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# PROPANIL RESISTANCE IN SPRANGLETOP (*LEPTOCHLOA CHINENSIS* [L.] NEES) CAUSED BY ENHANCED PROPANIL DETOXIFICATION

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**Abstract**

The current study was conducted to elucidate the resistance mechanism of *Leptochloa chinensis* by comparing the difference in metabolism, aryl acylamidase activity, and chlorophyll fluorescence between the propanil-resistant (R) and the propanil-susceptible (S) biotypes of *L. chinensis,* using 4-week old seedlings. The concentration of propanil in the leaf and culm extracts of the R and S biotypes was found to increase after propanil treatment. The concentration of propanil in the leaf and culm extracts of the S biotype at 48 h was 12.23 and 5.49 μg $mL^{-1}$, respectively. However, a lower concentration of propanil was observed in the R biotype, compared to that in the S biotype. No residue of 3, 4-dichloroaniline was observed in the S biotype. In contrast the residue of 3, 4-dichloroaniline was detected in the leaf and culm extracts of the R biotype. The level of aryl acylamidase in the leaf tissue extracts of the R biotype was ~211% higher than that in the S biotype. The fluorescence studies showed that propanil inhibited the quantum efficiency of photosystem II in both the R and S biotypes after 2 h of incubation. However, when the leaf discs were transferred and incubated in deionized water for 48 h, the quantum efficiency increased in the R biotype but decreased in the S biotype. The results of the study suggest that propanil metabolism, enhanced by aryl acylamidase activity, is the most likely factor conferring the mechanism of propanil resistance in *L. chinensis* plants at the 4-week growth stage.

## Introduction

Propanil is a highly selective post-emergence herbicide that is used extensively to control barnyard grass (*Echinochloa crus-galli*) and jungle rice (*Echinochloa colona*) in rice fields. Propanil inhibits the photosynthetic electron transfer system of thylakoids in the chloroplast by binding the plastoquinone in the D1 protein of photosystem II (Wakabayashi & Boger, 2004). As a result, NADPH is depleted and active oxygen is generated, thereby injuring the photosynthetic apparatus in the chloroplasts (Usui, 2001). Although this herbicide provides excellent control of the susceptible biotypes of grassy weeds, extensive use has led to the development of propanil resistance in barnyard grass (Baltazar & Smith, 1994) and jungle rice (Garro *et al*., 2003.

The propanil-resistant barnyard grass (R-BYG) biotype was first discovered in Poinsett County, Arkansas, USA, in 1990 (Baltazar & Smith, 1994). Since then, the occurrence of R-BYG biotype had also been reported in a number of countries such as Sri Lanka (Marambe *et al.,* 1997), Thailand (Maneechote & Krasaesindhu, 1999), and Italy (Busi *et al*., 2003). Propanil-resistant jungle rice has been confirmed to be present in the rice-producing areas of Costa Rica (Garro *et al*., 2003), Columbia (Fisher *et al*., 1993), Venezuela, and Mexico (Hoagland *et al*., 2004). Two hypotheses have been suggested on the mechanism of propanil resistance (Carey *et al*., 1995; Leah *et al*., 1994) and these include enhanced metabolism of propanil and enzymatic detoxification of propanil. Some of these mechanisms contribute to propanil resistance in weeds such as barnyardgrass (Carey *et al*., 1997; Hirase & Hoagland, 2006) and jungle rice (Leah *et al*., 1997; Martinez *et al*., 2001).

The seasonal grass weed, *L. chinensis* (L.) Nees is one of the most successful colonisers in rice fields. It is abundant and extensively disseminated in the rice growing regions of the world (Abeysekera & Wickrama, 2005) and ranked third in the rice weed ecosystems. It is present in more than 50% of the fields in rice growing areas of Malaysia (Begum *et al*., 2005). ). *Leptochloa chinensis* shows great adaptability and are essential component of all type of ecosystems (Anjum *et al*., 2006). Propanil, quinchlorac, molinate, fenoxaprop-p-ethyl, benthiocarb (Ismail *et al.,* 2011) and cyhalafop in combination with sethoxydim (Chuah *et al.,* 2006a) have been used to control *L. chinensis* in rice crops. However, propanil has been preferred by the rice farmers for weed control because of its high selectivity. However, a propanil-resistant *L. chinensis* biotype was first reported in the rice fields of Pasir Mas in Kelantan, Malaysia, in 2006 (Chuah *et al*., 2006b) due to the frequent use of propanil. The resistance test was conducted using leaf discs from 4-week old seedlings of the R and S biotypes. Based on visual assessment, the degree of retention of the green coloration of the leaf discs in various concentrations of propanil was scored. The leaf disc tests indicated that the resistant (R) biotypes were two-fold more resistant than their respective susceptible (S) biotypes (Chuah *et al*., 2006a). Whole plant bioassay of 8 week-old plants further confirmed that the R biotype showed a 2.5-fold resistance to propanil, compared to that of the S biotype (Ismail *et al*., 2011).

The objective of the current study was to elucidate the resistance mechanism of *L. chinensis* by comparing the difference in metabolism, the specific activity of aryl acylamidase, and the effect of propanil on the photosynthetic electron transport chain of the propanil-resistant (R) and the propanil-susceptible (S) biotypes of *L. chinensis*.

## Materials and Methods

**Plant materials:** The seeds of *L. chinensis* were collected from rice fields (06°05'N, 102°10'E) under the supervision of the Kemubu Agriculture Development Authority at Kubang Sepat, Pasir Mas, in Kelantan, Malaysia. The seeds of the putative R biotypes were sampled from the rice fields where propanil had been applied at least twice a year for five consecutive years, while the seeds of the putative S biotypes were sampled from areas around the rice fields that were rarely sprayed with propanil.

**Herbicides:** Commercial as well as analytical-grade propanil formulations were used in the study. The commercial propanil formulation was Wham EZ (Zeenex, Kuala Lumpur, Malaysia), containing 420 g ai $L^{-1}$. The analytical-grade propanil (Pestanal>95% purity,) was supplied by Reidel da Haën (Seezle, Germany) and was used after being dissolved in methanol to produce the required 200 µmol $L^{-1}$ stock solution.

**Dose–response tests:** Before conducting the dose–response tests, a preliminary study was carried out, using the putative R and S seedlings of *L. chinensis* and spraying them with propanil at 6.80 kg ai $ha^{-1}$ (twice the recommended rate), to verify propanil resistance in *L. chinensis* under glasshouse conditions. It was found that all the putative R seedlings survived, while all the putative S seedlings were killed after treatment. Then, the seeds of the R and S biotypes were germinated separately in (26 × 32 × 6) cm plastic trays that contained soil potting mixture (Florasca 801; TURBA Earth and Humus, Papenburg, Germany). After 2 weeks, the seedlings of each biotype were transplanted into 12 cm diameter pots, containing clay loam soil, and were grown in a greenhouse at 29 ± 6°C, with a 12 h photoperiod and photosynthetic photon flux density (PPFD) of 800 ± 200 µE $m^{-2}$ $s^{-1}$. The plants were watered twice daily to ensure the soil was kept moist. At the four-leaf stage (4 weeks old), the plants were sprayed with commercial propanil, using a compression sprayer (Matabi Style 7; Goizper S. Cooperative Company, Guipuzcoa, Spain) at 200 kPa pressure with a flat-fan nozzle delivering a spray volume of 450 L $ha^{-1}$. The propanil rates that were used for the dose–response tests were 0, 0.43, 0.85, 1.70, 3.40, 6.80, 13.60, and 27.20 kg ai $ha^{-1}$. The plants from each biotype were divided randomly into eight treatment groups, including control plants, with each treatment consisting of 5 plants. The shoot fresh weight was recorded 1 week after the herbicidal treatment. The data of the shoot fresh weight were fitted to a logistic regression model, as follows (Kuk *et al*., 2002):

$$Y = a / [1 + (x/x0)^{b}]$$

where Y = the fresh weight of the harvested plants, a = the coefficients corresponding to the upper asymptotes, b = the slope of the line, x0 = the herbicide rate necessary to inhibit the shoot growth by 50%, and x = the herbicide rate. Regression analyses were conducted and the herbicide rates that were necessary to reduce the shoot fresh weight by 50% ($GR_{50}$) were calculated from the regression equations. The resistance level was calculated as the $GR_{50}$ of the R biotype divided by the $GR_{50}$ of the S biotype.

## Metabolism

**Plant extraction**: Four-week old plants of both the R and S biotypes that were grown as described above were sprayed with commercial propanil at the rate of 0.85 kg ai $ha^{-1}$, using a compression sprayer as described above. Unsprayed plants were used as the control. The leaf and culm of each biotype were excised at 2, 24, 48, 72, and 96 h after being sprayed. Liquid extraction was used to draw out the herbicide from the leaf and culm samples, according to the technique of Mitsou *et al*., (2006) with some modifications. The plant samples (~2 g) were washed with deionized water before extraction was undertaken, using 25mL methanol, followed by shaking for 2 h in an orbital shaker (Model S1 02; Firstek Scientific, Taipei, Taiwan) at 160 rpm. The extract was filtered through filter paper (no. 4; Whatman, Maidstone, England) into a 100mL conical flask containing 3 g anhydrous sodium sulphate. After 5 min, a 5mL aliquot was taken and concentrated in a gentle stream of nitrogen to a final volume of 1mL and then subjected to chromatographic analysis.

**Chromatographic analysis:** The chromatographic analysis was carried out with a capillary gas chromatograph (4890N; Hewlett Packard, Waldbronn, Germany) that was equipped with an electron capture detector, at 250°C. The target compound was separated with a column (30 cm length, 0.25 mm i.d.) (HP-5; Hewlett-Packard) that was coated with phenylmethylsiloxane at a film thickness of 0.25µm. The temperature program and analysis were 100°C (1min) and 260°C (20°C $min^{-1}$) for 6 min. Helium was used as the carrier gas at a flow rate of 1 mL $min^{-1}$, while nitrogen was used as the make-up gas, with a flow rate of 50 mL $min^{-1}$. The injector temperature was set at 250°C. The splitless mode was used for injection, with an injection volume of 1 µL.

## Aryl acylamidase activity

**Enzyme extraction:** Plants of both the R and S biotypes were grown as described above and the 4 week old plants were used. The leaves were excised from 10 freshly harvested individual plants (~2 g) of both the R and S biotypes. The leaves were homogenized in 20 mL phosphate buffer (100 mmol $L^{-1}$ pH = 7.5, containing 1 mmol $L^{-1}$dithiothreitol and 2% insoluble polyvinylpyrollidone), using a chilled mortar and pestle in liquid nitrogen. Then, the homogenate was centrifuged at 7168 g for 30 min at 4°C and the supernatant was used directly as the crude enzyme preparation.

**Enzyme assay:** The protein concentration was measured in all the plant extracts, using the Bradford reagent (Bradford, 1976). Bovine serum albumin (Bio-Rad, Benicia, CA, USA) was used as the protein standard. The standard reaction mixture consisted of 0.3mL crude enzyme solution, 0.7mL analytical-grade propanil (10 µg $mL^{-1}$ dissolved in methanol), and 0.5 mL methanol (50 mL $L^{-1}$). The reactions were allowed to run for 3 h in a water bath at 35°C. The reactions were terminated with the addition of 0.5mL of a mixture of hydrochloric acid glacial acetic acid (9:1 v/v). After 15 min, 1.5mL p-dimethylaminocinnamaldehyde (p-DACA: 0.12% dissolved in ethanol) was added and mixed. After allowing it to stand for 15 min, the product of the enzyme reaction, 3,4-dichloroaniline (DCA), was quantified by measuring the absorbance at 565 nm, using a spectrophotometer (U-2000; Hitachi, Tokyo, Japan). One unit of the enzyme specific activity was defined as 1 µmol DCA that was produced per mg of protein $h^{-1}$ (mg protein $h^{-1}$). A standard curve of absorbance versus concentration of DCA was prepared using various concentrations of DCA (>95% purity: 10 µg $mL^{-1}$ dissolved in methanol), ranging from 0.4 to 100 µmol $L^{-1}$, mixed with 1.5 mL p-DACA and the absorbance was measured at 565 nm.

**Effects of propanil on photosynthesis:** Plants of both the R and S biotypes were grown as described above and 4 week old plants were used. The second leaf of the plants of the R and S biotypes was punched with a cork borer in order to obtain discs of 6 mm diameter. Five discs were placed in each of the 1.5 cm diameter test tubes that contained either 5 mL of 200 µmol $L^{-1}$ analytical-grade propanil or 5 mL of deionized water (the control). The test tubes were covered with aluminium foil in order to protect the leaf discs from light exposure during incubation at 25°C. After 2 h of incubation, the leaf discs were washed with distilled water and transferred to 9 cm diameter Petri dishes containing 5 mL deionized water. The leaf discs were then kept in a growth chamber at 25°C in darkness. The chlorophyll fluorescence was measured using a fluorometer (Plant Efficiency Analyser; Hansatech Instruments, Norfolk, UK) after 2, 24, 48, 72, and 96 h of incubation and after 60 s illumination time. For the fluorescence measurement, each leaf disc was removed from the treatment solution and blotted dry. A single disc was placed on the light electrode, with the adaxial side towards the light source. The red monochromatic light source had PPFD and wave length of 2.055 µE $m^{-2}$ $s^{-1}$ and 670 nm, respectively. The difference in peak and terminal fluorescence was used to calculate the maximum quantum efficiency of photosynthesis, compared to the measurement that was obtained for the untreated leaf discs that were incubated in deionized water (Schreiber *et al*., 1977).

**Statistical analysis:** Each of the above experiments was carried out using a randomized complete block design with three replications, except for the evaluation of the effect of propanil in the photosynthesis studies, which had five replications. The *t*-test at the 5% level of significance was used to compare the means between the R and S biotypes for both the metabolism and the enzyme-assay. All the data of the photosynthesis studies were subjected to an ANOVA and the means were compared using the Tukey's test at the 5% level of significance.

## Results and Discussion

**Dose–response tests:** Figure 1 shows the response of the R and S biotypes of *L. chinensis* to the propanil treatments. The shoot fresh weight of both biotypes decreased as the propanil rate increased, but the S biotype showed more decline in the shoot fresh weight, compared to that of the R biotype. The R biotype exhibited a 3.7-fold resistance to propanil, compared to that of the S biotype in the 4 week old seedlings. Recent findings of the authors have demonstrated that the resistance level was 2.5-fold when *L. chinensis* plants were 8 weeks old (Ismail *et al*., 2011). This result suggests that the propanil resistance level of *L. chinensis* declines with age as seen in paraquat resistance of *Crasocephalum crepidioides* (Benth.) S. Moore (Ismail *et al*., 2001). However, Koger *et al*., (2004) reported that the growth stage had little effect on the level of glyphosate resistance in *Conyza canadensis* L. Cronq.

**Metabolism:** The metabolism of propanil, to yield the non-toxic compound, DCA, is presumed to be the primary mechanism that is responsible for propanil tolerance in rice. This metabolism is mediated by the enzyme, aryl acylamidase. The inhibition of this enzyme reduces the metabolism of propanil in the R populations and the herbicide produces the same effect on the S populations, as demonstrated by the similarity of the thin layer chromatographic profiles of the metabolic products of propanil in these biotypes (Carey *et al.,* 1995; Carey *et al.*, 1997).

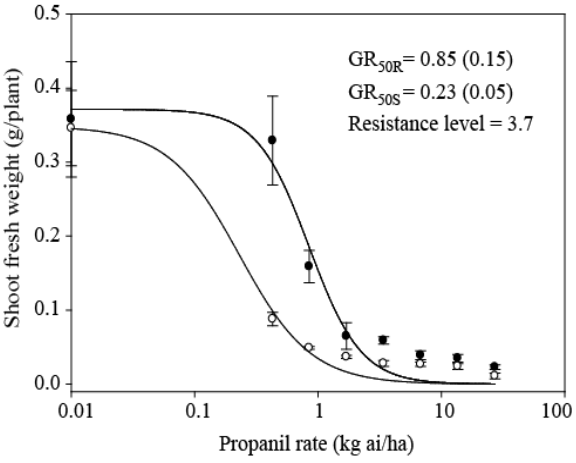


Fig. 1. Shoot fresh weight of the susceptible (○) and resistant (●) biotypes of *Leptochloa chinensis*, as affected by propanil in the whole-plant bioassay 7 days after treatment under glasshouse conditions. Every point is a mean of five replicates, each containing five plants. The vertical bars represent the standard error of the mean. The $GR_{50R}$ and $GR_{50S}$ are the herbicide rates that were required to reduce the shoot fresh weight of the resistant and susceptible biotypes by 50%, respectively. The values in parentheses are the standard error of the mean.

Propanil and its degradation product, 3,4-dichloroaniline (DCA) were identified based on gas chromatographic analysis (Fig. 2). The concentration of propanil in the leaf and culm extracts of the R and S biotypes increased 72 h after propanil treatment (Tables 1, 2). Likewise, a previous study conducted by Leah *et al*., (1995) demonstrated that the uptake of propanil by the leaves of *E. colona* for both biotypes increased with time within 3 days after treatment, although propanil resistance in *E. colona* was due to enhanced metabolism in the R biotype. In the current study, a lower concentration of propanil was observed in the R biotype, compared to that in the S biotype. In addition, it was clearly shown that some propanil was hydrolyzed into its metabolite, DCA, which was detected in the leaf and culm extracts of the R biotype. On the contrary, no residue of DCA was observed in the S biotype (Tables 2, 3). Therefore, the lower concentration of propanil that was detected in the R biotype probably was related to the metabolism of propanil. In the current study, the residues of DCA were detected both in the leaf and culm extracts of the R biotype, implying that the leaf and culm together played roles in metabolizing propanil into DCA at the 4 week growth stage. On the other hand, Ismail *et al*., (2011) documented that leaf tissues were more efficient in metabolising propanil into DCA since no DCA was detected in culm extracts at the 8 week growth stage of *L. chinensis*. This may be the reason why the propanil resistance level at the 4 week growth stage (3.7-fold) was higher than that at the 8 week growth stage (2.5-fold).

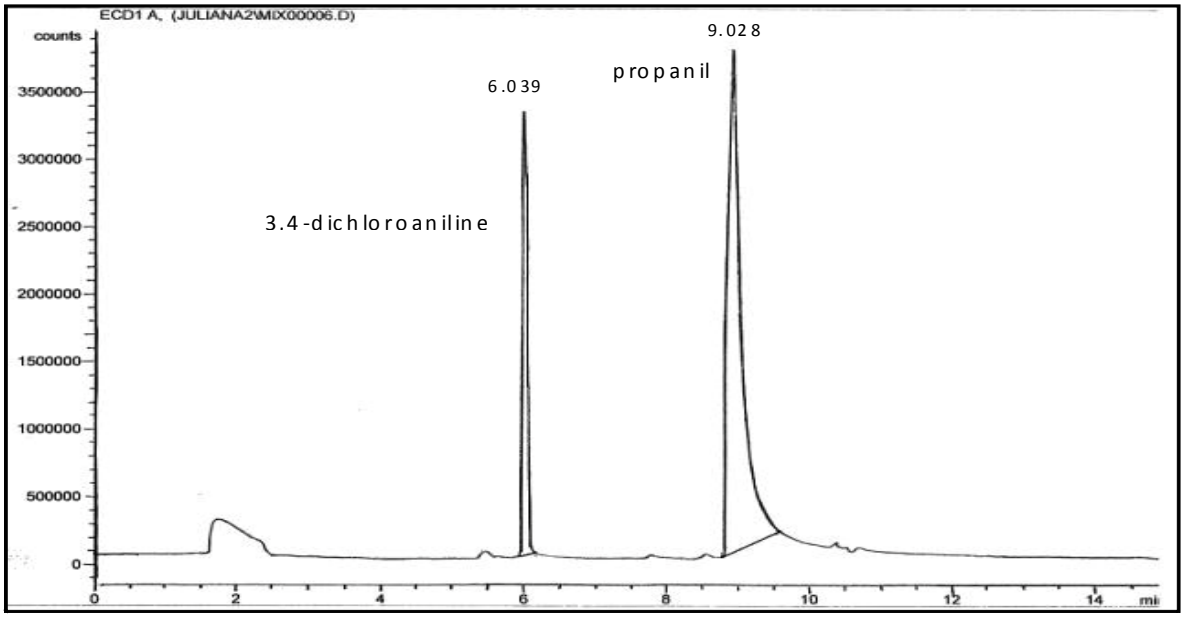


Fig. 2. Representative gas chromatography spectrum: analysis of propanil (>95% purity) and 3,4-dichloroaniline (>95% purity) dissolved in methanol.

**Table 1. Concentration of propanil and 3,4-dichloroaniline in the leaf extracts of the *Leptochloa chinensis* biotypes at different intervals of time after being sprayed with propanil.**

| Time(h) | Concentration (µg $mL^{-1}$ ± SD) | | | |
|---|---|---|---|---|
| | Propanil | | 3,4-Dichloroaniline | |
| | R | S | R | S |
| 2 | 4.0566 ± 0.0515a* | 4.0781 ± 0.0535a | ND** | ND |
| 24 | 4.2524± 0.2245b | 6.9842 ± 0.2712a | 0.2163 ± 0.1192 | ND |
| 48 | 4.4506 ± 0.0372b | 12.2329 ± 0.0786a | 0.7977 ± 0.3673 | ND |
| 72 | 6.0733 ± 0.1692b | 12.5087 ± 0.0285a | 0.8461 ± 0.5959 | ND |
| 96 | 5.5059 ± 0.2252b | 12.2985 ± 0.0508a | 0.4318 ± 0.2200 | ND |

*Means within a row are significantly different between the R and S biotypes at $p<0.05$, using the *t*-test. ND, not detected; R= Resistant; S= Susceptible; SD= Standard deviation

**Aryl acylamidase activity:** Aryl acylamidases, which are found to be important for endogenous nitrogen metabolism in plants (Hirase & Matsunaka, 1991), have been associated with the hydrolysis of propanil, to form DCA (Still & Kuzirian, 1968). Aryl acylamidase activity has been measured in a wide range of plants (Hoagland *et al*., 1974) and the enzyme has been partially isolated and characterized in several higher plants, including rice and *E. crus-galli* (Frear & Still, 1968). The measurement of the crude extracts from rice and *E. crus-galli* has shown that the aryl acylamidase level is an important factor in the differential hydrolysis, and hence the selective action, of propanil (Still & Kuzirian, 1967).

In the present study, the level of aryl acylamidase in the R biotype was substantially higher than that in the S biotype in the 4 week-old plants. The measurement of the crude extracts indicated that the R biotype exhibited a specific activity of aryl acylamidase that was ~3.1-fold higher than that of the S biotype (Fig. 3). This higher level of aryl acylamidase in the R biotype of *L. chinensis* is most likely responsible for the detoxification of propanil into DCA, as shown under metabolism, implying that propanil resistance in the R biotype of *L. chinensis* is related to increased aryl acylamidase activity. These results are in line with previous findings, where the mechanism of propanil resistance in barnyardgrass (Carey *et al*., 1997) and jungle rice (Leah *et al*., 1995) biotypes was found to be related to the increased activity of aryl acylamidase in the R biotypes. Ismail *et al*., (2001) demonstrated that the level of aryl acylamidase in the leaf tissue extracts of the R biotype was only 2.4 fold higher than that in the S biotype at the 8 week growth stage. It is possible that the decline of propanil resistance level in *L. chinensis* with age is partly due to a decrease in function of inducible aryl acylamidase as the plants mature.

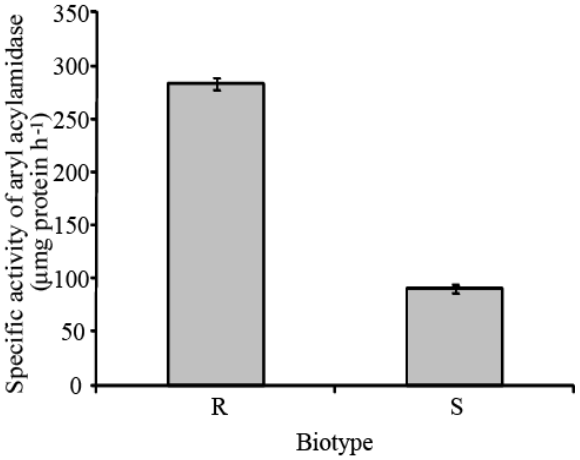


Fig. 3. Specific activity of aryl acylamidase in the leaf tissue extracts of the *Leptochloa chinensis* biotypes. The vertical bars represent the ± standard deviation of the means (n = 3). *The means are significantly different between the R and S biotypes ($p<0.05$), using the t-test. R, resistant; S, susceptible.

**Effects of propanil on photosynthesis:** The quantum efficiency or yield of the PS II (Fv/Fm) can be used to measure plants' photosynthetic performance, with optimal values of ~0.83 being measured for most plant species (Bjorkman & Demmig, 1987; Johnson *et al.,* 1993). Values that are lower than this level are observed when plants are subjected to stress, indicating the phenomenon of photo-inhibition, in particular. The Fv/Fm ratios of both the R (0.389 arbitrary units, a.u.) and S (0.354 a.u.) plants were lower than that of the control plants (0.768 a.u.) after 2 h of incubation (Table 3), indicating that propanil inhibited photosynthetic activity at the PS II level in both the R and S biotypes. These results strongly suggest that the propanil-binding site is not altered in both the biotypes, and this is in agreement with the results of previous studies (Martinez *et al.,* 2001). If the resistance mechanism of the R biotype was due to a change in the photosynthetic electron transport chain, there would be no initial effect (Ahrens *et al*., 1981; Gohbara *et al*., 1988). However, after the leaf discs were transferred to deionized water and incubated for 48 h, the Fv/Fm ratio increased in the R biotype (0.646 a.u.) and decreased in the S biotype (0.160 a.u.) (Table 3). The inhibition of photosynthesis decreased as the incubation time after treatment increased, implying that the R biotype was affected initially by propanil, but subsequently recovered. However, after 72 h of incubation, the Fv/Fm ratio decreased in both the R and S biotypes, with the S biotype appearing to show a more rapid decrease in the Fv/Fm ratio (Table 3). A similar trend was exhibited by both the R and S biotypes at the 8 week growth stage (Ismail *et al.,* 2001). The decreased Fv/Fm ratio that was observed in the R biotype probably is related to the incapability of the R leaf discs to metabolize all the absorbed propanil within the 72 h experimental period. Consequently, the remaining propanil in the leaf discs could still inhibit photosynthesis in the R biotype. These results strongly suggest that metabolism is the main factor contributing to the resistance to propanil by the R biotype. The results that have been obtained are in line with those of previous reports on propanil resistance of *E. crus-galli* (Martinez *et al*., 2001; Norsworthy *et al*., 1998) and *E. colona* (Martinez *et al*., 2001).

**Table 2. Concentration of propanil and 3,4-dichloroaniline in the culm extracts of the *Leptochloa chinensis* biotypes at different intervals of time after being sprayed with propanil (±SD).**

| Time(h) | Concentration (µg $mL^{-1}$ ± SD) | | | |
|---|---|---|---|---|
| | Propanil | | 3,4-Dichloroaniline | |
| | R | S | R | S |
| 2 | 3.3715 ± 0.4176a* | 3.5022 ± 0.3810a | ND** | ND |
| 24 | 3.7081 ± 0.4330a | 3.8654 ± 0.3487a | ND | ND |
| 48 | 3.8640 ± 0.4168a | 5.4950 ± 0.3206a | 0.1916 ± 0.0353 | ND |
| 72 | 4.1329 ± 0.2113b | 6.1349 ± 0.1398a | 0.2023 ± 0.0595 | ND |
| 96 | 4.0794 ± 0.3010b | 5.5058 ± 0.0305a | 0.2498 ± 0.0398 | ND |

*Means within a row are significantly different between the R and S biotypes at $p<0.05$, using the *t*-test. ND, not detected; R= Resistant; S= Susceptible; SD= Standard deviation

**Table 3. Quantum efficiencies of the photosystem II (PS II) in the leaf discs of the *Leptochloa chinensis* biotypes following incubation in propanil and measurement at 2, 24, 48, 72, and 96 h after being transferred to deionized water.**

| Time (h) | Quantum efficiencies of PS II, Fv/Fm (a.u. ± SD) | | |
|---|---|---|---|
| | Control | R | S |
| 2 | 0.768 ± 0.006a* | 0.389 ± 0.065b | 0.354 ± 0.063b |
| 24 | 0.725 ± 0.003 a | 0.530 ± 0.039 b | 0.258 ± 0.049c |
| 48 | 0.696 ± 0.018 a | 0.646 ± 0.026 a | 0.160 ± 0.019c |
| 72 | 0.495± 0.072 a | 0.417 ± 0.037a | 0.074 ± 0.029b |
| 96 | 0.335± 0.035 a | 0.227 ± 0.047 b | 0.023 ± 0.059c |

*Means followed by a different letter within a row are significantly different at $p<0.05$, using the Tukey's test. The data represent the means of five replicates. R, resistant; S, susceptible; SD, standard deviation

## Conclusion

The metabolite of propanil degradation, DCA, was found in the leaf and culm extracts of the R biotype. However, such metabolites were not detected in the S biotype. The level of aryl acylamidase in the leaf tissue extracts of the R biotype was significantly higher than that in the S biotype. The fluorescence studies demonstrated that propanil inhibited photosynthetic activity at the PS II level in both the R and S biotypes after 2 h of incubation, implying that there was no difference in the propanil site of action for both biotypes. The results of the present study suggest that enhanced propanil metabolism and the higher level of the enzyme, aryl acylamidase, probably account for the resistance mechanism of *L. chinensis* to propanil.

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