

Resistance status and physiological responses of *Dactyloctenium aegyptium* to diuron herbicide in pineapple plantation

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Abstract. Diuron herbicide has been used in the pineapple plantation in Lampung, Indonesia, for more than 35 years. It has been realized that the use of herbicides with the same mode of action intensively can speed up the evolution of resistant weeds over a long period of time. This study aimed to determine whether *Dactyloctenium aegyptium* from pineapple plantation has evolved resistance to diuron and to examine whether the resistance correlates with the weed physiological activities. The study was conducted at the University of Lampung, from September 2018 to March 2019. The study consisted of two stages, i.e. Stage 1: Weed resistance test, and Stage 2: Physiological activity test on resistant weed. The study used a split-plot design. The main plot was the origins of weeds (exposed and unexposed to diuron) and the subplot was the diuron dose consisting of 0; 1,200; 2,400; 4,800; and 9,600 g ha⁻¹. The result showed that *D. aegyptium* exposed has high-level resistance to diuron with a Resistance Index (RI) value of 16.70. The physiological activities of *D. aegyptium* which has a high level of diuron resistance exhibited higher carbon assimilation, stomatal conductance, and transpiration rates than the sensitive *D. aegyptium*. These findings can be used as the basis for policy formulation in determining appropriate prevention and control methods for handling resistant weeds.

Keywords: carbon assimilation, resistant, stomatal conductance, transpiration, weed

INTRODUCTION

Pineapple (*Ananas comusus* L.) is one of the leading tropical fruit commodities in Indonesia, especially in Lampung which has great potential in world trade. In 2017 pineapple exports were in second place after bananas as the largest contributor to foreign exchange from fruit commodities. Pineapple exports reached around 8.02 thousand tons or US\$4,969,234. Most of the pineapples are exported to the United Arab Emirates, Korea, Japan, Saudi Arabia and Hong Kong [1]. However, in the pineapple cultivation there are several problems and one of them is the presence of weeds. The damages caused by weeds among others are reduction in crop yield, weeds can become hosts palnt pests and diseases, also make maintenance and harvesting processes to be difficult. The

presence of weeds in pineapple plantations can reduce fruit yields by 20-42% [2]. It was reported that *Dactyloctenium aegyptium* in Central Lampung pineapple plantations was dominant and difficult to control [3].

Weed control typically consists of many methods which are divided into biological, chemical, cultural, and physical/mechanical control and integrated weed control. However, the main weed control method in the pineapple plantation is chemical control using herbicides, one of which is diuron. Diuron has been used in the pineapple plantation for more than 35 years. The use of herbicides that have the same mode of action intensively over a long period of time can accelerate the emergence of resistant weeds that can not be controlled with herbicides at recommended dosages. Weeds that survived after the application of herbicides may have genetic mutations that are passed on to their progeny [4]. Losses due to weed resistance to herbicides is an increase in the dose of herbicide used to overcome resistant weeds, so that the cost of purchasing herbicides increases. Ecologically, increasing the dose of herbicide

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Received: March 2022 | Revised: September 2022 | Accepted: October 2022

can certainly increase the risk of environmental pollution due to residual herbicides. In addition, the resistance of weeds to herbicides causes farmers to lose an important, effective and inexpensive weed control device [5].

The herbicide application causes differences in physiological activity that occurs between resistant and sensitive weeds [6]. The plant metabolism process was correlated with gas exchange that occurs. Gas exchange in plants can be measured to determine the rate of photosynthesis that occurs by measuring carbon assimilation and transpiration rate [7].

Weed resistance trends in the world are increasing from year to year. It is known that worldwide in 1995 there were 192 resistant weed biotypes, increased to 337 biotypes in 2005, then to 498 biotypes in 2018. Specifically, the number of weed species that are resistant to diuron herbicides (PSII inhibitors) had increased from 11 in 1990, to 29 in 2018. Although the trend of weed resistance to herbicides in the world has increased sharply, the information about the development of weed resistance to herbicides in Indonesia is still very little. This certainly raises many questions because herbicides have been used intensively in Indonesia for more than 30 years. Based on data from The International Survey of Herbicide Resistant Weeds, there were only two species of weeds resistant to herbicide that were reported in Indonesia, namely *Limnocharis flava* and *Eleusine indica* [8].

The herbicide dose used in this study was the recommended dose of 1,200 g ha⁻¹, up to four times the recommended dose. In previous research has been used up to six times the recommended dose of diuron in testing weed resistance in pineapple plantations [9]. In the preparation of the weed resistance test protocol, the recommended herbicide dose was used and the dose was three times the recommended dose [10]. This is to determine the level of resistance that occurs. This study aimed to identify the resistance of *D. aegyptium* in pineapple plantations in Central Lampung, Indonesia that was suspected to be resistant to diuron and confirm the resistance by physiological activity test.

METHODOLOGY

Stage 1: Resistance Status Test

Field Survey and Sampling. The study was arranged in a split-plot design with 6 replications in Stage 1. The main plot was the origins of weeds (exposed and unexposed weeds to diuron). Subplot was the dosage levels of diuron (0; 1,200; 2,400; 4,800; and 9,600 g

ha⁻¹). Field surveys were carried out in locations that had long been exposed to diuron (pineapple plantation) and in locations that were never exposed to herbicides in Terbanggi Besar, Central Lampung. The coordinate point of sampling for exposed weeds is at 4049'10.9 "S 105011'31.4" E and for unexposed weeds at the coordinate point 4053'33.5 "S 105012'55.2" E in Terbanggi Besar. The weeds were taken in the form of seedlings and strived for plant height and the number of leaves to be uniform. Total number of required weeds for the sample are 60 weeds/species. But, we collected weeds from the field five times from the required amount as an effort to prevent shortages of planting material.

Planting and Maintenance. Weed seedlings that have been taken from the field were transplanted to a plastic pot filled with soil media that has been added with manure. Weeds were maintained until the vegetative phase, when the weeds already had 3-6 leaves.

Herbicide Application. Weeds that have been planted and maintained, then selected to get the same level of uniformity. Before the herbicide applications, the calibration was done first. This was done so that each trial unit got the same amount of herbicide according to treatment. Calibration was done by the spacious method using a red nozzle with a spray width of 2 m. The calibration results obtained a spray volume of 500 l ha⁻¹. Spraying was done in the morning with several dosage levels, starting from the lowest dose to the highest dose using a knapsack sprayer. The applied weeds were arranged randomly on an area of 10 m². The dosage levels of diuron are (0; 1,200; 2,400; 4,800; and 9,600 g ha⁻¹).

Observation. The variables observed in stage 1 were percentage of poisoning and dry weight. Determination of percentage of poisoning was done by comparing weeds that were applied by herbicides to weeds without treatment (control). Comparisons observed were leaf color, leaf shape changes, and abnormal growth. From this comparison, we could get the percent value of weed poisoning. Observations were made at 2, 4, 6, 8, 10, 12, up to 14 days after treatment (DAA) based on recommendations from previous research [11]. Percentage of poisoning was conducted in two replications (two observers). Harvesting was done on 14 DAA. Weeds dried in an oven at 80°C for 48 hours until the weed dry weight was constant.

Data Analysis. Data analysis was performed to find out; (1) speed of poisoning weeds by 50% (LT₅₀). Percent poisoning data was processed by probit analysis to get the value of Lethal

Time (LT₅₀). Comparisons among the glutinous rice production systems used one-way analysis of variance (one-way ANOVA) and Fisher's Least Significant Difference (LSD). (2) ED₅₀ (Effective Dosage 50%) is a value that indicates the number of herbicide doses that cause suppression of weeds up to 50%. ED₅₀ values were obtained from the results of the conversion of weed dry weight data into percentage values of weed damage [12]; (3) Resistance Index (RI), which is the value of the comparison of ED₅₀ exposed weeds with unexposed weeds. Based on the value of RI weeds can know the status of weed resistance tested. The level of weed resistance can be determined by RI criteria, which are classified as sensitive if RI <2, low resistance if RI 2-6, moderate resistance if RI > 6-12, and high resistance if RI > 12 [13].

Stage 2: Physiological activity test on resistant weeds

Planting and Maintenance. Weed populations that have proven to be resistant on Stage 1 were newly collected from the same coordinate point is used on stage 1. Weeds were planted and maintained until early vegetative phase with the same technique on Stage 1.

Herbicide Application. In this stage, the treatment was repeated in 3 replications in the same technique, plot size and experimental design as Stage 1. On physiological activity test the dosage levels of diuron were 0; 600; 1,200; 2,400; 4,800 g ha⁻¹. In the second stage of the test, half the recommended dose of 600 g ha⁻¹ was used, because to know the physiological activity of weeds if it is less than the recommended dose. In the second stage, the dose of 9,600 g ha⁻¹ was not tested for its physiological activity. This is based on the LT₅₀ results in exposed and unexposed weeds to diuron not much different. So, to find the difference in physiological activity on sensitive and resistant weeds used up to a dose limit of 4,800 g ha⁻¹ diuron.

Observation. Physiological activity was observed at 4, 8, and 12 DAA. Weeds that were the object of observation for physiological activity were labeled with yellow labels on the second and third leaves that had been fully opened as a sign of leaf samples to be observed. Physiological activity testing was carried out using a Li-cor 6800 F (Portable Photosynthesis System) with observations of carbon assimilation, stomatal conductance, and transpiration rate.

Data Analysis. The physiological activity data of resistant and sensitive weeds were analyzed and the standard deviation was calculated, then a curve made to determine the differences in

physiological properties between resistant and sensitive weeds.

RESULTS AND DISCUSSION

Resistance Status Test

D. aegyptium exposed to diuron was more difficult to poison than unexposed (Fig.1). At a dose of 1,200 g ha⁻¹ *D. aegyptium* exposed and unexposed to diuron began to experience poisoning 2 days after application (DAA) at 2.33% and 4.75%, respectively (Fig. 1). The percentage increase in poisoning occurred up to 14 DAA. However, in *D. aegyptium* exposed to diuron, the increase in the percentage of poisoning up to 14 DAA was not significant compared to *D. aegyptium* unexposed which increased the poisoning percent very rapidly, namely 16.67% and 81.75%, respectively. The same trend occurred at doses of 2,400 g ha⁻¹ and 4,800 g ha⁻¹, the poisoning percent of *D. aegyptium* exposed to 2 to 14 DAA did not reach 50%, while for *D. aegyptium* unexposed, the poisoning percent was more than 50% and even reached 100% at a dose of 4,800 g ha⁻¹. At a dose of 9,600 g ha⁻¹, exposed *D. aegyptium* was poisoned by 7.33% at 2 DAA and increased by 84.17% at 14 DAA, while *D. aegyptium* did not cause poisoning by 8.75% at 2 DAA and increased to 100 % at 14 DAA.

D. aegyptium exposed to diuron was more difficult to poison than unexposed *D. aegyptium*. The trend of percent poisoning shows that the higher the dose, the higher the percentage of *D. aegyptium* poisoning and poisoning occurs more quickly. This was because resistant weeds have the ability to degrade herbicide molecules before the herbicide reaches its target side. The mechanism of weed resistance is an increase in metabolism in weeds so that weeds are able to degrade herbicides before destroying the enzyme composition [14].

Exposed weeds have ability to survive against the herbicide [15]. An increase in diuron metabolism (PS II inhibitor class) was thought to occur in *D. aegyptium* exposed to diuron. resistant weeds have increased their herbicide metabolism because they are able to degrade the herbicide before destroying the composition of weeds enzyme. Resistant weeds were able to degrade 50% of the applied herbicide within 60 hours, whereas sensitive weeds needed more than 120 hours to degrade 50% of the applied herbicide. As a result, the level of herbicide translocation was lower, so that the occurrence of poisoning can be prevented. That increased metabolism of the herbicide metribuzine (PS II inhibitor class) and mutation of the psbA gene

strongly contribute to metribuzine resistance in wild radish (*Raphanus raphanistrum*) [16]. Diuron herbicides block the electron pathway in PS II, thereby disrupting the formation of nicotinamide adenine dinucleotide phosphate (NADPH) and adenosine triphosphate (ATP) [17]. NADPH and ATP are needed to fix CO₂. Low electron flow causes oxidative stress and

chlorophyll oxidation [18]. This causes *D. aegyptium* unexposed to diuron to be unable to survive after diuron application. On Fig.2, it can be seen that *D. aegyptium* that exposed to diuron survive up to 14 DAT at all doses were applied. Meanwhile the unexposed weeds showed chlorosis, then necrosis or tissue death at 14 DAA.

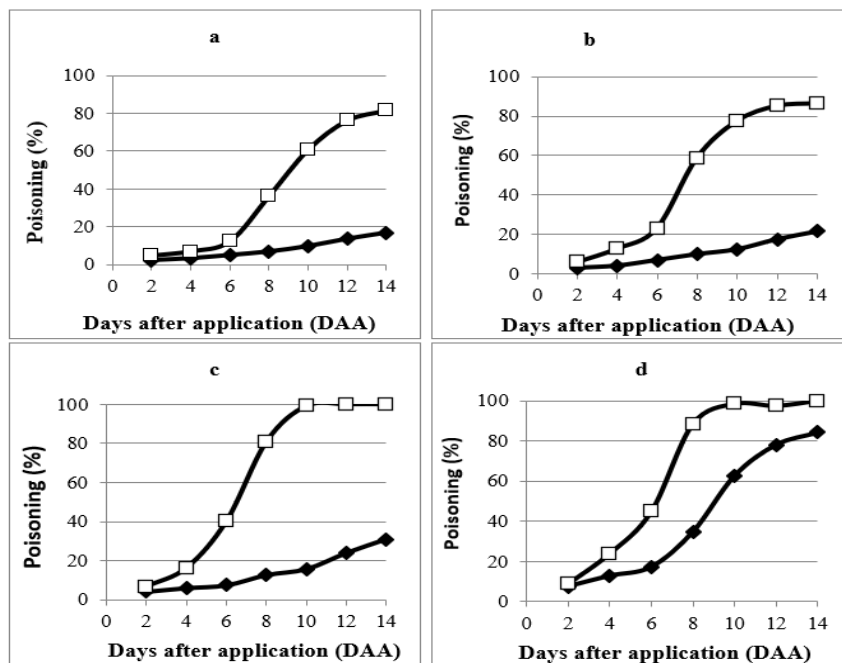


Figure 1. Percentage of poisoning of *D. aegyptium* due to diuron at 1,200 g ha⁻¹ (a); 2,400 g ha⁻¹ (b); 4,800 g ha⁻¹ (c); 9,600 g ha⁻¹ (d); □ unexposed; ◆ exposed.

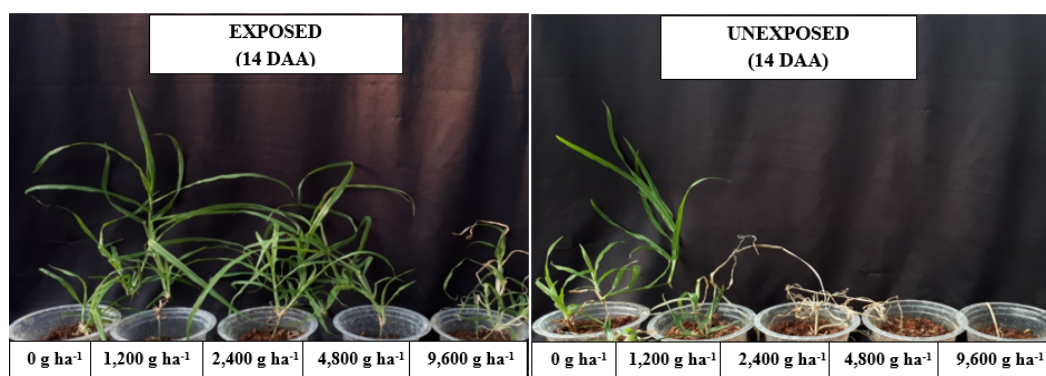


Figure 2. Response of exposed and unexposed *Dactyloctenium aegyptium* to diuron application at 14 DAA.

The dry weight results obtained were in line with the poisoning percent that occurs (Fig. 3, Fig. 4), both of them were inversely related. The higher the diuron dose, the lower the dry weight produced (Fig. 3). At a dose of 1,200 g ha⁻¹, dry weight of *D. aegyptium* exposed to diuron was 0.52 g, while at *D. aegyptium* unexposed was

0.22 g and it was lower and even reached 0 g at high doses. The dry weight of *D. aegyptium* exposed at dose level of 4,800 to 9,600 g ha⁻¹ was 0.46 g and 0.12 g, respectively, while the dry weight of *D. aegyptium* unexposed was 0 g. The dry weight results obtained were in line with the damage percent that occurs, both of

them were inversely related. The higher the treatment dose, the higher the toxicity and the lower the dry weight (Fig. 3). At doses of 4,800 and 9,600 g ha⁻¹, the percent damage to *D. aegyptium* exposed to diuron was 39.20% and 82.85%, respectively, while in *D. aegyptium* it was 100%. This was in accordance with the dry weight produced, the higher the dose of diuron, the lower the dry weight produced due to the high damage that occurs to weeds. The higher the treatment dose, the higher the toxicity and the lower the dry weight. The poisoning percent in *D. aegyptium* exposed to diuron was lower than *D. aegyptium* unexposed to diuron, so the dry weight value of *D. aegyptium* exposed to diuron was higher than the dry weight value of *D. aegyptium* unexposed to diuron. The percentage of damage was obtained by comparing the dry weight value of the herbicide treated weeds with the control weeds, so that it can be seen how severe the damage and the percentage growth reduction that occurred to the weeds due to the diuron application. Based on Figure 4, it can be seen that the higher the diuron dose level, the higher the weed damage. The higher the dose of diuron applied to weeds, the faster weeds were poisoned, causing damage to weed tissue [19].

Weeds that have been applied to herbicides will have a low electron flow, causing oxidative stress and chlorophyll oxidation [18]. The exposed weeds were able to degrade herbicides while those that were unexposed were unable to do so. Thus, in weeds exposed weeds, oxidation stress and chlorophyll oxide can be prevented. The exposed weeds were able to carry out normal photosynthetic activities, so that weeds were able to grow normally and have a higher dry weight than the unexposed weeds.

The LT₅₀ value is the time required for the herbicide to poison the tested weeds by 50%. From the LT₅₀ value, it can be seen that the time required for diuron to poison *D. aegyptium* was up to 50%. The LT₅₀ value of *D. aegyptium* exposed was higher than the LT₅₀ value of *D. aegyptium* unexposed (Table 1). This means that diuron takes a longer time to poison *D. aegyptium* exposed by 50% compared to *D. aegyptium* unexposed. There was no difference in herbicide absorption between resistant and susceptible (sensitive) weeds. However, resistant weeds were able to metabolize herbicides faster than sensitive weeds. This causes differences in the time required for the herbicide to poison resistant and sensitive weeds. Increased metabolism causes herbicide translocation to decrease, so the time needed to poison resistant weeds was longer than sensitive weeds [20]. The results of the study in Table 1

have shown that at a dose of 1,200 g ha⁻¹ (recommended dose), it can kill 50% of exposed weeds within 111.09 DAA, while unexposed weeds only take 8.87 DAA.

Resistance occurs due to repeated application of herbicides with the same mode of action, thereby placing strong selection pressure on the target weed population [21]. In Table 2, the ED₅₀ values of *D. aegyptium* exposed and unexposed to diuron were 3292.98 g ha⁻¹ and 197.17 g ha⁻¹, respectively, so that the *D. aegyptium* resistance ratio value was 16.70 and classified in the high resistance group [13]. This selection pressure causes the weeds to survive and reproduce normally even when applied with doses of herbicides that usually kill the weeds. The results showed that *D. aegyptium* from pineapple plantations in Central Lampung had high resistance (Table 2). The results of this study were in line with the previous research that *D. aegyptium* exposed from pineapple plantations in Central Lampung had developed resistance to diuron [9, 11].

The population of *D. aegyptium* which proved to be resistant was maintained until it produced weed saplings. The saplings then planted to be tested for physiological activity test and compared with sensitive weeds after the herbicide application. Measurement of gas exchange was very important to determine photosynthesis in leaves, including CO₂ assimilation, stomatal conductance, and transpiration or water use efficiency [7].

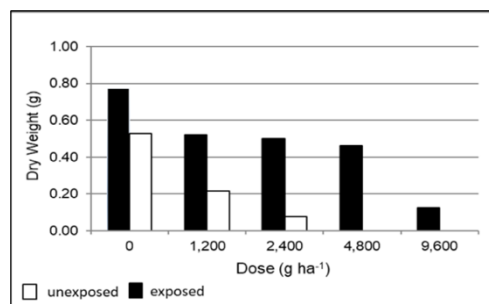


Figure 3. Dry weight of *D. aegyptium* due to diuron herbicide at 12 DAA

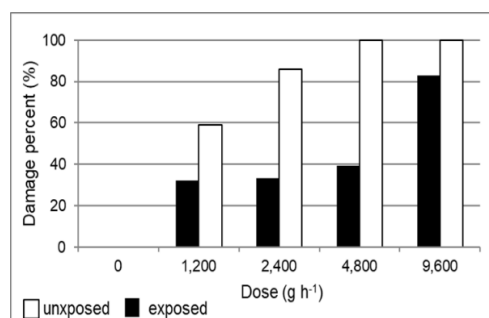


Figure 4. Damage percent of *D. aegyptium* due to diuron herbicide at 12 DAA

Physiological activity test on resistant weeds

CO₂ assimilation rate on *D. aegyptium* was always positive at 4, 8 and 12 DAA even though diuron was applied (Fig. 5). This means that gas exchange to carry out the photosynthesis process was still running normally, the amount of carbon needed was sufficient, so that the resistant *D. aegyptium* survives. CO₂ directly affects plant metabolism which has a very important role in photosynthesis, so an increase in CO₂ was expected to increase the rate of photosynthesis [22]. *D. aegyptium* belongs to C₄ plants [23]. Every C₄ plant has an internal biochemical pump to optimize the concentration of CO₂ at the carboxylation site which functions to remove oxygenase components [24]. Carbon fixation in C₄ plants is carried out by the enzyme phosphoenolpyruvate (PEP) carboxylase which has a high affinity for CO₂ and can not bind O₂. This causes no or very little photorespiration to occur, so that photosynthesis takes place optimally [25].

In sensitive *D. aegyptium*, the rate of CO₂ assimilation was low and negative (Fig. 5). This was the stress response of weeds to the applied diuron. The carbon fixed by sensitive *D. aegyptium* does not meet the carbon requirement for photosynthesis and other metabolic processes. A negative CO₂ assimilation flux occurs because the plant has run out of CO₂ supply or has lost the ability to take CO₂, as a result the photosynthesis process does not run normally [7]. In this case, it was suspected that the stomata *D. aegyptium* which was sensitive to diuron was no longer functioning normally, so gas exchange does not run smoothly in contrast to the condition of resistant *D. aegyptium*. At doses of diuron 600, 1,200, 2,400, and 4,800 g ha⁻¹, *D. aegyptium* unexposed to diuron (sensitive) experienced chlorosis, bleaching, wilting and drying.

Evaluation of photosynthetic parameters in herbicide-resistant plants makes it possible to verify the changes that occur in photosynthetic metabolism, one of which is stomatal conductance [6]. Stomatal conductance was further understood as a physiological mechanism to control transpiration, which plays a role in water absorption, nutrition and leaf temperature regulation [26]. In line with the rate of CO₂ assimilation, the rate of stomatal

conductance of resistant *D. aegyptium* at each application dose level of 600, 1,200, 2,400, and 4,800 g ha⁻¹ was higher than the stomatal conductance rate of sensitive *D. aegyptium* (Fig. 6). Stomata act as a means of gas traffic (CO₂ and H₂O) from outside into the plant. That was metabolic processes go hand in hand with the degree of opening and closing of stomata. If metabolism was inhibited, the stomatal conductance will decrease and even stop [27]. This happened in *D. aegyptium*, the application of diuron reduced the stomatal conductance of the weed. However, *D. aegyptium* from resistant populations was able to metabolize diuron herbicide rapidly, so that the herbicide's performance in interfering with photosynthesis decreased. This was illustrated by a decrease in the rate of stomatal conductance.

Transpiration rate in plants can be a determinant of selectivity or plant resistance after application of herbicides [28]. Based on Figure 7, it can be seen that in general at 4, 8, and 12 DAA the transpiration rate of resistant *D. aegyptium* was higher than the transpiration rate of sensitive *D. aegyptium*. Diuron application on sensitive *D. aegyptium* caused a decrease in guard cell turgor pressure. Loss of turgidity in guard cells causes stomata to close, resulting in inhibition of transpiration. This was in line with the observations of the stomatal conductance rate [29]. The stomatal conductance rate of sensitive *D. aegyptium* was lower than that of resistant *D. aegyptium* stomatal conductance. At the control treatment or a dose of 0 g ha⁻¹, the stomatal conductance of resistant weeds was almost four times higher than that of sensitive weeds. Metabolism increased in weeds encourages weed resistance to herbicide application [30].

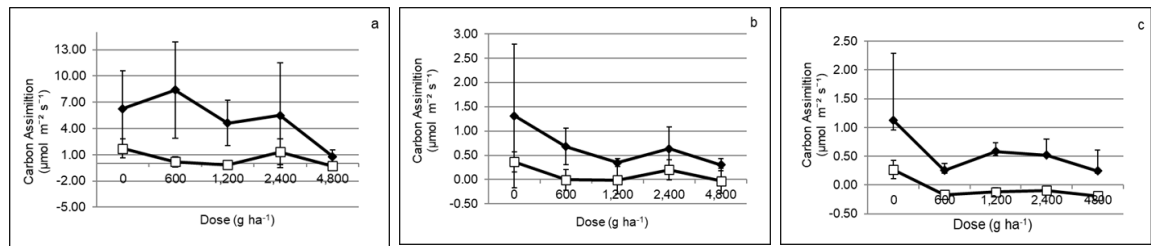


Figure 5. Carbon assimilation rate of *D.aegyptium* resistant to diuron herbicide. a: 4 DAA, b: 8 DAA, c: 12 DAA; □ unexposed; ◆ exposed

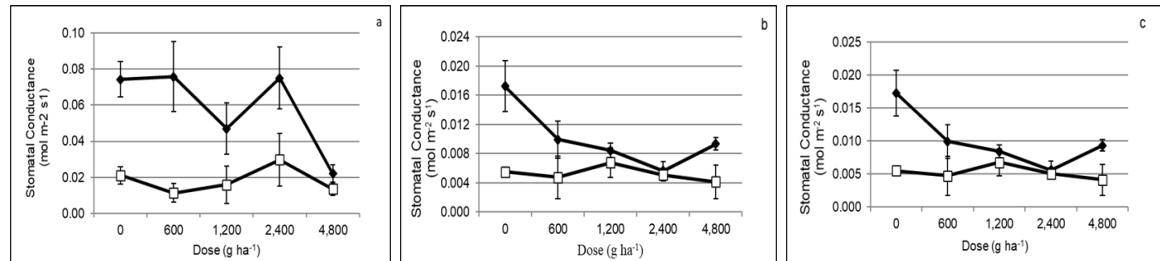


Figure 6. Stomatal conductance rate of *D.aegyptium* resistant to diuron herbicide. a: 4 DAA, b: 8 DAA, c: 12 DAA; □ unexposed; ◆ exposed

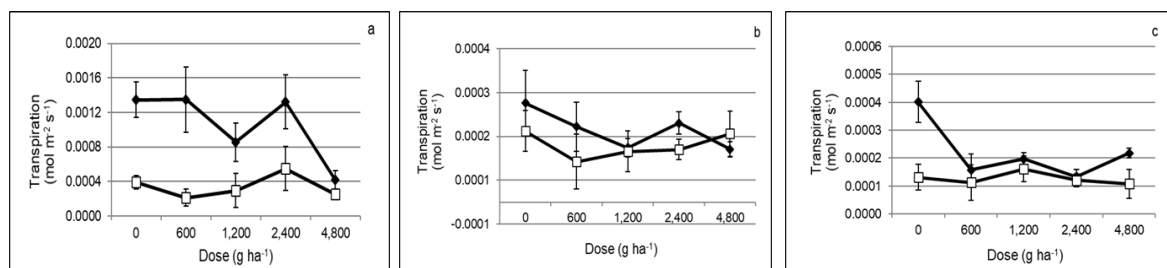


Figure 7. Transpiration rate of *D.aegyptium* resistant to diuron herbicide. a: 4 DAA, b: 8 DAA, c: 12 DAA; □ unexposed; ◆ exposed

CONCLUSION

D. aegyptium in pineapple plantations in Central Lampung is resistant to diuron due to continuous herbicide application. This was evident from the average rate of carbon assimilation, stomatal conductance, and transpiration in the *D. aegyptium* weed from pineapple plantations which was higher than weeds that had never been exposed to diuron. From the results of this study, it is hoped that there will be follow-up to confirm the molecular resistance mechanism.

ACKNOWLEDGMENT

This research was funded by University of Lampung, Indonesia through Graduate School Competitive Grant. Special thanks to the Weed Science Laboratory University of Lampung, and the Integrated Laboratory of the Technology Innovation Centers University of Lampung.

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