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# The Effects of the Growth Regulator Paclobutrazol on Physiological Characteristics of Rain Tree (*Albizia saman* Jacq. Merr.)

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## ABSTRACT

The increase of carbon dioxide ($CO_2$) has been identified since the industrial revolution era. *Albizia saman* is a tree species which can absorb excess $CO_2$ from the atmosphere in large quantities. This study was to identify the effect of spraying time and concentration of paclobutrazol on the growth of *A. saman* seedlings. This research using a completely randomized design, the first factor is spraying time which is divided into three levels: spraying at age of 25, 50 and 75 days. The second one is the concentration of paclobutrazol, consisting of four levels: control, 75, 150 and 225 $mol \cdot L^{-1}$. The further test used are DMRT at $p<0.05$. Paclobutrazol influences the higher rate of photosynthesis, namely 38.27 M $CO_2$ $m^{-2}s^{-1}$. There are varied stomatal conductance values, where the highest value is 0.35 mM $m^{-2}s^{-1}$ and the lowest carbon dioxide content in treatment is 56.86 $mol \cdot L^{-1}$. The slowest transpiration rate is the combination of 50 days after treatment, and the concentration of paclobutrazol is 0.24 mM $H_2O$ $m^{-2}s^{-1}$. The shortest growth of *A. saman* is shown from the 50-day treatment using paclobutrazol and 150 $mol \cdot L^{-1}$ concentration. The relationship between photosynthetic rate and stomatal conductance and transpiration concludes that the trend is similar to that of the curve, whereas the trend is not the same as the internal $CO_2$.

**Keywords:** *A. saman,* photosynthesis, paclobutrazol, inhibiting, physiological.

## INTRODUCTION

Global warming has triggered the irregular rainy and dry seasons which cause difficulties in predicting cropping patterns, agricultural production rate, and food stock supplies. Studies found that every 2°C increase in air temperature will reduce China and Bangladesh's agricultural production by 30% later in 2050 [Akbar, Silva 2013]. The increase in carbon dioxide is caused by coal, crude oil, livestock, forest fires, exhaust, and other gases in the air such as methane ($CH_4$), nitrogenous gases or compounds (NO, $NH_3$ and $N_2O$), sulfur components ($H_2S$ and $SO_2$), ozone ($O_3$) and chlorofluorocarbons (CFCs) [Meszaros et al. 2013]. One of the efforts to reduce the impact of global warming is through planting of certain plants which can absorb carbon dioxide ($CO_2$) significantly such as rain tree (*Albizia saman* Jacq. Merr.) [Dahlan 2010; Hanafi 2011; Fathurrahman et al. 2016]. Single rain tree plant may age over 10 years and it can absorb the $CO_2$ up to 28 ton. Human activities directly affect the carbon cycle by either increasing atmospheric $CO_2$ or reducing carbon sources, such as forests [Fathurrahman 2023]. However, it is not suitable to be planted in the house yard, because its roots can damage the foundation of the house if it is growing bigger uncontrollably. The shape of its wide canopy inhibits the growth of other plants that live under it to get sunlight. However, rain tree plant has become popular as a shade plant and a good oxygen producer.

Therefore, it is necessary to conduct specific research on how to slow down the growth of rain tree, so that it can be planted in a narrow yard. The most commonly used growth regulator is *Paclobutrazol* (PBZ). It is one of the retardants which can inhibit cell elongation in the sub-apical system, reducing the rate of stem elongation without affecting leaves growth. Paclobutrazol is a popular inhibitor of gibberellin phytohormone biosynthesis. Mutations can be carried out by soaking hot water at 80°C on *Albizia saman*, which causes the germination percentage to decrease. This method of soaking hot water can be used for gene mutations [Fathurrahman et al. 2015]. This research aims to determine the interaction of the effect of spraying time and paclobutrazol concentration on the growth and physiological traits of *rain tree* seedlings. Benefits used of metabolites in addition to inhibiting plant growth are also widely used. For examples include controlling the growth of toxigenic fungi for contamination of feed, food and agricultural stocks [Mohamed et al. 2022].

## MATERIALS AND METHODS

This research was carried out in an experimental garden of the Faculty of Agriculture, Universitas Islam Riau, Pekanbaru, Indonesia. The research was conducted from March to July 2021. Ultisol soil were used the planting media and the plants were grown in polybag. This research used a factorial completely randomized design consisting of two factors. The first factor is the spraying time (T) which is divided into three levels, and the second one is the concentration of Paclobutrazol (PBZ) (P) which consists of four levels so that there are 12 treatment combinations with 3 replications, with the total of 36 experimental units. Each unit consists of 4 plants per plot and 2 plants are observed as the samples for a total of 144 plants. The treatment factors are arranged as follows: Spraying time factor, consisting of three levels: T1 = 25 days, T2 = 50 days, and T3 = 75 days. Paclobutrazol factor, consisting of four levels: P0 = control, P1 = 75 ppm, P2 = 150 ppm and P3 = 225 ppm.

Physiological variables which are being measured include photosynthesis rate, stomatal conductance, $CO_2$ content, and plant transpiration rate among leaves cells. The measurements are made on full-width mature leaves samples from the fifth branch from the growing point using the LI-COR 6400XT Portable Photosynthesis System (LI-COR Inc., Lincoln, Nebraska, USA) at 1000 mol PAR (Photosynthetically Active Radiation) 1000 μmol $m^{-2}s^{-1}$. Photosynthetic observations are done from 8.00 – 11.00 am with sunny weather (no cloud or rain). The measurements of photosynthesis rate, stomata flow, $CO_2$ concentration among leaves cells, and transpiration rate are conducted once at the age of 105 days.

A randomized block design has been used and the data on physiological have been statistically analysed using Analysis of Variance with SAS 9.1.3 software. When the treatment had a significant effect, it was continued with the Duncan Multiple Range Test (DMRT) at $p< 0.05$. The linear model shall be as follows:

$$Ytp = \mu + Tt + Pp + \sum(tpn)$$

where: Ytp – the observed variable from spraying time the p level and the spraying time of the t level,
μ – the effect of the mean value,
Tt – effect of the P factor on thep p level,
Pp – effect of factor T on level – t,
TtPp – effect of interaction between factor T on level to t and the P factor at the p level,
(tpn) – error effect of factor T at the t level and the P factor at the p level and repetition up to n,
T – spraying time (1, 2, 3),
P – paclobutrazol (0, 1, 2, 3),
n – repeat (1,2, 3).

## RESULTS AND DISCUSSION

### Photosynthesis rate

The density of active light photon flow uses the Haniff [2006] method such as 1000 μmol to measure the photosynthesis rate that occurs in the leaves. The observation results of the photosynthesis rate on rain tree leaves after analysis of variance show that the spraying time and the concentration of paclobutrazol have a significant influence on the photosynthesis rate. The average photosynthetic rate observed after further testing for DMRT $p <0.05$ can be seen in Table 1.

Based on Table 1, the highest treatment interaction in the photosynthesis process is significantly different in the treatment of 50-day spraying time after planting and paclobutrazol concentration of 250 ppm with a photosynthetic rate of 38.27 μM $m^{-2}s^{-1}$. Meanwhile, the main effect is significantly different, where it can be seen at the paclobutrazol concentration of 225 ppm with the highest value of 33.64 μM $m^{-2}s^{-1}$. The main effect

**Table 1.** Average rain tree photosynthesis rate with the treatment of spraying time and paclobutrazol concentration

| Spraying time (days) | Paclobutrazol concentration (ppm) | | | | Average |
|---|---|---|---|---|---|
| | 0 | 75 | 150 | 225 | |
| 25 | 27.65 h | 28.35 g | 29.01 f | 32.25 b | 29.31 |
| 50 | 28.82 f | 29.75 e | 32.19 b | 38.27 a | 32.25 |
| 75 | 29.79 e | 29.67 e | 30.65 c | 30.40 d | 30.12 |
| Average | 28.75 b | 29.25 b | 30.61 ab | 33.64 a | |

**Note:** The numbers in the column followed by the same lowercase letter are not significantly different according to the DMRT test at $p < 0.05$.

of spraying time is not greatly different. However, the highest observation value is obtained from the 50-day spraying time with a photosynthetic value of 32.25 μM $m^{-2}s^{-1}$. The results of the observation show that the lowest photosynthesis rate is when the *rain tree* has been sprayed at the age of 25 days without any paclobutrazol application (27.65 μM $m^{-2}s^{-1}$). Higher result reading in the combined treatment at a concentration of 225 μM $m^{-2}s^{-1}$ may be influenced by paclobutrazol compounds affecting the photosynthetic values. The optimal concentration and best fertilization time is to combine paclotrazole treatment with a concentration of 225 ppm and spraying rain tree seedlings at the age of 50 days after planting, giving a high photosynthetic efficiency of 38.27.

As seen from physiological, quality and quantity aspects, the cells associated with photosynthesis are active in their maximum state. However, in this research, the photosynthesis results are likely to be distributed more to the tree stem, roots and C:N ratio. The higher photosynthesis rate of rain tree plant treated at 150 ppm paclobutrazol is thought to be caused by higher stomatal conductance in the leaves and increasing diffusion of $CO_2$ that enters through the stomata. Higher $CO_2$ diffusion allows the carboxylation reaction by the *rubisco* enzyme in the mesophyll to be more active. This can increase the photosynthesis rate [Kostopoulou, Karatassiou 2016]. The higher photosynthesis rate is followed by the increasing number of mitochondria to meet cellular energy in the respiration process [Sharma 2014]. Increased rate of photosynthesis is due to increased paclobutrazul concentration leading to increased ABA hormone content. An increase in the hormone ABA causes plants to grow slower, but the results of photosynthesis are directed towards the roots, so their growth is more dominant. An increase in photosynthetic rate also occurred in herbaceous peony treated with paclobutrazol [Kondhare et al. 2014; Xing et al. 2018]. Plants that use the most efficient chemicals, e.g. colchicine or paclobutrazol, are capable of producing polyploidy by an artificial method [Fathurrahman et al. 2023].

## Stomatal conductance

The results of observations on stomatal conductance in rain tree leaves after analysis of variance show that the interaction between spraying time and paclobutrazol concentration has a significant influence on stomatal conductance. The average stomatal conductance observations after further testing for DMRT $p < 0.05$ can be seen in Table 2.

Table 2 shows that the highest treatment interaction in stomatal conductance is in the 50-day spraying time and 250 ppm of paclobutrazol concentration with a value of 0.35 mM $m^{-2}s^{-1}$. Meanwhile, the effect is not significantly different, but the treatment of 225 ppm paclobutrazol

**Table 2.** Average of stomata conductance on *rain tree* leaves with the treatment of spraying time and paclobutrazol concentration

| Spraying time (days) | Paclobutrazol concentration (ppm) | | | | Average |
|---|---|---|---|---|---|
| | 0 | 75 | 150 | 225 | |
| 25 | 0.09 g | 0.14 d | 0.08 h | 0.11 g | 0.10 |
| 50 | 0.30 b | 0.04 k | 0.06 j | 0.35 a | 0.18 |
| 75 | 0.13 e | 0.02 l | 0.07 i | 0.19 c | 0.10 |
| Average | 0.17 | 0.06 | 0.07 | 0.21 | |

**Note:** The numbers in the column followed by the same lowercase letter are not significantly different according to the DMRT test at $p<0.05$.

concentration results in the highest stomatal conductance of 0.21 mM $m^{-2}s^{-1}$. The results of the observation indicate that the lowest stomatal conductance is not in the control but the combined treatment of 0.02 mM $m^{-2}s^{-1}$.

Monda et al. [2016] stated that higher $CO_2$ concentration in leaves cells can increase stomatal activity. There is also contrast from the results of previous studies that report reduced stomatal conductance [Gao et al. 2015]. In this research, there are some variations in stomatal conductance, presumably due to the interaction between exposure time and concentration of paclobutrazol which causes stomatal conductance parameters to experience instability. Increasing the size of the stomata can change its capacity and structure. Larger stomata size will open or close more slowly than the smaller one because of the low surface-to-volume ratio and the need for solute transport to drive the movement of carbon dioxide and oxygen [Drake et al. 2013]. Sufficient water availability and flexible stomata states (frequently open and close) tend to have a doubled transpiration rate. Transpiration rate and stomatal conductance are known to influence plant photosynthesis.

### Carbon dioxide content

The observation results on $CO_2$ content in *rain tree* leaves after analysis of variance show that the interaction between spraying time and paclobutrazol concentration has a significant effect. The average observed $CO_2$ content after further testing for DMRT $p < 0.05$ can be seen in Table 3.

Table 3 shows that the smallest treatment interaction on $CO_2$ content is the spraying time on day 25 and paclobutrazol concentration of 250 ppm and paclobutrazol treatment of 75 ppm with the values of 56.86 ppm and 79.15 ppm respectively. Meanwhile, the highest $CO_2$ content is in the combination of 150 ppm paclobutrazol and without paclobutrazol with a 25-day spraying time. The $CO_2$ concentration values are 943.90 ppm and 868.45 ppm, respectively. Meanwhile, the spraying time and paclobutrazol concentration do not influence $CO_2$ concentration. However, the treatment with 75 ppm paclobutrazol concentration results in a lower value than the other main treatments, namely 165.20 ppm.

The decrease in $CO_2$ content in plant cells is related to the photosynthesis process. If the photosynthesis rate is increased, $CO_2$ concentration in the leaves becomes low after being used in the photosynthesis process. A significant decrease in $CO_2$ concentration in the leaves of rice plants is marked with a higher growth rate than control samples that have higher $CO_2$ concentrations [Doni et al. 2014]. The stomata have complex and distinguished characters, depending on the light intensity and $CO_2$ concentration which play a role in opening and closing it

Closed stomata result in reduced $CO_2$ diffusion into the mesophyll space, whereas the curled leaves cause a narrower area for photosynthesis. The decreased $CO_2$ content in the mesophyll space certainly affects the decrease in the carboxylation reaction by the Rubisco enzyme and influences the decreasing photosynthesis rate. According to Yamori et al. [2013], if the temperature drops, the activity of *ribulose-1.5-bisphosphate carboxylase/oxygenase* (Rubisco) will also decrease, resulting in a build-up of $CO_2$ and the rubisco enzyme can be an inhibiting factor for the photosynthesis rate.

### Transpiration rate

The observation results of the transpiration rate in *rain tree* leaves after analysis of variance show that the interaction between spraying time treatment and paclobutrazol concentration has a significant effect. The average observed transpiration rate after further testing for DMRT $p < 0.05$ can be seen in Table 4.

**Table 3.** Average of $CO_2$ content on *rain tree* leaves with the treatment of spraying time and paclobutrazol concentration

| Spraying time (days) | Paclobutrazol concentration (ppm) | | | | Average |
|---|---|---|---|---|---|
| | 0 | 75 | 150 | 225 | |
| 25 | 868.45 a | 79.15 h | 943.90 a | 56.86 h | 487.10 |
| 50 | 557.72 d | 144.77 b | 421.33 f | 613.26 cd | 434.30 |
| 75 | 760.18 b | 271.68 g | 290.40 g | 652.82 c | 493.80 |
| Average | 728.8 | 165.20 | 551.90 | 441.00 | |

**Note:** The numbers in the column followed by the same lowercase letter are not significantly different according to the DMRT test at $p<0.05$.

**Table 4.** Average of transpiration rate in *rain tree* leaves with the treatment of spraying time and paclobutrazol concentration

| Spraying time (days) | Paclobutrazol concentration (ppm) | | | | Average |
|---|---|---|---|---|---|
| | 0 | 75 | 150 | 225 | |
| 25 | 1.05 d | 1.54 f | 1.11e | 1.13 e | 1.20 |
| 50 | 3.21 h | 0.53 b | 0.68 c | 4.49 i | 2.27 |
| 75 | 1.57 f | 0.24 a | 1.00 d | 2.20 g | 1.25 |
| Average | 1.94 | 0.77 | 0.93 | 2.60 | |

**Note:** The numbers in the column followed by the same lowercase letter are not significantly different according to the DMRT test at p<0.05.

The relationship between spraying time and paclobutrazol concentration seems to have no significant effect. The slowest transpiration rate is obtained in the combination of 50-day spraying time after treatment and the paclobutrazol concentration of 0.24 mM $H_2O$ $m^{-2}s^{-1}$. The combination of the two can inhibit the transpiration rate. Thus, the plant had grown slower and it is suspected that the synthesis of gibberellin phytohormones is inhibited. The paclobutrazol is a type of inhibitor that can suppress plant growth rate. Decreased transpiration rate is caused by slower opening and stomata aperture. This may be due to the effect of paclobutrazol on the $CO_2$ concentration and abscisic acid (ABA) and the low availability of potassium ions. The low $CO_2$ concentration causes the stomata to open, but because the opening and closing of the stomata are also influenced by other compounds, it seems that its activity will become slow down. ABA interacts with gibberellins, cytokinins, and IAA antagonistically. Hormones from plant secondary metabolites can stimulate or inhibit growth. Most of the chemical plants are classified as secondary plants which are biochemical processes from primary metabolites [Dyah et al. 2022] The decreasing gibberellins concentration triggers an increasing concentration of ABA so that it pushes the stomata to close and this causes the transpiration rate to decrease as well.

## Water use efficiency

The observation results of water use efficiency in *rain tree* leaves after analysis of variance show that the interaction between spraying time treatment and paclobutrazol concentration has a significant effect. The average values of water use efficiency after further testing by DMRT p <0.05 can be seen in Table 5.

Table 5 shows that the highest treatment interaction result is in the 50-day spray time combined with 75-ppm paclobutrazol concentration with an increase in WUE of 57.37%. Meanwhile, the application paclobutrazol at the age of 25 and 50 days with a concentration higher than 75 ppm resulted in a lower percentage value.

The water use efficiency at the growth stage must be considered carefully to avoid growth inhibition. In this treatment, it turns out that the application of paclobutrazol with a concentration of 150 ppm has been able to achieve water use efficiency so that the growth rate is getting slower. Previous studies have shown that the water use efficiency increases at higher $CO_2$ concentrations. Other experiments have demonstrated through $C_3$ plants that an increase in $CO_2$ will also enforce the photosynthesis rate [Loladze 2002]. However, in this research, the photosynthesis rate is decreasing due to paclobutrazol retardant treatment and this is under the research objective of

**Table 5.** Average of water use efficiency (%) in *rain tree* leaves with the treatment of spraying time and paclobutrazol concentration

| Spraying time (days) | Paclobutrazol concentration (ppm) | | | | Average |
|---|---|---|---|---|---|
| | 0 | 75 | 150 | 225 | |
| 25 | 26.03 d | 18.42 e | 26.10 d | 28.50 cd | 24.76 |
| 50 | 8.96 g | 57.37 a | 47.14 b | 8.51 g | 30.50 |
| 75 | 18.92 e | 19.41 e | 31.16 c | 13.85 f | 20.84 |
| Average | 17.97 | 31.73 | 34.80 | 16.95 | |

**Note:** The numbers in the column followed by the same lowercase letter are not significantly different according to the DMRT test at p<0.05.

inhibiting plant growth rate. The water use efficiency (WUE) can be used to identify the water needs of a plant in a specific area. *Rain tree* leaves treated using paclobutrazol show a positive response to WUE. WUE response in leaves is directly related to physiological processes, namely photosynthesis, and transpiration. Inhibitor can also inhibit growth rate so that assimilate translocation is concentrated for stem formation.

Although paclobutrazol is one of the inhibitors that becomes an antagonist to the gibberellin hormone, the retardant's responses in plants may vary widely. For example, differences in leaves, stems, and roots of different species for absorption and translocation of chemical compounds, the existence of deactivation mechanisms in some species, and differences in retardant interaction patterns among the plants.

The biosynthesis process may be inhibited due to paclobutrazol which causes a lower rate of cell division and elongation without triggering toxicity to the cells. The direct effect on plant morphology is the inhibition of plant vegetative growth. Under its working system, growth inhibitors with the active ingredient paclobutrazol have the effect of inhibiting stem elongation, shortening stem segments, and increasing leaf color to make them seem uniform, and making plants vegetative growth able to support the flower growth process. Normal photosynthesis and slow transpiration cause less evaporation from the leaves so that the water use becomes more efficient [Jerry, Christian 2019].

## Relationship between photosynthesis and stomatal conductance

The observation results of the relationship between photosynthesis and stomatal conductance can be seen in Figure 1A. Based on the observation of paclobutrazol treatment, the increase in photosynthesis started from a concentration of 75 to 225 ppm. On the other hand, the stomatal conductance causes the velocity to decrease to 0.06 $mM\ m^{-2}s^{-1}$ at 75 ppm of paclobutrazol concentration. However, at the concentrations of 150 ppm and 225 ppm, the stomatal conductance readings increase to 0.21. Tomato plants are reported to experience a decrease in their stomatal conductance and transpiration levels by 30–40% in a puddle treatment for 24 hours.

Some plants have mechanisms to tolerate the abiotic stress like paclobutrazol is the stomata opening and closing responses or the stomata conductance of *A. saman* leaves. It can be seen that stomata act as a tool for gases circulation ($CO_2$ and $H_2O$) from the air into the plant, or in other words, metabolic processes go hand in hand with the level of stomata opening and closing. If the metabolism process is inhibited, the stomatal conductance will decrease. Decreased in root conductance values are caused by abiotic factors followed by stomata. A decrease in stomatal conductance causes decreasing photosynthesis rate [Bertolde et al. 2012]. On the other hand, an increase in conductance can accelerate the photosynthesis rate.

The observations on the relationship between photosynthesis and stomatal conductance during spraying time application show that there is a similar trend (Figure 1B), where spraying time was applied at 25 days of age, the stomatal conductance and photosynthesis rate are low. However, there is an increase in the application of spraying time at the age of 50 days. The application at 75 days of age triggers a decrease in stomatal conductance and photosynthetic rate. It is suspected that 75 days is the most appropriate time for having spraying time treatment to inhibit the growth of *A. saman.* The results can be seen in

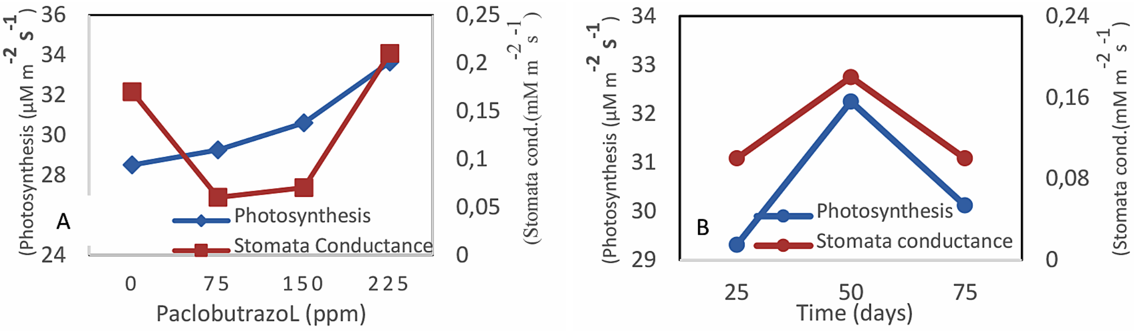

**Figure 1.** The relationship between photosynthesis and stomatal conductance in A) sample of paclobutrazol concentration; B) sample of spraying time application period

**Figure 2.** Rain trees plant growth based on paclobutrazol concentration and spraying time age: A = 25 days of spraying time without any paclobutrazol; B = 50 days of spraying time with 75 ppm paclobutrazol; C = 50 days of spraying time with 150 ppm paclobutrazol and D = 75 days of spraying time with 225 ppm paclobutrazol. Plant age 198 days of spraying time

Figure 2. Lower plant height growth can be seen at 50 days of plant age and the application of 150 ppm of paclobutrazol concentration and spraying age 75 days with 225 ppm paclobutrazol treatment interactions.

## Relationship between photosynthesis and internal $CO_2$

The observation on the relationship between photosynthesis and internal $CO_2$ in paclobutrazol treatment showed that increasing paclobutrazol concentration influences increasing photosynthetic rate (Figure 3A). Meanwhile, the internal state of $CO_2$ in the leaves decreases when it is given a concentration of 75 ppm paclobutrazol. However, the internal $CO_2$ content increases again after the application of 150 ppm paclobutrazol concentration. Increasing the concentration of paclobutrazol at 225 ppm, on the other hand, decreases the $CO_2$ content in the leaves and does not contradict the increasing photosynthesis rate.

Physiologically, the higher the concentration of carbon dioxide in the leaves, the faster the photosynthesis rate, because $CO_2$ is the main component in the photosynthesis process besides water and other elements. The effect of $CO_2$ concentration and stomatal opening has been recognized well. The stomata's guardian cells react to the internal $CO_2$ concentration of the leaves instead of the $CO_2$ concentration outside of the leaves. The hole opens when the internal $CO_2$ decreases and closes when it rises [Mott 2009]. This is presumably due to the biosynthetic activity of gibberellin with decreasing $CO_2$ concentration.

The relationship between photosynthesis and internal $CO_2$ concentrations on the *A. saman* treated with spraying time shows that there is a clear contradiction, where the application of paclobutrazol at 25 days causes the increasing photosynthesis rate, while the internal $CO_2$ decreases due to the photosynthesis rate (Figure 3B). Furthermore, the application of spraying time at the age of 50 days caused the decreasing in photosynthesis rate until 75 days, while the $CO_2$ concentration increases sharply at the age of 75 days. The application of spraying time based on the plant's age proves that there is an antagonism between photosynthesis and internal $CO_2$. The photosynthesis rate can increase along with the increasing

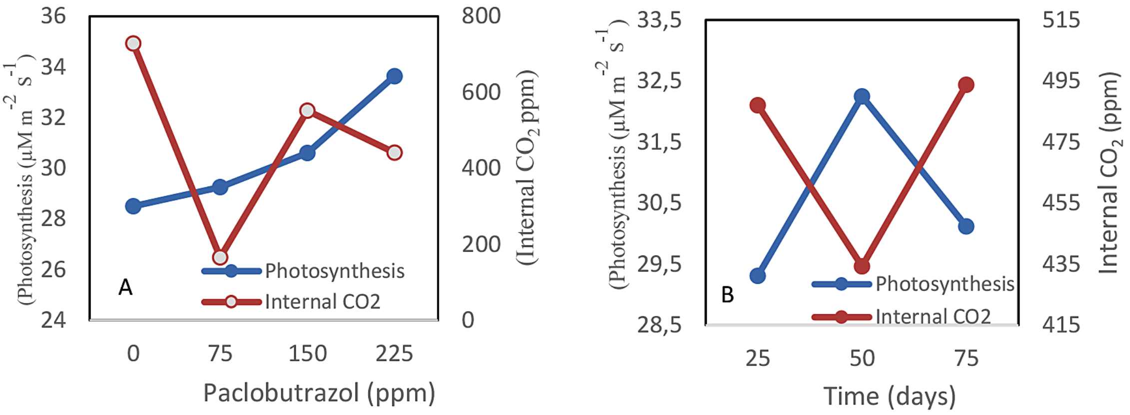

**Figure 3.** The relationship between photosynthesis and internal $CO_2$ in A) sample concentration of paclobutrazol; B) sample of spraying time application time

$CO_2$ concentration in the air. However, too much $CO_2$ concentration can poison or cause stomata to close and photosynthesis to be disrupted. The low $CO_2$ content in the leaves causes photosynthesis to increase in the morning because the availability of $CO_2$ is also high. The chemical reaction between the high $CO_2$ hydrogen ions causes decreasing $CO_2$ concentration in the leaves.

## Relationship between photosynthesis and transpiration rate

The relationship between photosynthesis and transpiration in the treatment of *A. saman* concentration shows that the increasing concentration of 225 ppm paclobutrazol caused an increasing photosynthesis rate (38.64 $\mu M\ m^{-2}s^{-1}$ (Figure 4A). Meanwhile, the transpiration decreases after the application of 75 ppm paclobutrazol. Transpiration increases after being treated paclobutrazol concentrations of 150 ppm and 225 ppm. Viewed from the trend graph, it shows that the higher the paclobutrazol concentration, the higher the photosynthesis and transpiration rate. Fluctuations in the photosynthesis rate and $CO_2$ absorption in plants are influenced by the amount of transpiration in the leaves which is received by the leaves and the size of stomata opening, so there is a similar correlation to the effect of paclobutrazol concentration.

The relationship between photosynthesis and transpiration in *A. saman* treated with spraying time showed a harmonious relationship, where spraying for 25 days increased the rate of photosynthesis and transpiration (Figure 4B). Furthermore, spraying at the age of 50 days caused a decrease in the rate of photosynthesis and transpiration until the age of 75 days. The rate of photosynthesis can increase with increasing $CO_2$ transpiration in the air. Transpiration also influences stomatal apperture and $CO_2$ exchange. Therefore, transpiration, directly and indirectly, affects net photosynthetic rate through changes in stomatal conductance, intercellular $CO_2$ concentration, water, and water potential status based on transpiration attraction. These factors are considered important and influential in growth differences [Yongchao et al. 2020] Plant growth characteristics are mainly influenced by genetic and environmental factors. Increasing the photosynthetic efficiency of plants can improve these characteristics [Ort et al. 2015; Eva et al. 2019; Lawson et al. 2020].

There is a correlation between photosynthesis and transpiration during the application of paclobutrazol treatment on *A. saman*. The application of paclobutrazol at the age of 50 days causes higher photosynthesis and transpiration rates, but the treatment at 75 days shows a decreasing trend. Therefore, photosynthesis and transpiration have the same response to the application of paclobutrazol at different times (days). Transpiration indirectly reflects the plants' ability to absorb or transport water and nutrients, thereby affecting photosynthesis and growth characteristics. Transpiration also affects stomata opening, $CO_2$ exchange, and evapotranspiration [Yongchao et al. 2020]. The increase in photosynthetic and transpiration rates with 50 days of paclobutrazol treatment indicates that the photosynthetic products are directed to stem growth so that the treatment sample has a bigger stem than the control one.

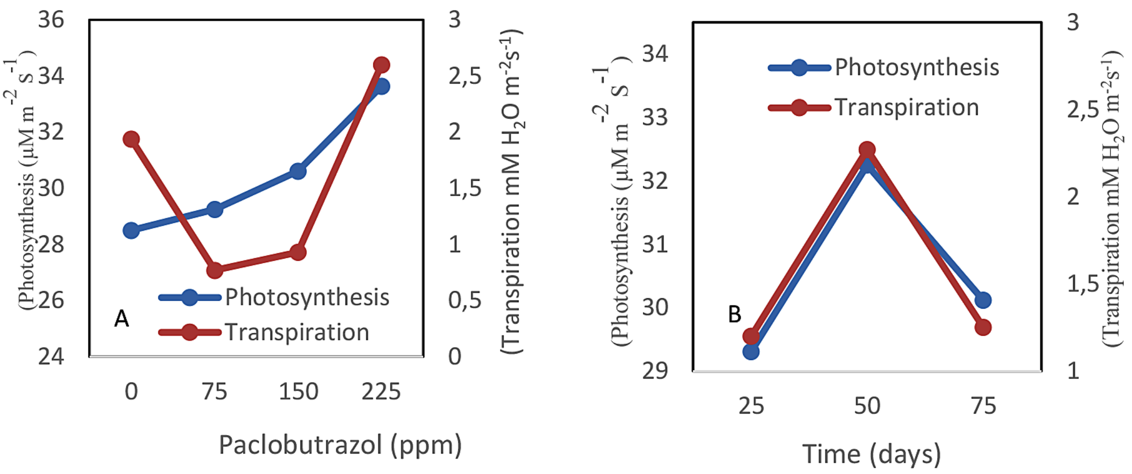

**Figure 4.** The relationship between photosynthesis and transpiration rate in A) sample concentration of paclobutrazol; B) sample of spraying time application time

## CONCLUSIONS

The interaction of photosynthetic rate increases in the treatment of spraying time and the paclobutrazol concentration. Higher paclobutrazol concentration results in a higher photosynthesis rate. However, the growth rate of the *A. saman* sample in the combination treatment is lower than the control one. Likewise, the stomatal conductance is also higher in the *A. saman* as the treated sample compared to the control one. On the other hand, the lowest internal $CO_2$ content in the treatment combination is compared to the control sample. The transpiration rate in the combination of treatment samples also decreases compared to the control one, resulting in a slower growth rate, higher stem diameter, but shorter *A. saman* plant. For water use efficiency, the percentage is higher in the treatment sample. The relationship between photosynthesis and stomatal conductance has a similar trend curve from low to high treatment. Meanwhile, the relationship between photosynthesis and internal $CO_2$ shows an antagonistic response curve towards each other. Finally, the relationship between photosynthesis and transpiration shows the same trend curve as in the relationship between photosynthesis and stomatal conductance. Paclobutrazol is characterized as an ecologically stable compound in soil and water with a half-life of more than one year.

The potential for paclobutrazol to contaminate groundwater at optimal concentrations is low, but the risk of exposure to aquatic life is high.

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