







## Article

RAPOSO JR., R.<sup>1</sup>  
PEREIRA, G.A.M.<sup>2</sup>   
MATOS, C.C.<sup>2</sup>   
BARCELLOS JR., L.H.<sup>2\*</sup>   
PAULA, D.F.<sup>2</sup>  
SILVA, A.A.<sup>2,3</sup> 

## USE OF 2,4-D IN MIXTURE WITH ETHEPHON AND CALCIUM CARBIDE IN PINEAPPLE CROP

*Uso do 2,4-D em Mistura com o Ethephon e Carbureto de Cálcio na Cultura do Abacaxi*

**ABSTRACT** - The 2-Chloroethylphosphonic acid (ethephon) and calcium carbide are used to induce and synchronize the flowering of pineapple crop. Some farmers have used the 2,4-D herbicide in combination with these flowering inducers. This practice is based on the hypothesis that 2,4-D would act as a growth regulator, increasing fruit size and weight. The objectives of this work was to verify if the use of 2,4-D, associated to calcium carbide or ethephon, influences the productivity and quality of the pineapple fruits and, also if the use of this herbicide leaves residues in the fruits. For this, two field experiments were carried out in two different farms. The treatments consisted of the application of ethephon and calcium carbide, alone, and in a mixture with two, four and six drops of 2,4-D in the center of the leaflet of the pineapple cv. "Pérola" at 10 months after planting. The use of 2,4-D mixed with calcium carbide or ethephon, regardless of the dose and culture environment, did not influence the size, weight, total soluble solids content, density and pH of the pineapple fruits. However, depending on the growing environment, ethephon + 2,4-D mixtures did not influence or reduce flowering and crop yield. No 2,4-D residues were found in the fruit pulp of pineapple. It was concluded that the use of 2,4-D as a growth regulator of pineapple under the conditions evaluated does not incorporate qualitative or quantitative benefits to the productive system of this crop. In addition, no 2,4-D residues were found in the fruit pulp of pineapple.

**Keywords:** *Ananas comosus*, dichlorophenoxyacetic acid, floral inducer, plant growth regulator.

**RESUMO** - O ácido 2-cloroetilfosfônico (ethephon) e o carbureto de cálcio são usados para induzir e sincronizar a floração da cultura do abacaxi. Alguns produtores têm utilizado, em associação com esses indutores do florescimento, o herbicida 2,4-D. Essa prática baseia-se na hipótese de que o 2,4-D atua como regulador de crescimento, aumentando o tamanho e peso dos frutos. Diante disso, os objetivos deste trabalho foram verificar se a utilização do 2,4-D, associado ao carbureto de cálcio ou ao ethephon, influencia a produtividade e qualidade dos frutos do abacaxizeiro e, também, se o uso desse herbicida deixa resíduos nos frutos. Para isso, foram realizados dois experimentos de campo em duas fazendas. Os tratamentos consistiram da aplicação de ethephon e carbureto de cálcio, isoladamente e em mistura com duas, quatro e seis gotas de 2,4-D no centro da roseta foliar do abacaxizeiro cv. Pérola aos 10 meses após o seu plantio. O uso de 2,4-D em mistura com o carbureto de cálcio ou o ethephon, independentemente da dose e do ambiente de cultivo, não influenciou o tamanho, peso, teor de sólidos solúveis totais, densidade e pH dos frutos de abacaxi. Todavia, dependendo do ambiente de cultivo, as misturas de ethephon + 2,4-D não influenciaram ou

\* Corresponding author:  
<[lucasherjngbrj@hotmail.com](mailto:lucasherjngbrj@hotmail.com)>

Received: January 30, 2019  
Approved: March 25, 2019

Planta Daninha 2019; v37:e019219257

**Copyright:** This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided that the original author and source are credited.



<sup>1</sup> Fiscal Estadual Agropecuário da Agência Estadual de Defesa Agropecuária do Maranhão, São Francisco, São Luís-MA, Brasil; <sup>2</sup> Universidade Federal de Viçosa, Viçosa-MG, Brasil; <sup>3</sup> Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Brasília-DF, Brasil.

*reduziram o florescimento e a produtividade da cultura. Não foram encontrados resíduos do 2,4-D na polpa dos frutos do abacaxi. Concluiu-se que o uso de 2,4-D associado ao carbureto de cálcio ou ao ethephon como regulador de crescimento do abacaxi, nas condições avaliadas, não incorpora benefícios qualitativos ou quantitativos ao sistema produtivo dessa cultura. Além disso, não foram encontrados resíduos de 2,4-D na polpa dos frutos de abacaxi.*

**Palavras-chave:** *Ananas comosus*, ácido diclorofenoxiacético, indutor floral, regulador de crescimento vegetal.

## INTRODUCTION

Artificial induction of flowering is a common cultural practice in commercial pineapple plantations [*Ananas comosus* (L.) Merrill]. The main purpose of this treatment is to induce early and uniform flowering, and to facilitate and concentrate the harvesting of the fruit in commercially favorable periods (Turnbull et al., 1999; Cunha, 2005). Chemical substances, growth regulators or phytohormones have been widely used in the induction of pineapple flowering (Das, 1965; Onaha et al., 1983; Cunha, 2005; Liu et al., 2018).

Ethephon (2-chloroethylphosphonic acid) and calcium carbide ( $\text{CaC}_2$ ) are the main artificial inducers of pineapple flowering. These chemicals decompose inside the plant tissue, releasing ethylene and acetylene, respectively, which induces flowering (Turnbull et al., 1999; Manica, 2000; Abeles et al., 2012).

2,4-Dichlorophenoxyacetic acid (2,4-D) is one of the oldest and widely used herbicides in the world. Similar to plant hormones, sublethal doses of 2,4-D can stimulate plant growth (Grossmann, 2010; Song, 2014). In addition, they present the same effect in regulating plant growth as endogenous natural auxins do (Velini et al., 2010). The doses are used to control ripening in fruits, reduce falls, increase fruit size, as well as improve crop yield and prolong harvesting, as reported in citrus (Velini et al., 2010; Roa et al., 2015; Mollapur et al., 2016; Nartvaranant, 2018), pomegranate (Gosh et al., 2009) and kiwi (Nazir et al., 2018).

2,4-D is also used in tissue cultures to induce somatic embryogenesis of plant cells (Gaspar et al., 1996; Mostafiz and Wagiran, 2018). Plant growth regulators such as 2,4-D induce pineapple flowering by increasing the content of ethylene in the meristematic zone of the plant (Burg and Burg, 1966; Abeles, 1968; Grossmann, 2010), where the absorption of products is faster (Cunha, 2005).

2,4-D has been used in combination with calcium carbide or ethephon. This mixture has been applied in the center of the pineapple leaf rosette ("eye" of the plant) during the artificial induction process of flowering performed by some producers in Maranhão state, Brazil. This procedure aims to increase crop productivity by using the 2,4-D as a growth regulator, leading to an increase in size and weight of the pineapple fruits. However, there is no scientific evidence of the effects caused by 2,4-D on the pineapple or the presence of this herbicide residues in the fruits after the treatments.

According to the Brazilian plant defense system, the presence of 2,4-D residue in pineapple fruit is not allowed, because this herbicide is not registered to be used in this crop, either as a growth regulator or herbicide, set out in the Ministry of Agriculture, Livestock and Supply (MAPA). Therefore, this work aims to investigate the effects on the productivity and quality of pineapple fruits containing a mixture of 2,4-D with calcium carbide or ethephon, and also if this mixture leaves residues in the fruits after its use.

## MATERIAL AND METHODS

### Experimental conditions

The experiments were conducted in two pineapple farms, located in São Domingos do Maranhão, in Maranhão state, Brazil. Condurú farm (farm I), and Baixa da Areia farm (farm II) presented similar productive management systems. The region where the farms are located is

dominated by Red-Yellow Latosol, a soil with medium fertility (Moura, 2004). The physical and chemical properties of the soil samples collected in the farms are shown in Table 1. According to Köppen and Geiger, the climate is classified as Aw. The average annual temperature is 26.7 °C, and about 1,174 mm of *precipitation* falls *annually*.

**Table 1** - Physical and chemical properties of soils of Condurú farm (I) and Baixa da Areia farm (II)

Soil attribute	Unit	Farm I	Farm II
	Chemical		
Organic Matter	g dm <sup>-3</sup>	26.0	18.0
pH	pH	4.5	4.1
Phosphorus	mg dm <sup>-3</sup>	6.0	4.0
Potassium	mmol <sub>c</sub> dm <sup>-3</sup>	3.3	1.5
Calcium	mmol <sub>c</sub> dm <sup>-3</sup>	24.0	15.0
Magnesium	mmol <sub>c</sub> dm <sup>-3</sup>	9.0	6.0
Sum of Bases	mmol <sub>c</sub> dm <sup>-3</sup>	36.3	22.5
potential acidity	mmol <sub>c</sub> dm <sup>-3</sup>	25.0	34.0
Cation Exchange Capacity (CEC)	mmol <sub>c</sub> dm <sup>-3</sup>	61.3	55.5
base saturation	%	59.0	40.0
Potassium in CEC	%	5.4	2.7
Magnesium in CEC	%	14.7	10.6
	Physical		
Coarse sand (2 - 0.2 mm)	%	4.0	20.0
Thin sand (0.02 - 0.05)	%	40.0	43.0
Silt (0.05 - 0.002)	%	26.0	15.0
Clay (< 0.002)	%	30.0	22.0
Conductivity at 25 °C	dS m <sup>-1</sup>	0.16	0.11
H <sub>2</sub> O in saturation paste	%	38.4	32.4

\* Soil samples collected on the flowering induction day of pineapple plants, by farm.

In these areas, pineapple cultivated plots in a development stage (7-15 months of age) were selected in order to induce artificial flowering (Cunha, 2005). In both farms, the planting pits were dug at a spacing of 1.00 x 0.40 x 0.30 m, in two rows. Areas measuring 16 x 6 m were demarcated in each of the farms. The area corresponding to each location was 2 x 2 m.

### Experimental design

The experiments were carried out in a randomized block design with eight treatments and three replications. The treatments consisted of: calcium carbide (T1), calcium carbide + 7.62 g ha<sup>-1</sup> of 2,4-D (T2), calcium carbide + 15.24 g ha<sup>-1</sup> of 2,4-D (T3), calcium carbide + 22.86 g ha<sup>-1</sup> of 2,4-D (T4), ethephon (T5), ethephon + 7.62 g ha<sup>-1</sup> of 2,4-D (T6), ethephon + 15.24 g ha<sup>-1</sup> of 2,4-D (T7) and ethephon + 22.86 g ha<sup>-1</sup> of 2,4-D (T8).

### Preparation of the solutions and their application

The preparation of the solutions containing calcium carbide and ethephon, as well as the dose applied per plant, was performed according to Reinhardt and Cunha (2013). 25 g of calcium carbide was firstly diluted in 5 L of clean and cold water. 2.5 mL of Ethrel® (ethephon) was diluted in a solution containing 5 L of water, 100 g of urea (2%) and 1.75 g of calcium hydroxide. Two, four or six drops of Aminol® (670 g and L<sup>-1</sup> of 2,4-D) was added to the water in the treatments that presented 7.62, 15.24 and 22.86 g ha<sup>-1</sup> of 2,4-D, respectively. This was then followed by the addition of calcium carbide and ethephon as described. The doses of 2,4-D used in the treatments were the same as commonly used by producers from São Domingos do Maranhão-MA.

After the dilution of the calcium carbide or ethephon, 50 mL of this solution was applied per plant, depending on the treatment. The solution jets were applied in the center of the pineapple rosette centre ("eye") (Reinhardt and Cunha, 2013), using a 20 L using hand sprayer with a piston pump.

The Induction treatments on flowering in pineapple plants were performed 10 months after planting, between 5h05 and 7h07.

### **Harvesting and fruit sampling**

The pineapple crop was kept free from weed competition and irrigated using a micro sprinkler system. The fruit harvesting was done 155 and 149 days after the application of the treatments (DAT), at farms I and II, respectively.

Four pineapple fruits were harvested in each farm to perform physico-chemical analysis. The sample size was worked out using the sample calculation for a finite population, using Win Episcopo 2.0 software (Blas et al., 2004). A maximum error of 5% and a population standard deviation of 0.25, and a confidence level of 95% were determined for this calculation.

The fruits at the internal planting rows were randomly collected, alternating from four to six plants to each fruit collected. During fruit harvesting, the stalk length was determined using a tape measure. The fruits were then identified, stored in expanded polystyrene thermal boxes containing recyclable ice bars and transported to the Laboratory of Technology for Physical and Chemical Analysis of Water and Food of the State University of Maranhão (UEMA), to perform the physico-chemical analysis.

### **Physico-chemical analysis of the fruits**

The physicochemical characteristics of the pineapple fruits evaluated were: crown length, shape, weight, average length and diameter of the fruit, pH, density, soluble solids content and concentration of 2,4-D residues.

Fruit and crown weights were measured using an analytical balance (5 g). Fruit and crown lengths were determined using a tape measure. The average diameter of the fruit was measured using a caliper.

After physical analysis, the fruits were fractionated and 250 g portions of pulp were collected and refrigerated. Part of the pulp was previously processed according to AOAC (1997), followed by measurements of pH, total soluble solids (SST) and fruit density. The fruit pulps from the treatments with 22.86 g ha<sup>-1</sup> of 2,4-D (T4 and T8) from farm I (Condurú farm) were used for the analysis of herbicide residues.

The soluble solids content was quantified using a digital refractometer with an integrated temperature correction model HI96822 from HANNA/INST. Fruit density was determined using a handheld refractometer, model Q767-5, from QUIMIS (Instituto Adolfo Lutz, 2008). The pH was measured using a pH meter, model HI 221/HANNA INST (Instituto Adolfo Lutz, 2008).

The presence of 2,4-D residues was determined by the multi-waste method, which is based on the extraction of different pesticide residues in food by organic solvents. A liquid-liquid phase separation followed by evaporation and determination of pesticides were performed by gas chromatography coupled to an electron capture detector. For these analyses, an HP 6890 gas chromatograph (Agilent) equipped with an electron capture detector (Ni63), automatic injection system and workstation - ChemStation (Cardoso et al., 2010) was used.

### **Statistical analysis**

Data were submitted to analysis of variance (ANOVA), and the significant factors were submitted to Tukey test ( $p < 0.05$ ). The statistical analyses were performed using R software. The data from the pH, TSS and density of pineapple fruits did not have a normal distribution. A descriptive analysis was also performed on them.

## RESULTS AND DISCUSSION

The effect between the location and the flowering induction treatments on inflorescence formation, stalk length, fruit crown length and pineapple yield (Table 2) was significant (Table 2). This allowed a comparison between the averages within each location and flowering inducers used.

**Table 2** - Pineapple Fruit Crowns Length cv. Pérola, cultivated at Condurú Farm (I) and Baixa da Areia Farm (II), after application of flowering inducers, calcium carbide and ethephon, alone or in combination with 2,4-D

Treatment	Farms	
	I	II
	Inflorescence formation (%)	
T1- Calcium carbide	89.00 aA	100.00 aA
T2- Calcium carbide + 7.62 g ha <sup>-1</sup> of 2,4-D	98.92 aA	95.19 abA
T3- Calcium carbide + 15.24 g ha <sup>-1</sup> of 2,4-D	98.81 aA	96.32 aA
T4- Calcium carbide + 22.86 g ha <sup>-1</sup> of 2,4-D	96.55 aA	96.66 aA
T5- Ethephon	90.48 aA	91.07 abA
T6- Ethephon + 7.62 g ha <sup>-1</sup> of 2,4-D	98.72 aA	58.89 cB
T7- Ethephon + 15.24 g ha <sup>-1</sup> of 2,4-D	94.00 aA	77.44 bB
T8- Ethephon + 22.86 g ha <sup>-1</sup> of 2,4-D	91.75 aA	45.91 cB
VC (%)	7.79	
	Stalk length (cm)	
T1- Calcium carbide	51.48 aA	51.68 aA
T2- Calcium carbide + 7.62 g ha <sup>-1</sup> of 2,4-D	35.00 cB	40.60 bA
T3- Calcium carbide + 15.24 g ha <sup>-1</sup> of 2,4-D	31.06 cdB	40.70 bA
T4- Calcium carbide + 22.86 g ha <sup>-1</sup> of 2,4-D	29.00 dB	40.51 bA
T5- Ethephon	44.35 bA	47.06 aA
T6- Ethephon + 7.62 g ha <sup>-1</sup> of 2,4-D	28.96 dB	40.56 bA
T7- Ethephon + 15.24 g ha <sup>-1</sup> of 2,4-D	28.58 dB	39.55 bA
T8- Ethephon + 22.86 g ha <sup>-1</sup> of 2,4-D	33.55 cdB	39.90 bA
VC (%)	5.25	
	Fruit crown length (cm)	
T1- Calcium carbide	17.70 aA	17.45 abcA
T2- Calcium carbide + 7.62 g ha <sup>-1</sup> of 2,4-D	13.20 bB	18.70 abA
T3- Calcium carbide + 15.24 g ha <sup>-1</sup> of 2,4-D	14.83 abB	18.87 abA
T4- Calcium carbide + 22.86 g ha <sup>-1</sup> of 2,4-D	14.08 abB	19.96 aA
T5- Ethephon	16.87 abB	20.00 aA
T6- Ethephon + 7.62 g ha <sup>-1</sup> of 2,4-D	15.62 abA	15.91 bcA
T7- Ethephon + 15.24 g ha <sup>-1</sup> of 2,4-D	14.25 abA	16.58 abcA
T8- Ethephon + 22.86 g ha <sup>-1</sup> of 2,4-D	14.29 abA	14.61 cA
VC (%)	8.74	

Means followed by the same letter, uppercase letter on the line and lowercase letter on the column, are not significantly different by Tukey's test at 5% probability. VC = Variation of coefficient.

At farm I, the use of 2,4-D in combination with flowering inducers did not influence the inflorescence formation of pineapple plants, with an average flowering rate of 95% (Table 2). At farm II, the use of 2,4-D in combination with ethephon reduced the pineapple inflorescence formation (Table 2). In this farm, the lowest flowering percentages were observed in plants after the application of ethephon + 7.62 g ha<sup>-1</sup> of 2,4-D (58.89%) and ethephon + 22.86 g ha<sup>-1</sup> of 2,4-D (45.91%). On the other hand, the association between herbicide and calcium carbide provided a flowering rate similar to the control treatment (T1).

The inflorescence formation in pineapple was greater after the application of a mixture of ethephon with 2,4-D (T6, T7 and T8) in the pineapples from farm I (Table 2).

The length of the pineapple peduncle varied from 28.58 to 51.68 cm, depending on the treatment applied (Table 2). The results showed that 2,4-D caused a reduction in the peduncle length of the plant on both farms, regardless of the dose applied.

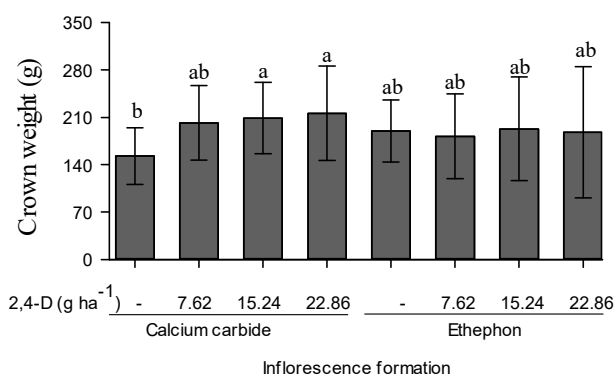
After treatment with the mixtures of 2,4-D with calcium carbide or ethephon, plants from farm I presented a smaller peduncle (Table 2). Plants after the application of 2,4-D (5 to 10 mg L<sup>-1</sup>, 50 mL per plant), in the center of the rosette, prior to ethephon use, produced fruits with a short peduncle (Castro, 1998).

Although our study described the application of 2,4-D in a mixture and not prior to the application of ethephon or calcium carbide as reported by Castro (1998), this study presented similar results to the ones currently obtained.

The crown length was longer in pineapple fruits from farm II when the following treatments were used: calcium carbide + 7.62 g ha<sup>-1</sup> of 2,4-D, calcium carbide + 15.24 g ha<sup>-1</sup> 2,4-D, calcium carbide + 22.86 g ha<sup>-1</sup> of 2,4-D and ethephon (Table 2).

In farm I, only the use of carbide + 7.62 g ha<sup>-1</sup> of 2,4-D (T2) caused a reduction in crown length when compared to calcium carbide (T1) (Table 2). In farm II, it was observed that plants which were submitted to the application of ethephon (T5) produced fruits with a longer crown length when compared to the pineapple plants after being treated with ethephon + 7.62 g ha<sup>-1</sup> (T6) and ethephon + 22.86 g ha<sup>-1</sup> of 2,4-D (T8).

The mixture of 2,4-D with calcium carbide or ethephon had little influence on the pineapple crown weight (Figure 1). Plants treated with calcium carbide (T1) resulted in fruits with the lowest crown weight. However, this treatment was only statistically different to the results obtained after the treatments with calcium carbide + 15.24 g ha<sup>-1</sup> of 2,4-D (T3) and calcium carbide + 22.86 g ha<sup>-1</sup> of 2,4-D (T4) (Figure 1).



Bars represent mean  $\pm$  standard deviation ( $n = 3$ ). Different letters indicate significant differences between means by Tukey's test ( $p < 0,05$ ).

**Figure 1** - Crown weight of the pineapple fruit cv. Pérola submitted to application of flowering inducers: calcium carbide (T1), calcium carbide + 7.62 g ha<sup>-1</sup> of 2,4-D (T2), calcium carbide + 15.24 g ha<sup>-1</sup> of 2,4-D (T3), calcium carbide + 22.86 g ha<sup>-1</sup> of 2,4-D (T4), ethephon (T5), ethephon + 7.62 g ha<sup>-1</sup> de 2,4-D (T6), ethephon + 15.24 g ha<sup>-1</sup> de 2,4-D (T7) e ethephon + 22.86 g ha<sup>-1</sup> of 2,4-D (T8).

(Table 1). Higher contents of N, P and K increases the average weight of the pineapple fruit (Guarçoni and Ventura, 2011). In addition, depending on the stage of crop development, variations in temperature and precipitation may also influence the growth of the pineapple fruit (Azevedo et al., 2007; Williams et al., 2017).

Pineapple yield varied from 22.74 to 61.77 t ha<sup>-1</sup>, based on the treatment applied and location of its production (Table 4). Mixtures of 2,4-D with flowering inducers showed no effect on the

The application of ethephon and calcium carbide with or without the addition of 2,4-D did not influence the length, diameter and weight of the pineapple fruits (Table 3). Similarly, the application of these flowering inducers, alone and at different concentrations, did not affect the growth of the pineapple fruit (Cunha, 1980). On the other hand, the application of 2,4-D on pomegranate and *Citrus sinensis* plants increased the size and weight of their fruits, probably due to the action of this herbicide in stimulating the cell division and elongation (Ghosh et al., 2009 Roa et al., 2015). The lack of effect of 2,4-D doses on pineapple fruit growth may be a result of the doses applied and/or the time of application of the product. However, these hypotheses still require further investigation.

Regardless of the treatment used for floral induction, the pineapple fruits produced in farm I showed greater length, diameter and weight (Table 3). This may be a result of the higher contents of P, K, Ca and Mg in the soil of the farm, when compared to the soil from farm II

**Table 3** - Length, diameter and weight of the pineapple fruits cv. Pérola, cultivated at Condurú Farm (I) and Baixa da Areia Farm (II), after application of flowering inductors, calcium carbide and ethephon, alone or in combination with 2,4-D

Farm	Length of fruits (cm)	Diameter of fruits (cm)	Weight of fruits (g)
I	42.97 a	10.85 a	1227.9 a
II	35.72 b	10.02 b	1126.4 b
VC (%)	15.30	12.11	14.36

VC = Variation of coefficient.

**Table 4** - Pineapple yield cv. Pérola, cultivated at Condurú Farm (I) and Baixa da Areia Farm (II), after application of flowering inductors, calcium carbide and ethephon, alone or in combination with 2,4-D

Treatment	Yield (t ha <sup>-1</sup> )	
	Farms	
	I	II
T1- Calcium carbide	43.79 aA	50.66 aA
T2- Calcium carbide + 7.62 g ha <sup>-1</sup> of 2,4-D	52.71 aA	49.79 aA
T3- Calcium carbide + 15.24 g ha <sup>-1</sup> of 2,4-D	61.77 aA	53.17 aA
T4- Calcium carbide + 22.86 g ha <sup>-1</sup> of 2,4-D	58.28 aA	56.69 aA
T5- Ethephon	53.91 aA	53.71 aA
T6- Ethephon + 7.62 g ha <sup>-1</sup> of 2,4-D	62.57 aA	29.57 bB
T7- Ethephon + 15.24 g ha <sup>-1</sup> of 2,4-D	56.57 aA	39.84 abB
T8- Ethephon + 22.86 g ha <sup>-1</sup> of 2,4-D	53.88 aA	22.74 bB
VC (%)	13.26	

Means followed by the same letter, uppercase letter on the line and lowercase letter on the column, are not significantly different by Tukey's test at 5% probability. VC = Variation of coefficient.

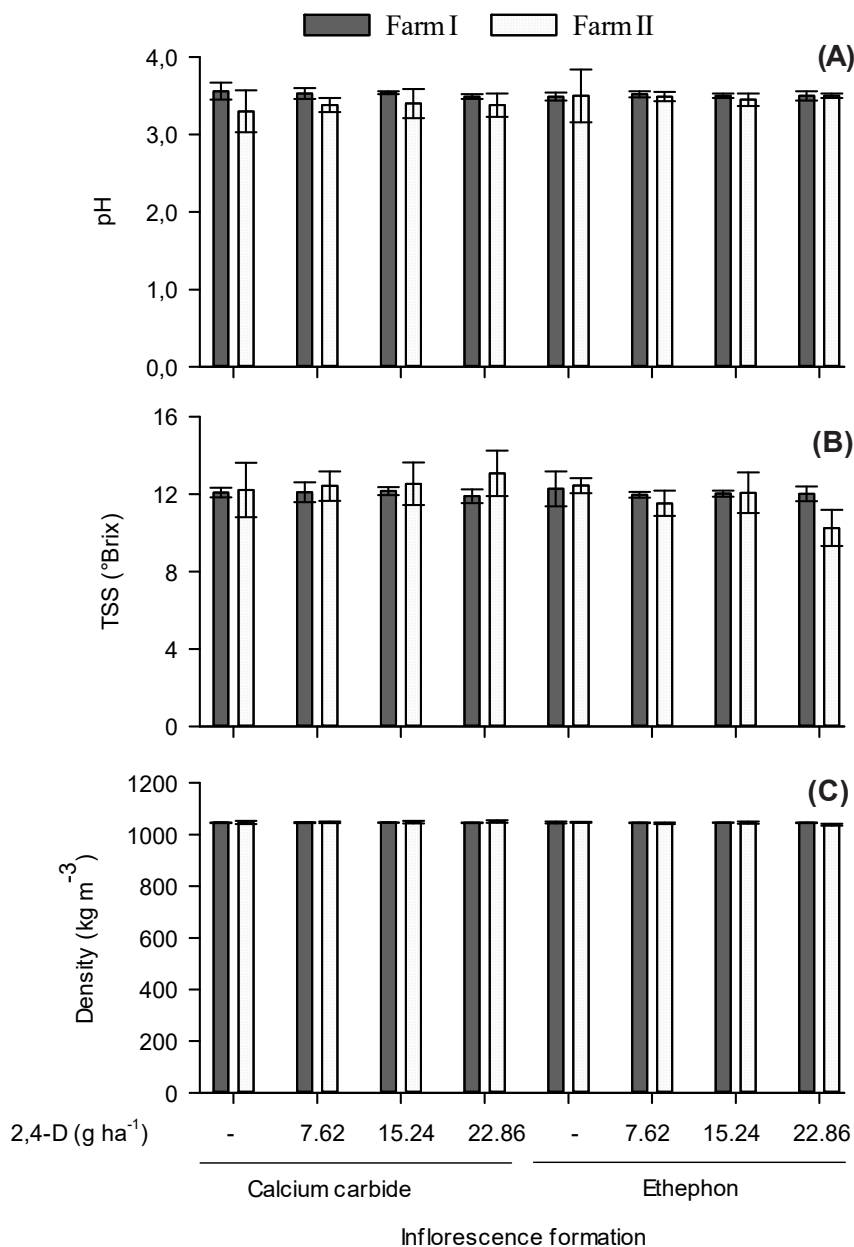
pineapple yield from farm I. However, in farm II, the application of ethephon + 7.62 g ha<sup>-1</sup> of 2,4-D (T6) and ethephon + 22.86 g ha<sup>-1</sup> of 2,4-D (T8) caused a reduction in the yield of this crop (Table 4).

The pineapple yield was higher in farm I, when compared to farm II after treatments with the mixture of ethephon with 2,4-D (Table 4). This could be a result of the differences in the chemical and physical properties of both soils (Table 1) at the climatic conditions and crop treatments applied. These differences in properties of the soils may have increased the pineapple sensitivity to the 2,4-D, leading to reductions in inflorescence formation and, consequently, the productivity of this crop. Furthermore, differences in the chemical interaction between 2,4-D mixtures and the flowering inductors were also observed, since unlike ethephon, the association of this herbicide with calcium carbide did not influence flowering and the yield of this crop, regardless of the growing environment.

The location and the application of flowering inductors with or without 2,4-D did not cause any effect on the pH of the pineapple fruits (Figure 2A). The average pH value of fruits from farm I was 3.52, and 3.43 from the fruits of farm II, which are in the range of pH values usually found on pineapple fruits from variety "Pearl" (3.55-3.97) (Bengozi et al., 2007; Berilli et al., 2014).

The results showed SST content of the pineapple fruits ranging from 10.25 to 13.08 °Brix (Figure 2B). This low variation in the TSS content corroborates with the results observed by Cunha (1980) after single applications (without mixtures) of 2,4-D, calcium carbide and ethephon in pineapple Pérola cultivar. Similarly, these flowering-inducing substances did not affect the SST levels of pineapple cultivars Sugarloaf (Norman, 1975). On the other hand, the 2,4-D increased the TSS levels in pomegranate fruits (Ghosh et al., 2009) and kiwi cv. Hayward (Nazir et al., 2018). It also increased sugar levels in sweet oranges of the *Salustiana* cultivar (*Citrus sinensis*) (Roa et al., 2015).

The use of calcium carbide, ethephon and a mixture with 2,4-D did not affect the pineapple fruit density produced in both farms (Figure 2C). Values of density can be used to measure fruit ripening (Bengozi et al., 2007). The fruit is still green when it has a density value close to



Bars represent mean  $\pm$  standard deviation ( $n = 3$ ).

**Figure 2** - pH (A), total soluble solids - TSS (B) and density (C) of pineapple fruits pulp cv. Pérola, cultivated at Condurú Farm (I) and Baixa da Areia Farm (II), submitted to application of flowering inducers: calcium carbide (T1), calcium carbide + 7.62 g ha<sup>-1</sup> of 2,4-D (T2), calcium carbide + 15.24 g ha<sup>-1</sup> of 2,4-D (T3), calcium carbide + 22.86 g ha<sup>-1</sup> of 2,4-D (T4), ethephon (T5), ethephon + 7.62 g ha<sup>-1</sup> de 2,4-D (T6), ethephon + 15.24 g ha<sup>-1</sup> de 2,4-D (T7) e ethephon + 22.86 g ha<sup>-1</sup> of 2,4-D (T8).

974.0 kg m<sup>-3</sup>. Furthermore, it is ripe when a density value of around 1,012.0 kg m<sup>-3</sup> is recorded (Pantastico, 1975). The results showed an average of 1,046.31 and 1,046.30 kg m<sup>-3</sup> of the pineapple fruits produced on farms I and II, respectively (Figure 2C). This suggests that the fruits were in an appropriate stage of maturation. Therefore, the use of 2,4-D did not influence the ripening stage of the pineapple fruits.

Safety is the most desirable quality attribute in food. It is very important for any food product to be free of any contamination that may compromise the consumer's health (Gonçalves and Carvalho, 2000). The results showed that no 2,4-D residues were found in the pineapple fruit pulp (Table 5). The absence of residues of this herbicide showed that its use in a mixture with calcium carbide or ethephon does not contaminate the fruit. However, 2,4-D did not improve the



**Table 5** - 2,4-D residues in the pineapple fruit pulp cv. Pérola after application of flowering inductors: T4- Calcium carbide + 22.86 g ha<sup>-1</sup> of 2,4-D and T8- Ethephon + 22.86 g ha<sup>-1</sup> of 2,4-D. Maximum residue limits (MRL) nationally accepted by Anvisa and Codex Alimentarius for 2,4-D in other crops

2,4-D* (mg kg <sup>-1</sup> )		MRL (mg kg <sup>-1</sup> )		
T4	T8	Crops	Anvisa**	Codex***
< 0.001	< 0.001	rice, corn, soybean, wheat etc.	0.1 a 0.2	0.01 a 2.00

\* Active ingredient not registered with the Ministério da Agricultura, Pecuária e Abastecimento for pineapple crop. \*\* Agrochemical regularization - Authorized monographs, available at <http://portal.anvisa.gov.br/registros-e-autorizacoes/agrotoxicos/produtos/monografia-de-agrotoxicos/autorizadas>. \*\*\* Pesticide Database. MRL of 2,4-D - Codex Alimentarius. Available at: [http://www.fao.org/fao-who-codexalimentarius/codex-texts/dbs/pestres/pesticide-detail/es/?p\\_id=20](http://www.fao.org/fao-who-codexalimentarius/codex-texts/dbs/pestres/pesticide-detail/es/?p_id=20).

growth and quality of the pineapple fruits. In addition, depending on the growing conditions, the mixture of 2,4-D and ethephon could cause delays in flowering, reducing the crop yield, as observed in farm II (Baixa da Areia farm). From this research, it can be concluded that the use of 2,4-D as a growth regulator of pineapple, under the evaluated conditions, does not improve the quality and quantity of the productive system of this crop.

### ACKNOWLEDGMENT

This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) -Finance Code 001, by the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) and Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG). We thank to Agência de Defesa Agropecuária do Estado do Maranhão - AGED/MA for encouraging research.

### REFERENCES

- Abeles FB. Herbicide-induced ethylene production: role of the gas in sublethal doses of 2,4-D. *Weed Sci.* 1968;16:498-500.
- Abeles FB, Morgan PW, Saltveit Jr ME. Ethylene in plant biology. San Diego: Academic Press; 2012. 339p.
- Association of Official Agricultural Chemists – AOAC. Official methods of analysis of AOAC International. Gaitheersburg: 1997.
- Azevedo PV, Souza CB, Silva BB, Silva VPR. Water requirements of pineapple crop grown in a tropical environment, Brazil. *Agric Water Manag.* 2007;88:201-8.
- Bengozi FJ, Sampaio AC, Spoto MHF, Mischan MM, Pallamin ML. Qualidades físicas e químicas do abacaxi comercializado na CEAGESP São Paulo. *Rev. Bras Frutic.* 2007;29(3):540-5.
- Berilli SS, Freitas SJ, Santos PC, Oliveira JG, Caetano LCS. Avaliação da qualidade de frutos de quatro genótipos de abacaxi para consumo in natura. *Rev Bras Frutic.* 2014;36:503-8.
- Blas I, Ortega C, Frankena K, Noordhuizen J, Thrusfield M. Win Episcopa 2.0. EPIDICON: Borland and Delphi®, 2004.
- Burg SP, Burg EA. Auxin-induced ethylene formation: its relation to flowering in the pineapple. *Science.* 1966;152:1269.
- Cardoso MHW, Gouvêa AV, Nóbrega AW, Abrantes SMP. Validação de método para determinação de resíduos de agrotóxicos em tomate: uma experiência laboratorial. *Cienc Tecnol Aliment.* 2010;30:63-72.
- Castro PR. Utilização de reguladores vegetais na fruticultura, olericultura e plantas ornamentais. Piracicaba: ESALQ/USP; 1998. 91p.
- Cunha GAP. Applied aspects of pineapple flowering. *Bragantia.* 2005;64:499-516.
- Cunha GAP. Efeitos de fitorreguladores na abertura de flores e aspectos qualitativos e quantitativos do abacaxizeiro 'Pérola'. *Pesq Agropec Bras.* 1980;15:423-9.
- Das N. Control of flower and fruit formation of pineapple by applications of auxin and anti-auxin alone and in mixtures. *J Indian Bot Soc.* 1965;9:498-507.

- Gaspar T, Kevers C, Penel C, Greppin H, Reid DM, Thorpe TA. Plant hormones and plant growth regulators in plant tissue culture. *Vitr Cell Develop Biol - Plant*. 1996;32:272-89.
- Ghosh SN. Effect of plant growth regulators in yield and fruit quality in pomegranate cv. Ruby. *J Hortic Sci*. 2009;4:158-60.
- Gonçalves NB, Carvalho VD. Características da fruta. In: Gonçalves NB, editor. *Abacaxi pós colheita*. Brasília: Embrapa Agroindústria de Alimentos; 2000. p.13-27.
- Grossmann K. Auxin herbicides: current status of mechanism and mode of action. *Pest Manag Sci*. 2010;66:113-20.
- Guarçoni A, Ventura JA. Adubação NPK e o desenvolvimento, produtividade e qualidade dos frutos do abacaxi 'Gold' (MD-2). *Rev Bras Cienc Solo*. 2011;35:1367-76.
- Instituto Adolfo Lutz. Métodos físico-químicos para análise de alimentos. São Paulo: 2008. 1020p.
- Liu CH, Liu Y, Shao X-H, Lai D. Comparative analyses of the transcriptome and proteome of comte de Paris and Smooth Cayenne to improve the understanding of ethephon-induced floral transition in pineapple. *Cell Physiol Biochem*. 2018;50:2139-56.
- Manica I. *Abacaxi, do plantio ao mercado*. Porto Alegre: Cinco Continentes; 2000. 122p.
- Mollapur Y, Miri SM, Hadavi E. Comparison of foliar fertilizers and growth regulators on pre-harvest drop and fruit quality of 'Thompson Navel' orange. *Open Agric*. 2016;1:112-7.
- Mostafiz SB, Wagiran A. Efficient callus induction and regeneration in selected indica rice. *Agronomy*. 2018;8:1-18.
- Moura EG. Agroambientes de transição avaliados numa perspectiva de agricultura familiar. In: Moura EG, editor. *Agroambientes de transição – entre o trópico úmido e o semiárido do Brasil*. atributos; alterações; uso na produção familiar. São Luís: UEMA, 2004. p.15-51.
- Nartvaranant P. The influence of exogenously applied 2,4-D and NAA on fruit drop reduction in pummelo cv. Thong Dee. *Int J Fruit Sci*. 2018;18:215-25.
- Nazir N, Sharma MK, Khalil A. Effect of exogenous application of plant growth regulators on vine growth, yield and quality attributes in kiwifruit cv. Hayward. *Indian J Hortic*. 2018;75:153-6.
- Norman JC. The influence of flowering compounds on "sugarloaf" pineapple (*Ananas Comosus* (L.) Merr.) in Ghana. *Acta Horti*. 1975;157-66.
- Onaha A, Nakasone F, Ikemiya H. Induction of flowering with oil-coated calcium carbide in pineapples. *J Japan Soc Hortic Sci*. 1983;52:280-5.
- Pantastico ER. Structure of fruits and vegetables. In: Pantastico EB, editor. *Postharvest physiology handling and utilization of tropical and subtropical fruits and vegetables*. Westport: The AVI Publishing; 1975. p.1-24.
- Reinhardt DHRC, Cunha GA. Controle da floração, in: Sanches N, Matos AP, editores. *Abacaxi, o produtor pergunta, a embrapa responde*. Brasília-DF: Embrapa; 2013. p.102-14.
- Roa AR, García-Luís A, Barcena JLG, Huguet CM. Effect of 2,4-D on fruit sugar accumulation and invertase activity in sweet orange cv. Salustiana. *Aust J Crop Sci*. 2015;9:105-1.
- Song Y. Insight into the mode of action of 2,4-dichlorophenoxyacetic acid (2,4-D) as an herbicide. *J Integr Plant Biol*. 2014;56:106-3.
- Turnbull CGN, Sinclair ER, Anderson KL, Nissen RJ, Shorter AJ, Lanham TE. Routes of ethephon uptake in pineapple (*Ananas comosus*) and reasons for failure of flower induction. *J Plant Growth Regul*. 1999;18:145-52.
- Velini ED, Trindade MLB., Barberis LRM, Duke SO. Growth regulation and other secondary effects of herbicides. *Weed Sci*. 2010;58:351-4.
- Williams PA, Crespo O, Atkinson CJ, Essegbey GO. Impact of climate variability on pineapple production in Ghana. *Agric Food Secur*. 2017;6:1-14.