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Article

# Physiological Variables in Pineapples Submitted to the Application of Diuron

*Variáveis Fisiológicas em Abacaxizeiros Submetidos à Aplicação do Diuron*

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ABSTRACT - Diuron is a widely used herbicide on pineapple cultivations. However, its application is recommended only for the implementation of the crop. The objective of this work was to evaluate the gas exchanges of four pineapple cultivars submitted to the application of the herbicide diuron at their reproductive stage. The experiment was conducted with the IAC Fantástico, Vitória, MD2 and Pérola pineapple cultivars using the active ingredient diuron at a dose of 7.5 mL $L^{-1}$. Measurements of $CO_2$ assimilation rate, stomatal conductance, transpiration, vapor pressure deficit and water use efficiency were carried out weekly, by the hour, on two plants per cultivar, over a 24 hour period. These determinations were carried out two days before the application of the herbicide and 8, 15, 22, 29 and 36 days after application of the herbicide. Measurement of photochemical efficiency was performed weekly on the same dates and all these determinations were made on the 'D' leaf. Measurements of gas exchange were performed with an infrared gas analyzer (IRGA) and of chlorophyll *a* fluorescence with a fluorometer. The application of diuron reduced the assimilation of $CO_2$ in the pineapple varieties IAC Fantástico, Vitória, MD2 and Pérola, at their reproductive phase, in all CAM metabolism stages. Diuron promoted a temporary reduction of the stomatal conductance and a temporary increase in water use efficiency. The photochemical efficiency of the studied pineapple cultivars was negatively affected by the herbicide diuron, with the recovery of the initial values by cultivar IAC Fantástico.

**Keywords:** *Ananas comosus* var. *comosus*, herbicide, gas exchange, weeds, chemical control.

*RESUMO - O diuron é um herbicida amplamente utilizado na cultura do abacaxizeiro. No entanto, sua aplicação é recomendada apenas na implantação da cultura. Objetivou-se com este trabalho avaliar as trocas gasosas de quatro cultivares de abacaxizeiros submetidos à aplicação do herbicida diuron na fase reprodutiva. O experimento foi realizado com os cultivares de abacaxizeiro IAC Fantástico, Vitória, MD2 e Pérola, utilizando o princípio ativo diuron na dose de 7,5 mL $L^{-1}$. As medições da taxa de assimilação de $CO_2$, condutância estomática, transpiração, déficit de pressão de vapor e eficiência do uso da água foram feitas semanalmente, a cada hora, em duas plantas por cultivar, num período de 24 horas. Essas determinações foram realizadas aos dois dias antes da aplicação do herbicida e aos 8, 15, 22, 29 e 36 dias após aplicação do herbicida. A medição da eficiência fotoquímica foi realizada semanalmente nas mesmas datas, e todas essas determinações foram feitas na folha 'D'. As medições das trocas gasosas foram realizadas com medidor de gases no infravermelho (IRGA), e as da fluorescência da clorofila a, com fluorímetro. A aplicação do diuron reduziu a assimilação de $CO_2$ nas variedades de abacaxizeiro IAC Fantástico, Vitória, MD2 e Pérola, na*

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*fase reprodutiva, em todas as fases do metabolismo CAM. O diuron promoveu redução transitória da condutância estomática e aumento transitório na eficiência do uso da água. A eficiência fotoquímica dos cultivares de abacaxizeiro estudados foi afetada negativamente pelo herbicida diuron, com recuperação dos valores iniciais pelo cultivar IAC Fantástico.*

**Palavras-chave:** *Ananas comosus* var. *comosus*, herbicida, trocas gasosas, plantas daninhas, controle químico.

## INTRODUCTION

The productivity of the pineapple crop is negatively affected, among other factors, by the competition of weeds. Since it is a small crop with a very slow initial vegetative development, weeds are favored, competing for water, light and nutrients (Catunda et al., 2005). The use of herbicides is among the management strategies. The management of weeds in pineapple crops is practically based on chemical and mechanical methods, isolated or combined (Maia et al., 2012).

The herbicide 3-(3,4-dichlorophenyl) -1,1-dimethylurea, known as diuron, acts as an electron transfer inhibitor of the photosystem II (PSII), which prevents the reduction of quinone A ($Q_A$), by competing with quinone B ($Q_B$) by the $D_1$ protein binding site, causing the output of $Q_B$ and an interruption in the electron flow, not allowing the reduction of NADPH (Fuerst and Norman, 1991).

The application of diuron is recommended in the pre- and post-emergence period of weeds; crop and weed pre-emergence; post-planting of seedlings and during the pre-emergence of weeds; and the post-emergence of the crop (AGROFIT, 2017). However, pineapple is a long-cycle crop, and the registered herbicides only contemplate the vegetative phase. Thus, there is a need to control weeds throughout the cycle, including in the planting rows, where competition for water, light and nutrients occurs.

Despite the careful application of the herbicide, unwanted contact with pineapples is common, especially at high planting densities, which could cause phytotoxicity to the crop, causing losses in productivity. Thus, it is necessary to evaluate the effect of the herbicide when applied at the reproductive phase, through interference in the physiology of pineapple plants.

Works involving the possibility of injury by diuron on pineapple crops evaluated growth, productivity, fruit quality (Maia et al., 2012) and photosynthetic activity when diuron was applied at the vegetative phase of the crop (Catunda et al., 2005). However, there is a lack of studies that address the physiological responses of plants when the herbicide is applied at the reproductive phase.

Chlorophyll fluorescence emission variables have been used in photosynthesis studies (Catunda et al., 2005), since it is a non-destructive evaluation method and it allows to evaluate qualitatively and quantitatively the absorption and use of light energy through PSII and the probable relations with the photosynthetic capacity (Torres Netto et al., 2005).

According to Galon et al. (2010), there may be marked differences between the genetic materials of the same species, which can be evaluated as for differences in stomatal conductance, transpiration rate, leaf temperature and dry matter accumulation when they are treated with herbicides. According to these authors, the genotype/herbicide association can determine those that stand out and, thus, guarantee better crop yields. Therefore, this research was conducted with the objective of evaluating the gas exchange in Pérola, Vitória, IAC Fantástico and MD2 pineapple plants submitted to the application of diuron at their reproductive phase.

## MATERIAL AND METHODS

The experiment was conducted in a greenhouse built in the chapel model, consisting of antechamber, shade curtain, fixed table, control panel, lighting system, drip irrigation system and clay cooling system. Through the control of the climatological variables, the air relative humidity was maintained close to 65%, and the air temperature varied between 26 and 29 $^{o}$C. During the experiment period, plants received an equal and daily volume of water, just enough to keep the soil moist, but without leach loss. Irrigation was done by dripping, placing a dripper in each pot, with a flow rate of 2 L $h^{-1}$.

The experiment consisted of four pineapple cultivars: Pérola, Vitória, IAC Fantástico and MD2. Micropropagated seedlings were obtained from the company SBW Brasil, in the city of Holambra, São Paulo state. These seedlings were acclimated until reaching the average size of 30 cm, in order to be suitable for planting, as well as to obtain the homogeneity of the material. For each variety studied, two replications were used, totaling eight plants. Seedlings were planted in plastic pots with a capacity of 5 $dm^3$, containing a mixture of subsurface soil, sand and hardened bovine manure as substrate, in the ratio of 3:1:1.

The amounts of used fertilizers were 4 g per plant of $P_2O_5$ during planting, incorporated into the substrate, and 10 g per plant of N and 10 g per plant of $K_2O$, as topdressing, around the plant, in five applications. The first plot was applied 60 days after planting, and the other applications were at intervals of 60 days. Artificial floral induction was performed 360 days after planting, using the commercial product Etrel® at a concentration of 240 g $L^{-1}$ a.i. The used dose was 1,000 mg $L^{-1}$. To prepare the solution, distilled water was added and the amount of 400 mL solution was prepared for eight pineapple plants. Fifty 50 mL of the solution were applied on the center of the leaf rosette to stimulate flowering.

Four days after the floral induction, diuron was applied. The active ingredient diuron (DIURON NORTOX) was used at a dose of 7.5 mL $L^{-1}$. It was placed in the sprayer with water up to 3/4 of its capacity, and then the volume was completed by stirring constantly. Plants were removed from the greenhouse for the application of diuron, which was done using a 20 L manual backpack sprayer, equipped with fan-type nozzles, calibrated for a 5 km $h^{-1}$ flow. Spraying was performed on the total area of each plant.

Measurements of $CO_2$ assimilation rate (A, μmol $CO_2$ $m^{-2}$ $s^{-1}$), stomatal conductance (Gs, mol $m^{-2}$ $s^{-1}$), transpiration (E, mmol $H_2O$ $m^{-2}$ $s^{-1}$) and vapor pressure deficit (VPD, kPa) were made by placing the central part of the "D" leaf in an LI-6400 XT infrared gas analyzer (IRGA) (LI-COR,Lincoln, NE, USA). Through A and E values, the water use efficiency (WUE, mol $CO_2$ mol $H_2O^{-1}$) was calculated, defined by the A/E ratio. Measurements were made using artificial light, with photosynthetically active radiation (PAR) fixed at 500 μmol photons $m^{-2}$ $s^{-1}$, according to the light saturation point of pineapple, considering 12 hours of light and 12 hours of darkness.

Gas exchange measurements were made hourly, on two plants from each variety, in a 24 hour interval, every seven days, totaling six evaluations. Six readings per plant were carried out at each evaluation hour, with an average time of five minutes per plant. The first evaluation was carried out under normal cultivation conditions (without stress application), two days before the application of the herbicide. After assessing and collecting data on IRGA, the rate of $CO_2$ assimilation was determined at four temporal phases of the crassulacean acid metabolism (CAM) [I, II, III, IV], according to Osmond (1978). They were: phase I: night – 6 p.m. to 6 a.m.; phase II: early morning – 6 to 7 a.m.; phase III: day – 7 a.m. to 3 p.m.; and phase IV: late afternoon – 3 p.m. to 6 p.m. Values from each accumulated evaluation refer to the sum of the value found in the phases. The ± standard deviation function was used to measure the dispersion degree of the values in relation to the mean. These values were integrated by calculating the area below the $CO_2$ curve using the Sigma Plot 12.0 software.

The fluorescence measurement was performed after 30 minutes of chloroplast adaptation to the dark so that all PSII reaction centers had the maximum opening condition and to avoid heat loss. Darkening was done with clamps fixed to the leaves, preventing the incidence of light. In the maximum fluorescence emission, a saturating light pulse of 0.3 s was used, under a 0.8 kHz frequency. Measurements were performed at 8 o'clock in the morning on the 'D' leaf, on the same leaf and dates on which gas exchange evaluations were made. Data were interpreted through a descriptive analysis.

## RESULTS AND DISCUSSION

Stress, due to the application of the herbicide, caused a reduction in the photosynthetic rate (Table 1) over the days. On the first evaluation day (under non-stressing conditions), the average value of A for the four evaluated cultivars was 123.72 µmol $CO_2$ $m^{-2}$ accumulated in 24 hours. However, at 36 DAA, $CO_2$ assimilation rate values were lower than those observed in previous evaluations in all cultivars, with a mean of 27.56 µmol $CO_2$ $m^{-2}$. This reduction in the photosynthetic rate values, observed for all cultivars, confirms the indirect effect that diuron has on the $CO_2$ assimilation of pineapple, reinforced by the fact that all studied cultivars showed the same behavior. This indirect effect occurs due to the action of this herbicide on the inhibition of electron transport of the photosynthesis phase (Catunda et al., 2005), which in turn influences the biochemical phase, that is, carbon fixation (Breitenbach et al. , 2001).

Diuron acts on the blocking of the PSII electron passage, reducing the formation of NADPH and ATP (Fuerst and Norman, 1991), necessary for the fixation of $CO_2$ (Breitenbach et al., 2001). The lower flow of electrons can overwhelm the energy in chlorophylls and carotenoids, causing oxidative stress and chlorophyll oxidation (Streit et al., 2005), thus reducing the photosynthetic activity. Similar results were verified by Galon et al. (2010), who observed a reduction of A in sugarcane plants after the application of ametryn, an herbicide that acts on the inhibition of PSII.

It was observed that, up to 15 DAA (Table 1), diuron did not affect the photosynthetic rate, with $CO_2$ fixation occurring normally, presenting values above those observed in the first evaluation at 8 DAA. From 22 DAA, plants presented physiological stress in response to the application of the herbicide, and $CO_2$ fixation gradually decreased until the last evaluation, at 36 DAA.

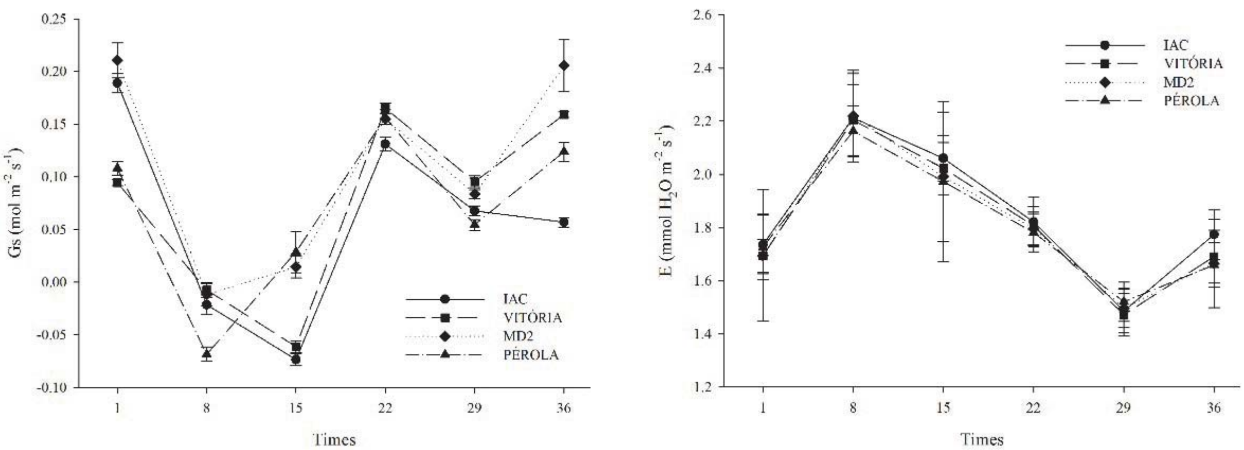
***Table 1*** - Daily $CO_2$ assimilation rates (A, µmol $CO_2$ $m^{-2}$) at each CAM Metabolism phase (I, II, III and IV) for pineapple cultivars IAC, MD2, Vitória, and Pérola, before and after the application of diuron at their reproductive phase (4 days after floral induction). Janaúba - Minas Gerais, 2017

| Cultivars | Days after the application of the herbicide | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 'IAC' | i* | ±SD | 8 | ±SD | 15 | ±SD | 22 | ±SD | 29 | ±SD | 36 | ±SD |
| Phase I | 54.24 | ±0.2 | 103.68 | ±0.64 | 73.94 | ±00.33 | 41.9 | ±0.74 | 37.05 | ±0.37 | 13.37 | ±0.87 |
| Phase II | 2.19 | ±0.59 | 10.6 | ±0.33 | 4.55 | ±3.24 | 2.74 | ±2.79 | 2.4 | ±1.72 | 1.2 | ±0.18 |
| Phase III | 54.41 | ±2.07 | 51.58 | ±2.22 | 41.14 | ±2.16 | 21.36 | ±0.79 | 20.89 | ±0.78 | 8.92 | ±1.23 |
| Phase IV | 2.19 | ±1.23 | 9.29 | ±0.15 | 8.64 | ±0.94 | 5.01 | ±0.85 | 4.14 | ±0.19 | 0.46 | ±1.08 |
| Total | 113.03 | | 175.15 | | 128.27 | | 71.01 | | 64.48 | | 23.95 | |
| 'MD2' | i* | ±DP | 8 | ±DP | 15 | ±DP | 22 | ±DP | 29 | ±DP | 36 | ±DP |
| Phase I | 68.97 | ±0.87 | 107.09 | ±0.48 | 85.7 | ±1.26 | 60.16 | ±0.80 | 50.71 | ±0.77 | 23.37 | ±0.76 |
| Phase II | 6.89 | ±0.18 | 10.51 | ±0.35 | 5.19 | ±4.04 | 2.98 | ±2.32 | 2.66 | ±1.14 | 1.07 | ±1.85 |
| Phase III | 55.36 | ±0.72 | 54.71 | ±1.95 | 39.43 | ±1.83 | 20.8 | ±0.34 | 21.86 | ±1.00 | 7.37 | ±0.88 |
| Phase IV | 4.21 | ±1.08 | 9.25 | ±0.33 | 9.23 | ±0.22 | 3.96 | ±0.29 | 4.78 | ±0.12 | 1.42 | ±0.86 |
| Total | 135.43 | | 181.56 | | 139.56 | | 87.9 | | 80 | | 33.23 | |
| 'Vitória' | i* | ±DP | 8 | ±DP | 15 | ±DP | 22 | ±DP | 29 | ±DP | 36 | ±DP |
| Phase I | 54.52 | ±0.28 | 108.05 | ±0.75 | 77.31 | ±0.40 | 46.13 | ±0.67 | 43.93 | ±0.35 | 16.44 | ±0.42 |
| Phase II | 6.33 | ±0.93 | 10.82 | ±0.00 | 4.71 | ±3.78 | 2.58 | ±1.97 | 2.69 | ±1.41 | 1 | ±1.84 |
| Phase III | 52.65 | ±1.84 | 52.48 | ±2.27 | 41.24 | ±1.93 | 22.88 | ±0.78 | 22.47 | ±1.17 | 7.7 | ±0.83 |
| Phase IV | 2.41 | ±1.45 | 9.22 | ±0.50 | 9.11 | ±0.11 | 3.88 | ±0.35 | 4.4 | ±0.65 | 0.54 | ±0.21 |
| Total | 115.92 | | 180.57 | | 132.37 | | 75.46 | | 73.5 | | 25.67 | |
| 'Pérola' | i* | ±DP | 8 | ±DP | 15 | ±DP | 22 | ±DP | 29 | ±DP | 36 | ±DP |
| Phase I | 63.11 | ±0.90 | 104.99 | ±0.40 | 75.29 | ±0.36 | 62.29 | ±0.75 | 43.07 | ±0.54 | 18.35 | ±0.48 |
| Phase II | 6.88 | ±0.51 | 10.43 | ±0.57 | 4.87 | ±2.96 | 3.72 | ±2.88 | 2.62 | ±1.19 | 1.1 | ±1.76 |
| Phase III | 58.51 | ±1.38 | 55.18 | ±1.89 | 43.03 | ±1.89 | 21.6 | ±0.44 | 23.05 | ±0.97 | 7.09 | ±0.82 |
| Phase IV | 2.04 | ±1.66 | 8.55 | ±0.41 | 7.99 | ±1.07 | 4.15 | ±0.07 | 4.78 | ±0.59 | 0.87 | ±0.08 |
| Total | 130.53 | | 179.15 | | 131.18 | | 91.76 | | 73.52 | | 27.4 | |

i* = initial evaluation, performed two days before the application of diuron. SD = standard deviation. Phase I (night - 6 p.m. to 6 a.m.), phase II (early morning - 6 a.m. to 7 a.m.), phase III (day - 8 a.m. to 3 p.m.) and phase IV (late afternoon - 4 p.m. to 6 p.m.).

In evaluations made before 22 DAA, the ratio of $CO_2$ assimilated in phases I and III was similar for all cultivars. From that date on, proportionally, the assimilation of $CO_2$ in phase I was higher than that of phase III in all cultivars. This indicates that, under a stress condition due to the application of diuron, phase I is more important for the assimilation than phase III (greater assimilation at night). A similar fact was observed by Keller and Luttge (2005) under conditions of water stress. In the last evaluation (36 DAA), there was a sharp reduction in A, with a predominance of $CO_2$ fixation in phase I. It is important to observe that in phase I, cultivars IAC Fantástico, MD2, Vitória and Pérola fixed 55, 82, 70.32, 64.04 and 66.97%, respectively, of the accumulated total during 24 hours of evaluation. The proportion of $CO_2$ captured by PEPCase at night or by Rubisco during the day (net $CO_2$ assimilation) is adjusted by the stomatal behavior, by fluctuations in the accumulation of organic acids and reserve carbohydrates, by the activity of primary (PEPCase) and secondary (Rubisco) carboxylation enzymes, by the activity of decarboxylation enzymes, and by the synthesis and breakdown of carbon $C_3$ skeletons (Taiz and Zeiger, 2013). Keller and Luttge (2005) found an average of 98% of the total amount of $CO_2$ fixed during the night period (phase I) in *Ananas ananassoides* and *Ananas comosus* cv Panare. These authors reported that water stress could be the main reason for the lack of $CO_2$ fixation in phases II and IV.

In the evaluation performed at 8 DAA, a reduction in the stomatal conductance was observed after the application of the herbicide (Figure 1), presenting values close to zero at 8 and 15 DAA. These values increased again at 22, 29 and 36 DAA. In the last evaluation, at 36 DAA, Gs returned to the initial condition in cultivars Vitória, MD2 and Pérola. The application of herbicides reduces stomatal conductance in sensitive plants and, often, in tolerant plants (Torres et al., 2012). This is usually due to a decrease in stomatal opening, which is influenced by several factors, such as VPD, solar radiation, $CO_2$ levels in the mesophyll, relative humidity, water availability, pollution and herbicides (Dias and Marenco 2007; Galon et al. 2010; Torres et al., 2012). As in this experiment water deficit and shading were not limiting factors for the metabolism of pineapple, the results can be attributed to the effect of VPD and herbicide.




Data are the means of two plants per evaluated cultivar and six readings per plant. Janaúba - Minas Gerais, 2017.

***Figure 1*** **-** Stomatal conductance (Gs) and transpiration (E) in IAC, MD2, Vitoria, and Pérola pineapple plants according to the evaluation periods. Period 1: non-stressful condition. Periods 8, 15, 22, 29, and 36 days after the application of diuron.

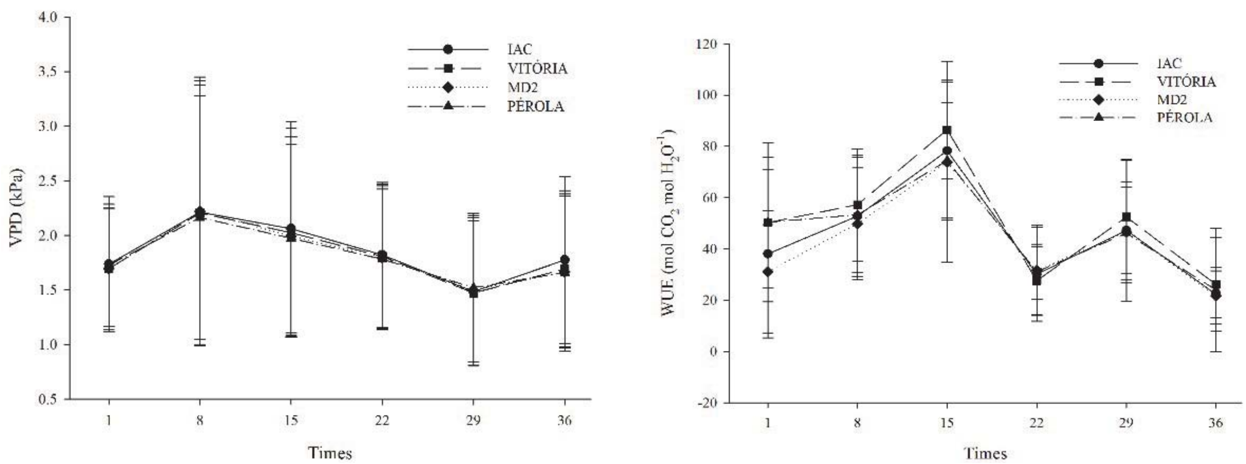
A reduction in stomatal conductance was also observed in RB855156 sugarcane, after the application of the herbicides tembotrione, MSMA, diuron + hexazinone, sulfentrazone, trifloxysulfuron-sodium, tebuthiuron and clomazone (Torres et al., 2012).

Transpiration (E) had the same behavior as stomatal conductance (Figure 1). At 8 DAA, a reduction was observed, with a subsequent increase in the last evaluation at 36 DAA. According to Merotto Junior and Fischer (2004), herbicides that are absorbed by the roots and translocated by the xylem are accumulated in the transpiration organs of plants, reaching sufficient concentrations to circulate through the membrane and exert their phytotoxic action. Thus, diuron is expected to interfere indirectly with transpiration, as it acts on the photosynthetic apparatus (Torres et al., 2012).

In order for the herbicides that are absorbed by the roots to be transported to the leaves, it is necessary that plants are actively transpiring (Araldi et al., 2011), since the transpiration flow is the most important phenomenon in the translocation of herbicides applied onto the soil (Merotto Junior and Fischer, 2004). According to these authors, this process depends on the water potential of soil, plant and atmosphere and has the control of the opening and closing of stomata as a regulating element.

In the last evaluation, the transpiration rate values of pineapple cultivars Vitória, MD2 and Pérola were very close to the non-stressing condition of the first evaluation. This may indicate a lower sensitivity of these cultivars for this characteristic, due to the behavior observed in the studied cultivars. Villalobo et al. (2012), with cultivar MD2, found values ranging from 1.42 to 6.0 mmol $H_2O$ $m^{-2}$ $s^{-1}$. Araldi et al. (2011) observed that the transpiration rate and the water consumption rate were determinant in the absorption of amicarbazone, imazapic, tebuthiuron and hexazinone by plants from different weed species and sugarcane cultivars.

VPD presented an inversely proportional behavior to that of Gs and E (Figure 2). Probably, the increase in VPD caused a decrease in Gs and in E. At 8 DAA, there was a peak in VPD, with values close to 2.2 kPa, and a sharp drop at 29 DAA. Generally speaking, under natural conditions, when air temperature increases, VPD also increases (Machado et al., 2005). Thus, a high VPD can reduce the photosynthetic rate, due to its effect on the closure of stomata, which leads to a reduction in the amount of internal carbon (Dias and Marenco, 2007). During these evaluations, air temperature ranged from 28.8 °C (8 DAA) to 26.5 °C (29 DAA) and, together with the action of the herbicide, it probably contributed to the reduction of stomatal opening.

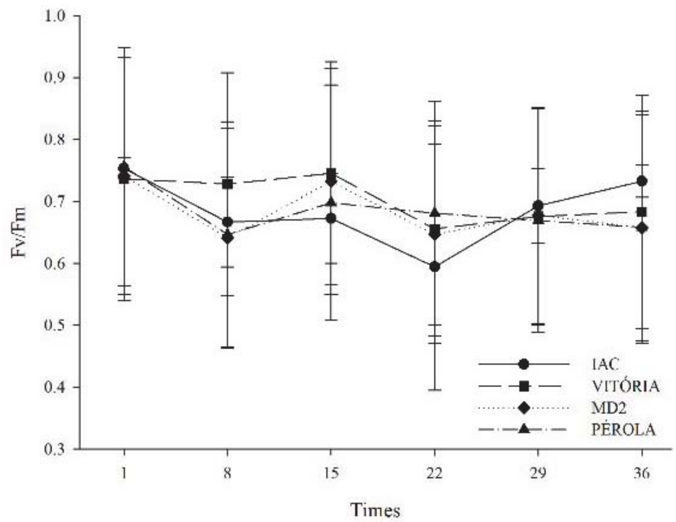



Data are the means of two plants per evaluated cultivar and six readings per plant. Janaúba - Minas Gerais, 2017.

***Figure 2 -*** Vapor pressure deficit (VPD) and water use efficiency (WUE) in IAC, MD2, Vitoria, and Pérola pineapple plants according to the evaluation periods. Period 1: non-stressful condition. Periods 8, 15, 22, 29, and 36 days after the application of diuron.

Water use efficiency (WUE) varied according to the pineapple cultivar (Figure 2). It was observed that plants had a higher capacity to revert the volume of consumed water in dry matter production at 15 DAA. At that time, one of the lowest transpiration rates was recorded. Since water use efficiency is a physiological parameter that depends on the $CO_2$ assimilation rate and on transpiration, and during the experimental period the A value still remained low without indicating recovery, the reduction in water use efficiency can be explained by the reduction in the photosynthetic rate of plants. From 22 DAA, the average QUE value was 30 mol $CO_2$ mol $H_2O^{-1}$ among the tested cultivars. Generally, a CAM plant loses from 50 to 100 g of water for each g of gained $CO_2$ (Taiz and Zeiger, 2013). A lower WUE was also observed after the application of ametryn on sugarcane, in a study by Galon et al. (2010). Stress depends on the characteristics of the product (mode of application and classification), culture (cultivar or hybrid, development phase, nutrition, water balance) and the environment (Oliveira Júnior, 2001; López-Ovejero et al., 2003).

Photochemical efficiency values ($F_v/F_m$) showed a similar tendency among the cultivars tested in the first evaluation (Figure 3). During this period, plants were under normal cultivation conditions and presented average values of 0.74 to 0.76. Some authors state that, under optimum conditions, these $F_v/F_m$ values range from 0.75 to 0.85. Other authors claim that the ideal values are close to 0.80. However, the decrease of this ratio is an indicator of photoinhibitory effect in plants subjected to chemical stress (Araus and Hogan, 1994); this was evidenced in this work.




Data are the means of two plants per evaluated cultivar and six readings per plant. Janaúba - Minas Gerais, 2017.

***Figure 3*** **-** Mean values of photochemical efficiency ($F_v/F_m$) in IAC, MD2, Vitória, and Pérola pineapple plants according to the evaluation periods. Period 1: non-stressful condition. Periods 8, 15, 22, 29, and 36 days after the application of diuron.

After the application of diuron, there was a reduction in the photochemical efficiency of PSII in pineapple plants. In the IAC cultivar, the fall occurred on day 8, recovering from day 29 on and returning to the initial values in the last evaluation. In the Vitoria cultivar, the reduction occurred from day 22, without recovery. Cultivar MD2, with the exception of day 15, presented a decrease on the other dates in the $F_v/F_m$ ratio. Cultivar Pérola presented a decrease in all evaluations.

It was possible to observe that cultivar Vitoria had values of 0.74, close to those of the period without stress, until 15 DAA, when the other cultivars already presented values that were well below. The lowest values of the $F_v/F_m$ ratio occurred at 8 DAA in MD2 and Pérola plants, with values of 0.64 and 0.65, respectively, while in cultivars IAC and Vitória the lowest values (0.59 and 0.66, respectively) were observed at 22 DAA.

Evaluations made after the application of diuron presented mean values of the quantum yield of photosystem II ($F_v/F_m$) that were lower than what was observed initially (under non-stressing conditions), suggesting a reduction in the electron flow; this means that there was a reduction in the amount of energy used by the plant to perform the photochemical processes, such as NADPH reduction (Catunda et al., 2005). Moreover, the binding site on $D_1$ protein is sensitive to many classes of chemical products with different structural characteristics, such as substituted ureas, diuron chemical group (Camilleri et al., 1987).

At the end of the experimental period, cultivar IAC recovered its maximum quantum efficiency of photosystem II, reaching values that were close to the one observed in the first evaluation. This result indicates a probable recovery of this cultivar. Although there was no recovery of the carbon assimilation rates, the $F_v/F_m$ values observed in the last evaluation of cultivar IAC Fantástico indicate a probable tolerance of this cultivar and possibly that its carbon assimilation rates will tend to return to normal values quicker than the other studied cultivars.

In the pineapple crop, Cruz et al. (2014) found that the application of diuron and sulfentrazone at high doses reduced the efficiency of the photosystem II of Imperial pineapple seedlings not inoculated with the *Piriformospora indica* fungus. Vieira et al. (2010) found $F_v/F_m$ values between 0.71 and 0.74 in plants from the cultivar Pérola submitted to 0, 15, 30 and 60 mg $kg^{-1}$ of ammonium sulphate. Catunda et al. (2005) found $F_v/F_m$ values of 0.8 in pineapple plants under conditions of no stress and a ratio of 0.66 and 0.19 when applying amicarbazone and diuron + paraquat on plants, respectively. These authors observed a reduction in the $F_v/F_m$ ratio five days after the application of the herbicide, but after 10 days, the $F_v/F_m$ ratio increased again.

Generally speaking, the physiological variables that were most affected by the application of the herbicide on pineapple were the $CO_2$ assimilation rate, the water use efficiency and the photochemical efficiency ($F_v/F_m$). It was possible to observe that the stomatal conductance and transpiration variables were also affected by VPD and, probably, the action of the herbicide was temporary. Cultivar IAC shower a greater reduction in E, Gs, and A values. In this cultivar, however, the physiological variable $F_v/F_m$ returned to its initial condition on the last day of evaluation (36 DAA).

Results showed that the application of diuron reduces the assimilation of $CO_2$ in the IAC Fantástico, Vitória, MD2 and Pérola pineapple varieties at their reproductive phase, at all CAM metabolism phases. The application of diuron promotes a temporary reduction in stomatal conductance and transpiration and a temporary increase in water use efficiency in the IAC Fantástico, Vitória, MD2, and Pérola pineapple varieties at their reproductive phase. It was observed that the photochemical efficiency of most of the studied pineapple cultivars is negatively affected by diuron. However, cultivar IAC Fantástico reestablishes the initial values of its photochemical efficiency 36 days after the application of diuron.

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