lesions showing damage, leaf epinasty, and reduced leaf and plant size were all considered when evaluating the visual lesions.

At 253 DAA, the following were evaluated: plant height (PH - cm), measured with the aid of a ruler graduated in centimetres, from the ground to the longitudinal end of the largest leaf; length of the 'D' leaf (LDL - cm) from the base to the apex of the most

Table 1. Chemical characteristics of the substrate

pH	P	K^+	Ca^{2+}	Mg^{2+}	Na^+	Al^{3+}	H+Al	t	T	SB	V
H_2O	mg dm^{-3}		------------------------------------			$cmol_c\ dm^{-3}$	------------------------------------				%
5.93	170.63	0.68	8.81	3.08	0.12	-	2.00	12.69	15.39	12.00	82.40

P: Mehlich[1] extractor; Ca^{2+}, Mg^{2+}, K^+ and Na^+: Mehlich[3] extractor; SB: sum of exchangeable bases; t: effective cation exchange capacity; T: cation exchange capacity at pH 7(CEC); V: base saturation.

developed leaf, measured with a ruler graduated in centimetres; stem diameter (SD - mm), measured with the aid of a digital calliper, considering a distance of 5 cm from ground level. The aerial part and the roots were then separated. The number of leaves (NL) was counted, and, using a precision analytical balance, the following were determined: the fresh weight of the 'D' leaf (FWDL, g); the total fresh weight (TFW, kg), from the sum of the weight of the leaves, stems and roots; and the root fresh weight (RFW, g).

These variables were chosen since they are normally used as parameters by pineapple growers to indicate the best time to carry out artificial flower induction in the pineapple, as they show a positive correlation with larger fruit (≥ 1000 g) once the plant in the VGP reaches a suitable size: PH ≥ 1 m, FWDL ≥ 0.70 g, and SD ≥ 85 mm (Caetano et al., 2013; Vilela et al., 2015).

Statistical analysis

The data were submitted to analysis of variance (ANOVA) by F-test, and when significant (P≤ 0.05), to non-linear regression analysis, with the regression models chosen based on the significance of the equation parameters, biological phenomenon and coefficient of determination.

To analyse the dose-response hormesis effect, the log-logistic regression model by Brain and Cousens (1989), adapted by Ritz et al. (2015), was tested (eq 1).

$$\hat{y} = c+(d-c+fx)/(1+\exp(b(\log(x)-\log(e)))) \qquad \text{eq.1}$$

where: y = treatment yield; x = herbicide dose; e = ED50, defined as the dose that provides 50% of the total achievable effect; d = upper limit; c = lower limit; b = denotes the relative slope around e; f = linear term.

The SIGMAPLOT software (SIGMAPLOT, 2012) was used for analysing the data and for producing the graphs of the variables that showed no hormesis, together with the phytotoxicity data. For any variable that showed the effects of hormesis, the analysis and graphs were made with the aid of the R software (R Core Team-R version 3.6.2), including the 'drc' v3.0-1 additional package developed by Ritz & Streibig (2005). In plotting the graphs, the model proposed by these authors was adopted.

Results and Discussion

The results referring to phytotoxicity (%) in pineapple plants treated with doses of glyphosate and 2,4-D were better represented by the three-parameter sigmoidal log-logistic equation, as seen from the adjusted R^2 and significance of the parameters for each period of evaluation (Figure 1A and B).

Pineapple plants treated with glyphosate and 2,4-D showed a pattern of phytotoxicity, with increasing values of parameter a proportional to the period of evaluation: 7, 42, 77 or 147 DAA (Figure 1 A and B). During the periods of evaluation, plants treated with doses ≤ 72 g a.e. ha^{-1} glyphosate showed phytotoxicity with mean values of 8%, 14%, 16%, 14% and 11%; in plants treated with doses ≤ 67 g a.e. ha^{-1} 2,4-D, the mean values found for phytotoxicity were 6%, 15%, 19%, 21% and 18%. For concentrations ≥ 180 g a.e. ha^{-1} glyphosate, the phytotoxicity showed mean values of 24%, 51%, 54%, 77% and 64%, while for doses of 2,4-D ≥ 167.5 g a.e. ha^{-1} the values for phytotoxicity were 23%, 42%, 45%, 50% and 44%. These results partially corroborate those seen in plants of Ananas erectifolius, a species of family Bromeliaceae (Maciel et al., 2009), in which phytotoxicity was proportional to the increase in glyphosate dose (Roundup; 360 g L^{-1}); however, over the periods of evaluation up to 60 DAA, mean toxicity at the maximum dose (360 g a.e. ha^{-1}) was 6%, far lower than that seen in the present study.

The phytotoxicity of glyphosate and 2,4-D at doses ≤72 and 67 g a.e. ha^{-1}, respectively, are almost the same for all the periods of evaluation (**Figure 1** A and B). However, for doses ≥ 180 and 167.5 g a.e. ha^{-1} for glyphosate and 2,4-D, respectively, phytotoxicity was generally higher in plants treated with glyphosate for all periods longer than 7 DAA (Figure 1 A). A similar result was found at doses ≥ 20% commercial glyphosate (Roundup; 360 g L^{-1}) and 2,4-D (2,4-D Nortox; 670 g L^{-1}) up to 120 DAA in the forest species *Croton floribundus*, *Heliocarpus americanos* and *Myrsine coriácea* (Monquero et al., 2016). However, the results of the present research partially corroborate the results seen in plants of the common grape vine (*Vitis vinifera*) under the effect of a simulated derivative of glyphosate (840 g a.i. ha^{-1}), 2,4-D amine (840 g a.i. ha^{-1}) and Dicamba (560 g a.i. ha^{-1}) (Mohseni-Moghadam et al., 2016), where the greatest value (35%) and longest duration (357 DAA) for phytotoxicity was seen in plants treated with 2,4-D amine. In contrast to the phytotoxicity found in this research for the effect of glyphosate, Mohseni-Moghadam et al. (2016) found that in the grape vine, even at the highest dose (28 g ha^{-1}) of glyphosate, phytotoxicity only lasted up to 42 DAA, well below our findings, where, at a similar dose, the effect was longer-lasting (217 DAA).

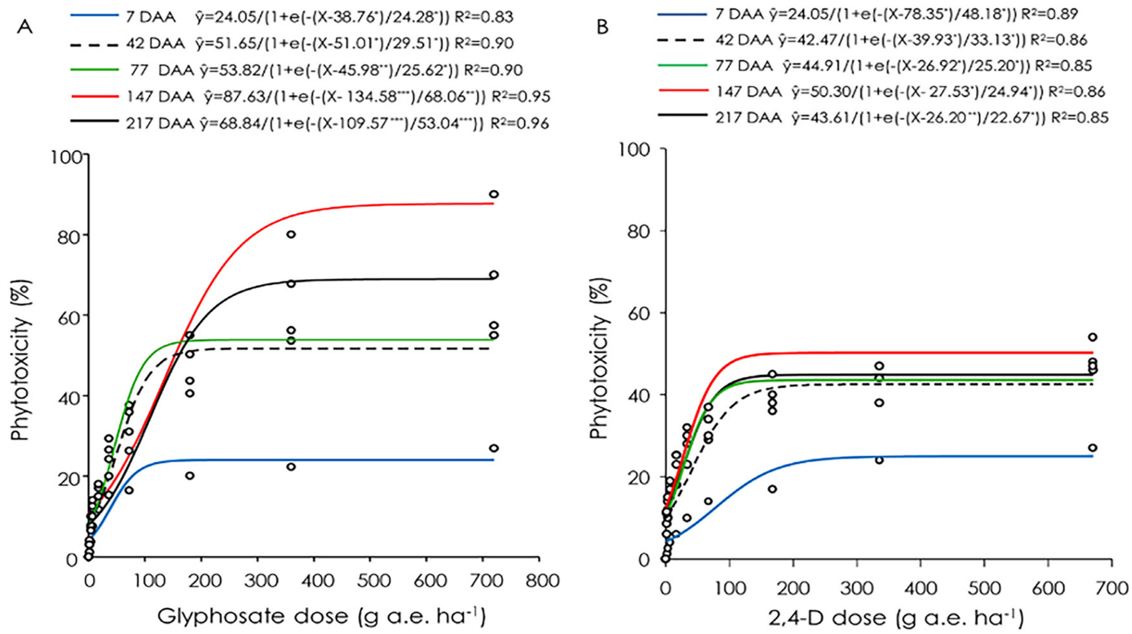

Figure 1. Phytotoxicity (%) in 'Pérola' pineapple plants submitted to doses of the herbicides glyphosate (A) and 2,4-D (B), evaluated at 7, 42, 77, 147 and 217 DAA. Three-parameter sigmoidal log-logistic model: ŷ=a*/(1+e(-(X-Xa50%)/β); a = estimated maximum phytotoxicity when the dose tends towards infinity; X = herbicide dose; $X_{a50\%}$ = dose capable of causing 50% of the estimated maximum phytotoxicity for a; β = the slope at the inflection point

At 7 DAA, the symptoms initially seen in plants treated with glyphosate were chlorosis and slight damage to the younger leaves ('C' and 'D': leaves with greater physiological activity). For the plants treated with 2,4-D, visual phytotoxicity included the onset of epinasty in the younger leaves, chlorosis and slight damage. Similar symptoms were observed in plants of *Ananas erectifolius* (Maciel et al., 2009), *Caryocar brasiliense* and *Coffea arabica* treated with glyphosate (Silva et al., 2016; Barbosa et al., 2020), and in cotton, soya and *Caryocar* Brasiliense treated with 2,4-D (Byrd et al., 2016; Manuchehri et al., 2019; Peres-Oliveira et al., 2017; Tavares et al., 2017).

In subsequent evaluations, at 42 and 77 DAA (Figure 1 A), it was found that for plants treated with glyphosate at doses in the range of 72 to 720 g a.e. ha^{-1} the symptoms evolved into more intense depigmentation, occupying a larger area of the leaf, especially at the base of the older leaves ('A' and 'B': leaves with less physiological activity); a reduction in PH, LDL and NL; and an increase in damaged area, showing necroses greater than 3.5 cm^2, this latter type of lesion particularly seen in plants treated with ≥ 360 g a.e. ha^{-1}. For the same period of evaluation (42 and 77 DAA) (Figure 1B), plants treated with doses ≥ 167.5 g a.e. ha^{-1} 2,4-D, and which showed the onset of chlorosis at 7 DAA, began to show more acute damage, more obvious epinasty, and a reduction in plant height and length of the 'D' leaf, albeit with a lower average phytotoxicity (45%). This happens because glyphosate and 2,4-D, once absorbed, are translocated to meristematic tissue, with the early symptoms of phytotoxicity seen in the younger parts of the plant and only later affecting the older leaves (Pazmiño et al., 2011; Silva et al., 2016), as seen in the present study.

For the same period (77 DAA), it was found that plants treated with doses of 360 or 720 g a.e. ha^{-1} glyphosate required less force for abscission of the younger leaves. It may be that the larger leaves, being more physiologically active, absorb a greater amount of the herbicide solution and are more affected than the smaller, less physiologically active leaves, as reported by Pazmiño et al. (2011) and Silva et al. (2016). This could be seen from the loss of leaves over the following days, with phytotoxicity reaching 87.63% at 147 DAA at the highest doses (360 and 720 g a.e. ha^{-1}) (Figure 1 A). During this period, despite the high phytotoxicity seen in the pineapple plants treated with glyphosate, there were signs of recovery, with the emission of new leaves, recovering to 21.44% phytotoxicity at 217 DAA compared to the previous period (147 DAA) (Figure 1A).

The symptoms of chlorosis and foliar lesions, as well as a reduction in the variables evaluated in the plants treated with glyphosate in this study, are possibly related to the fact of the herbicide acting as a chelate, a kind of chemical agent that blocks the absorption of macro- and micronutrients by the plant, causing immobilisation of cations that are important for various physiological processes, such as photosynthesis, which depends on Mg and Mn to capture light energy and produce chlorophyll

(Zobiole et al., 2011; Mertens et al., 2018). Other possible causes are chloroplast degeneration (Costa et al., 2012) and the accumulation of aminomethylphosphonic acid (AMPA), one of the main degraded phytotoxic metabolites of glyphosate (Reddy et al., 2008). These authors report that the AMPA produced by glyphosate degradation can reduce the chlorophyll and the weight of the leaves, and that the symptoms of phytotoxicity that can be seen even in transgenic plants tolerant to the herbicide are due in part to the accumulation of AMPA.

The pineapple plants treated with 2,4-D were again evaluated at 147 DAA (Figure 1B), when a mean phytotoxicity of 13.17%, 13.83%, 15.22%, 19.80%, 28.14% and 41.73% was found at doses ranging from 1.68 to 67 g a.e. ha^{-1}. For the range of 167.5 to 670 g a.e. ha^{-1}, phytotoxicity presented values very close to those estimated for parameter *a*, with a difference of 0.06%. This means that for this range of doses the symptoms of phytotoxicity were stable, generally marked by a reduction in PH, LDL, and necrotic damage to the edges of the leaves. For doses of less than 167.5 g a.e. ha^{-1}, the phytotoxicity is reduced on average by 24.94% from the inflection point of the curve for each unit (g a.e. ha^{-1}) less in the dose of 2,4-D. A similar fit of the model for phytotoxicity was found by Monquero et al. (2016) at 30, 60, 90 and 120 DAA of 2,4-D (2,4-D Nortox; 670 g L^{-1}) in plants of *Heliocarpus americanos*. However, the estimated values, especially for $X_{a50\%}$, were far lower than those of the present study.

The recovery of the plants seen at 217 DAA, showed little difference to the phytotoxicity seen at 147 DAA, as can be seen by the estimated values of the parameters *a* (7%) and $X_{a50\%}$ (1.33%) in the equation (Figure 1B). In general, at 217 DAA, low phytotoxicity (mean ≤ 18%) was seen in the pineapple plants treated with doses ≤ 67 g a.e. ha^{-1} 2,4-D; for doses ≥167.5 g a.e. ha^{-1} nearly all the plants presented a toxicity of ≈ 43%. These data corroborate other results for phytotoxicity seen in forest species (*Schisolobium amazonicum* and *Ceiba pentandra*) treated with 2,4-D DMA (806 g a.e. L^{-1}) (Yamashita et al., 2009), and in *Carya illinoinensis* treated with 2,4-D (455 g a.e. L^{-1}) and Dicamaba (480 g a.e. L^{-1}) (Wells et al., 2019), which showed a phytotoxicity similar to those found in the present study, at both lower and higher doses of these herbicides.

The phytotoxicity seen in the pineapple plants treated with doses of glyphosate was reflected significantly in all the variables under study (PH, LDL, SD, FWDL, TFW, NL and RFW). In turn, there was no significant difference for FWDL (mean of 22.65 g), NL (mean of 49 per plant) and RFW (mean of 83 g) in the plants treated with 2,4-D.

With an increase in the herbicide doses, the pineapple plants showed less height starting at doses ≥ 72 g a.e. ha^{-1} glyphosate and ≥ 167.5 g a.e. ha^{-1} 2,4-D (**Figure 2**A and B). The maximum tested dose of 720 g a.e. ha^{-1} glyphosate and 670 g a.e. ha^{-1} 2,4-D, inhibits growth in the pineapple plants by an average of 24.6% and 23.3%, compared to the height of the plants treated with the respective control treatment (zero dose).

Plant height is one of the most studied variables in relation to the hormesis effect caused by herbicides, however, the results in the literature are conflicting (Silva et al., 2012). The hormesis effect of glyphosate stimulated growth in the height of *Schisolobium amazonicum* of around 40% in relation to the control, at a dose of 9 g a.e. ha^{-1} (Marques et al., 2020). In other studies, which include maize, eucalyptus, soya (Velini et al., 2008) and sugarcane (Pincelli-Souza et al., 2020), there was a beneficial effect on plant height in the range of 1.8 to 3.6 g a.e. ha^{-1}. On the other hand, the same did not occur in plants of *Pyrus*

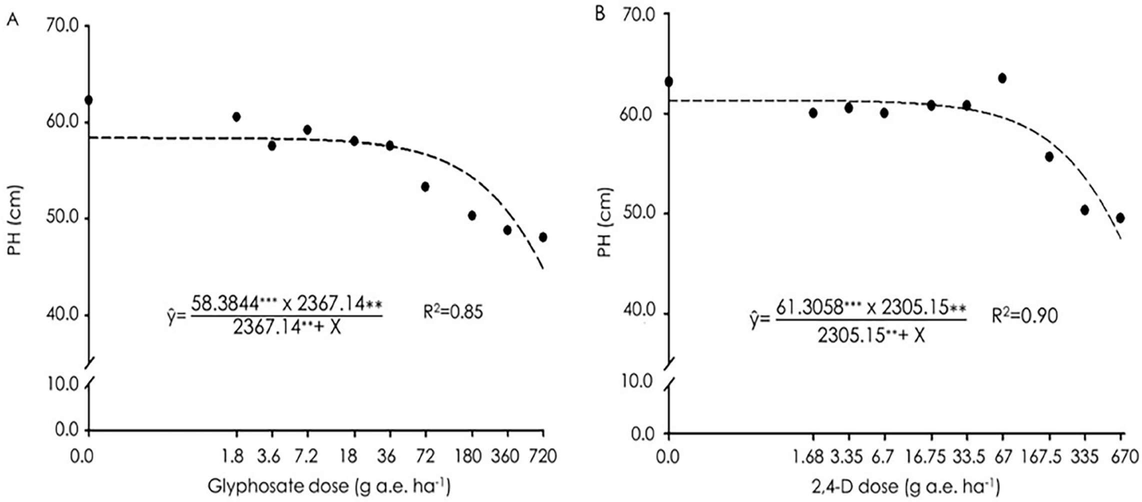

Figure 2. Plant height (PH-cm)in the 'Pérola' pineapple submitted to doses of glyphosate (A) and 2,4-D (B) herbicides.

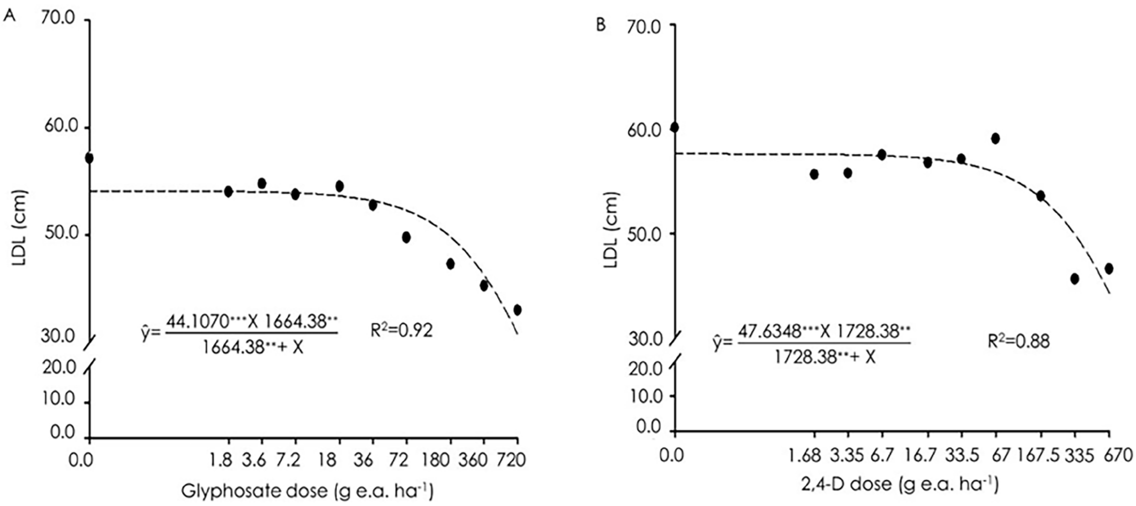

Figure 3. Length of the 'D' leaf (LDL-cm) in the 'Pérola' pineapple submitted to doses of glyphosate (A) and 2,4-D (B) herbicides.

communis (Carvalho et al., 2016) or coffee (Barbosa et al., 2020), where an increase in the glyphosate dose caused a reduction in plant growth, similar to the results of the present study.

For the effect of 2,4-D, the results also differed, showing a beneficial effect, stimulating growth in the height of the FMT 701 and Fibermax 966 cultivars of cotton (Américo et al., 2016), and the M-Soy 8866 and HO Cristalino Ipro cultivars of soya (Pinheiro et al., 2021); however, in the cotton, the FMT 701 cultivar was more responsive to the hormesis effect, while in the soya during the V2 and V5 vegetative stages (2 and 5 leaves), the M-Soy 8866 cultivar had the best height response for the range of doses from 0.20 to 36.01 g e.a. ha^{-1}. However, there was no effect on height in rice plants subjected to a range of 0.0 to 5.44 g e.a. ha^{-1} 2,4-D (Marsala et al., 2022), as in the present study.

The results for LDL relating to the glyphosate and 2,4-D doses were represented by the two-parameter hyperbolic decay model (**Figure 3**A and B), showing the LDL inhibited by 29.7% and 26.5% at the highest dose of glyphosate (720 g a.e. ha^{-1}) and 2,4-D (670 g a.e. ha^{-1}) respectively, compared to the controls (zero dose).

The reduction in LDL is a characteristic of the sensitivity of the crop to these herbicides, since, even at low doses, plant growth is affected, as seen for plant height, with a more marked effect for the glyphosate doses (Figure 2A). The LDL data from the present research corroborate the results seen by Maciel et al. (2009), in which glyphosate toxicity at doses ≥ 90 g a.e. ha^{-1} reduce the LDL in plants of *Ananas erectifolius*.

For SD and FWDL, the four-parameter sigmoidal model and the exponential decay model satisfactorily fitted the data, as seen from the adjusted R^2 (0.98, 0.77 and 0.97), relative to the effects of the glyphosate (**Figure 4**A and C) and 2,4-D (Figure 4 B).

Lower values were seen for SD when the plants were exposed to doses of 18 to 720 g a.e. ha^{-1} glyphosate, and at doses of 167.5 to 670 g a.e. ha^{-1} 2,4-D (Figure 4 A and B), with a reduction of 34.31% and 18.21% in SD, in relation to the control treatment (zero dose), relative to the effect of the maximum dose of the glyphosate and 2,4-D herbicides.

On the other hand, FWDL (Figure 4 C) showed a reduction, which was only due to the effect of the glyphosate doses, particularly in the range of 18 to 720 g a.e. ha^{-1}, with an average reduction of 29.63% compared to the control treatments (zero dose). Our data corroborate the results found in other plant species with the application of glyphosate, including coffee (França et al., 2010; Barbosa et al., 2020) and *Ananas erectifolius* (Maciel et al., 2009) - where biomass accumulation in the leaf was reduced, and apple and pear (Carvalho et al., 2016) - with a reduction in stem diameter; also the results of Dintelmann et al. (2020), who found a reduction in SD in several plant species subjected to doses of 2,4-D and dicamba.

The effect of glyphosate and 2,4-D on TFW (**Figure 5**A and B) fitted the hyperbolic three- and two-parameter decay models, as seen from the adjusted R^2 (0.99 and 0.80). The lowest values for TFW were seen in plants treated with glyphosate, with an average reduction of 11.14% at doses ≤ 36 g a.e. ha^{-1} and 41.24% at doses ≥ 72 g a.e. ha^{-1}. For TFW in plants treated with 2,4-D, there was a reduction of 8%, 16% and 28%, starting with the dose of 167.5 g a.e. ha^{-1}, compared to the control treatment (zero dose).

Due to the action mechanism of these herbicides, limitations on biomass accumulation (TFW), as well as PH,

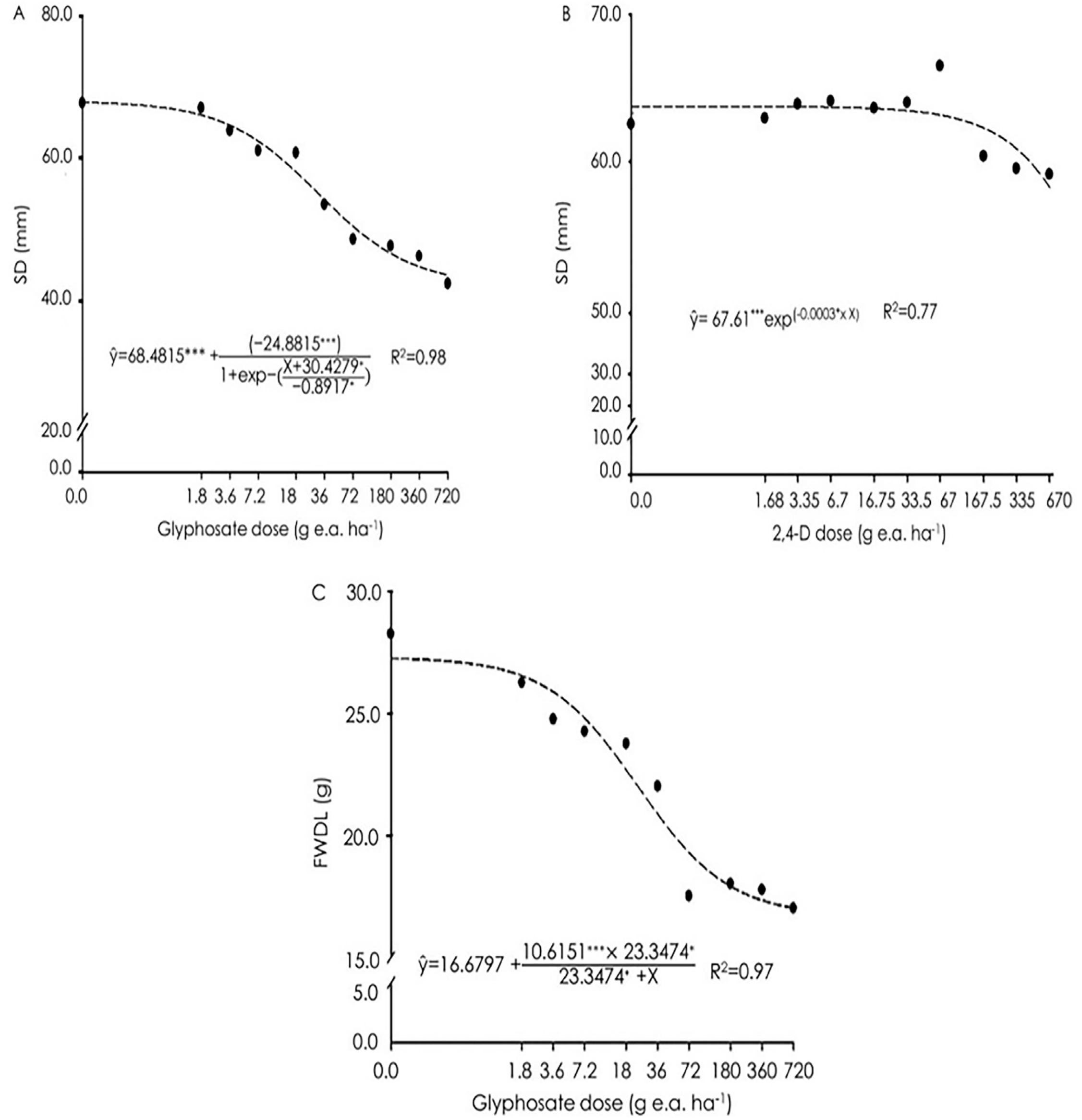

Figure 4. Stem diameter (SD – mm; A and B) and Fresh weight of the 'D' L (FWDL – g; C) in the 'Pérola' pineapple submitted to doses of glyphosate (A and C) and 2,4-D (B) herbicides.

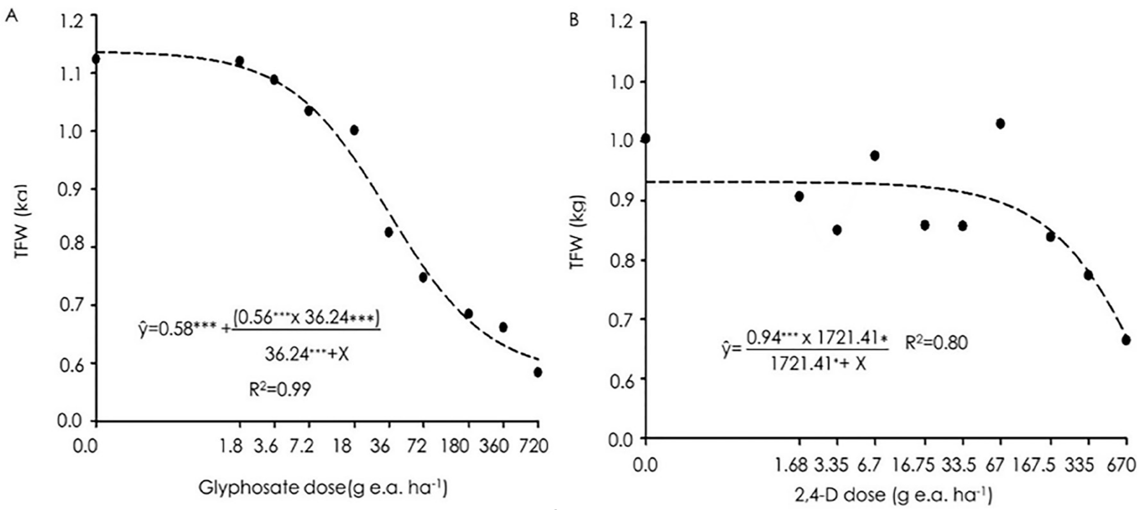

Figure 5. Total fresh weight (TFW- kg) in plants of the 'Pérola' pineapple submitted to doses of glyphosate (A) and 2,4-D (B) herbicides.

LDL and SD, may be associated with a reduction in the production of indole-3-acetic acid (IAA) (the main natural auxin of the plant) and CO_2 assimilation. As glyphosate acts by inhibiting the activity of the 5-enolpyruvylshikimate-3-phosphate synthase enzyme (EPSPs), it blocks the shikimic acid pathway and consequently the biosynthesis of aromatic amino acids (phenylalanine, tyrosine and tryptophan). These aromatic acids are required for various metabolic processes, including the synthesis of auxin, lignin and proteins, enzyme activity and other phenolic compounds that can represent up to 35% of plant biomass and 20% of the carbon fixed by photosynthesis (Cakmak et al., 2009; Gomes et al., 2014; Pincelli-Souza et al., 2020; KHAN et al., 2021). On the other hand, the 2,4-D herbicide, by mimicking the effect of natural auxin (AIA), acts on the perception pathways (TIR1/AFB receptor proteins), signalling pathways and genetic expression, which govern the biochemical and transcriptional responses of this hormone. Thus, at supra-optimal concentrations of 2,4-D, the overexpression of auxin-responsive genes leads to a successive series of biochemical and physiological events that induce the production of 1-aminocyclopropane-1-carboxylic acid (ACC), which then promotes the production of ethylene, and consequently the biosynthesis of abscisic acid (ABA). This accumulation of ethylene and ABA causes stomatal closure, which limits carbon assimilation and results in the production of plant biomass (Grossmann, 2009; Piasecki et al., 2017). In line with the observations of the above authors, it is presumed that by interfering in the shikimic acid metabolic pathway and in the biosynthesis of perception, signalling and gene expression, glyphosate (≥ 72 g a.e. ha^{-1}) and 2,4-D (≥ 167.5 g a.e. ha^{-1}) at the most toxic doses alter the homeostasis of the plant, affecting biomass accumulation and plant growth in the pineapple.

The doses of 2,4-D caused no interference in NL, where an effect was seen for the glyphosate doses only (**Figure 6**), with a more obvious reduction in this variable starting with doses ≥ 72 g a.e. ha^{-1} compared to the control. These results are in line with those reported for glyphosate treatments in *Ananas erectifolius* (Maciel et al., 2009) and coffee (Barbosa et al., 2020).

The toxicity of glyphosate on NL was possibly influenced by the reduction in leaf area and in plant growth structures, e.g. PH and LDL, supported by a lower photosynthetic rate, a nutritional imbalance, and a reduction in CO_2 incorporation. A good part of this carbon is directed to the shikimic acid metabolic pathway, albeit affected by the action mechanism of the glyphosate (Maeda & Dudareva, 2012; ZULET-GONZÁLEZ et al., 2020).

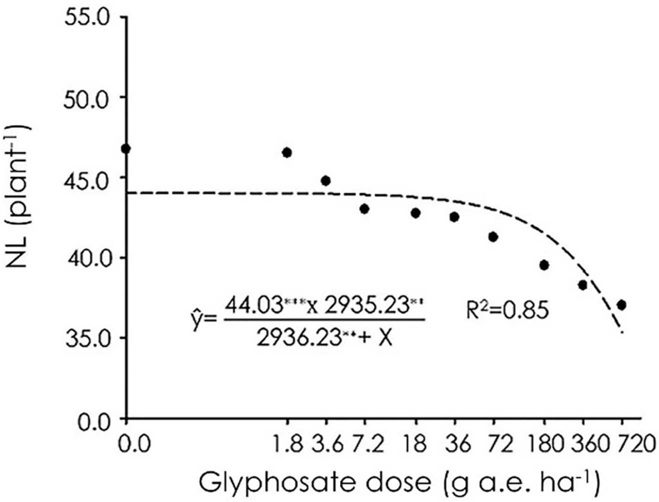

Figure 6. Number of leaves (NL) in plants of the 'Pérola' pineapple submitted to doses of glyphosate herbicide.

No changes were registered for RFW In pineapple plants treated with 2,4-D. However, for the effect of glyphosate on RFW (**Figure 7**), the results of the regression analysis revealed that the log-logistic model adequately fitted the data, as seen from the *p* value of the lack-of-fit test, agreeing with the prerequisites proposed for accepting the model, and indicating the occurrence of the hormesis effect (Knezevic et al., 2007).

A growth stimulus of 32.25% was seen in RFW in relation to the control, in pineapple plants treated with glyphosate at a dose of 1.8 g e.a. ha^{-1}. This is the first result demonstrating the hormesis effect of a sub-dose of glyphosate on RFW growth in the pineapple. Similarly, Velini et al. (2008) found an increase in root biomass in eucalyptus and pine plants submitted to doses of 3.7 g a.e. ha^{-1} and 4.6 g a.e. ha^{-1} glyphosate, respectively. However, the same effect was not detected in the root weight of soya or maize plants. Beneficial effects from low doses of glyphosate were also reported in several earlier studies, e.g. in maize (Barbosa et al., 2017), the common bean (*Phaseolus vulgaris*) (Rabello et al., 2012; Silva et al., 2013), and sugarcane (Pincelli-Souza et al., 2020).

Various studies attribute the occurrence of the hormesis effect from sub-doses of glyphosate to stimulating transpiration, conductance and CO_2 assimilation (Nascentes et al., 2018; Moraes et al., 2020); increased photosynthesis, due to electron transport in photosystem II (Silva et al., 2016); an increase in nutrients in the leaf tissue (Pincelli-Souza et al., 2020); a partial inhibition of the EPSPs enzyme; and a reduction in cell-wall lignification. The reduction in lignin levels allows greater elasticity and cell expansion, consequently, greater growth (Velini et al., 2008; Pincelli-Souza et al., 2020). Other studies argue that

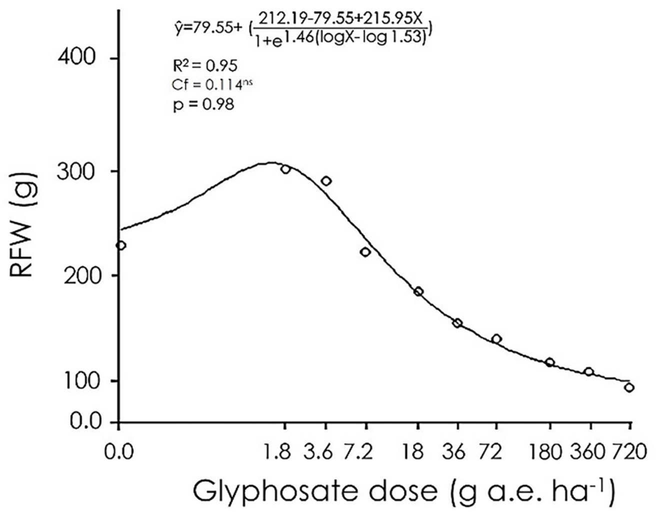

Figure 7. Fresh root weight (RFW-g) in the 'Pérola' pineapple submitted to doses of glyphosate herbicide. Cf.: calculated f; ^{ns} not significant; p value ≥ 0.5.

the hormesis effect occurs due to an adaptive response of the plant to a condition of stress, for example, low doses of herbicide, which trigger defence mechanisms in the plant, and which in turn stimulate growth (Agathokleous & Calabrese 2020; Berry & López-Martínez, 2020).

It has been suggested that the hormesis effect, by stimulating plant growth (Belz & Duke, 2017) and modifying the morphology and physiological and enzyme functions, might be caused by low doses of any herbicide (Brito et al., 2018). However, no hormesis effect was detected in any of the variables evaluated in pineapple plants treated with doses of 2,4-D; while, for the various growth parameters measured in pineapple plants treated with glyphosate, only RFW showed growth stimulation due to the hormesis effect caused by the low dose of glyphosate. This result partially confirms the hypothesis raised in this study, that the slow growth of pineapple during VGP would be overcome by the hormesis effect of subdoses of glyphosate and 2,4-D. Thus, these results were contrary to expectations, where pineapple plants of sufficient size, with PH ≥ 1 m, FWDL ≥ 0.70 g and DP ≥ 85 mm, would achieve floral induction after a shorter cultivation period.

For the growth stimulus relating to RFW, previous research shows that some herbicides can stimulate root growth at low doses, but have no stimulating effect on stem growth (Belz & Duke, 2014), i.e. the growth stimuli were stronger in the roots than in the leaves or stem (Silva et al., 2015). Other results show that using glyphosate at concentrations that are expected to produce a hormesis effect can result in harmful effects (Silva et al., 2012) or even the lack of any growth stimulus (Sousa et al., 2014), similar to the results seen in pineapple plants treated with 2,4-D in the present study. In addition, the growth stage of the plant and the time of application, the dose and type of drop that reaches the plant, and even the species, can influence the occurrence of the hormesis effect (Cedergreen, 2008; Carvalho et al., 2013; Cederlund, 2017).

The effects of growth stimuli on parts of the plant or even the whole plant, the lack of any stimuli, and the factors that influence the occurrence of the hormesis effect when using herbicides should be considered when evaluating the results, especially in the long term (Velini et al., 2008; Cedergreen, 2008; Carvalho et al., 2013; Belz & Duke, 2014; Sousa et al., 2014; Cederlund, 2017). It is, therefore, suggested that further study be needed to verify the viability of an increase in root weight in the pineapple, for example, in relation to the volume of soil exploited by the roots for water absorption and nutrient interception, and whether the uptake of resources from the soil can offset the drop in yield from photosynthesis, given the detrimental effect on the shoots of the same sub-dose.

Conclusions

Concentrations of glyphosate herbicide in the range of 18 to 720 g e.a. ha^{-1} and 2,4-D in the range of 167.5 to 670 g a.e. ha^{-1} were the most phytotoxic to the 'Pérola' pineapple, negatively affecting plant growth. The hormesis effect was evidenced by the 32.25% increase in root fresh weight at the dose of 1.8 g a.e. ha^{-1} glyphosate. The slow growth of the pineapple was not overcome by the hormesis effect of sub-doses of the glyphosate or 2,4-D herbicides.

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