Ecotypic Variation in *Echinochloa colona*

II. Intraspecific Variations in Sensitivity to Herbicides

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*Echinochloa colona*의 生態型 變異

II. 除草劑에 대한 種內 反應性 變異

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ABSTRACT

The intraspecific susceptibility of twelve *Echinochloa colona* (L.) Link ecotypes to herbicides was studied at three different stages of growth. At germination and post-germination stage, the Batangas ecotype was most susceptible to both butachlor (N-butoxymethyl-2-chloro-2', 6'-diethylacetanilide) and thiobencarb (S-4-dichloro -benzyldiethylthiocarbamate), whereas the Bukidnon ecotype was the most tolerant. The susceptibility of the ecotypes used was not associated with the seed weight and maturity. When the preemergence herbicides were applied at the 2-leaf stage of the ecotypes, the Nueva Ecija ecotype was least susceptible to butachlor at 1 ppm, but it was most susceptible to thiobencarb. The reverse was exhibited by the Cagayan ecotype. Differential susceptibility of the ecotypes to propanil (3', 4'-dichloropropionanilide) as determined by the necrotic length of the third leaf of 20-day-old seedlings was pronounced in the Camarines Sur, Iloilo, and IRRI (red) ecotypes. However, no necrotic tip appeared in the Cagayan and Batangas ecotypes. The differential responses varied with the type and the concentration of the herbicide studied and the growth stage of the ecotypes.

Key words : *Echinochloa colona*, Ecotype, Intraspecific susceptibility.

INTRODUCTION

Intraspecific responses of plant ecotypes to herbicides have been observed with various weed species (Sexsmith, 1964 ; Roche and Muzik, 1964 ; Whitworth and Muzik, 1967 ; Jacobsohn and Andersen, 1972 : Jenser et al., 1977). the differential response of ecotypes may account for survival of some usually susceptible plants following field application of herbicides for weed control.

Hamilton and Tucker (1964) found that there were different responses of *Sorghum halepense* L. ecotypes to dalapon (2, 2-dichloropropionic acid). McWhorter and Jordan (1976) reported that *S. halepense* ecotypes that were most tolerant to dalapon had longer rhizome internodes and higher water content in culms and rhizomes than susceptible ecotypes.

Convolvulus arvensis L. ecotypes also had different responses to 2, 4-D (2, 4-dichlorophenoxy acetic acid) (Whitworth, 1964). Many of the tolerant ecotypes showed incomplete topkill followed by reproduction from roots within 1 month after treatment, whereas those that were classified as most susceptible showed complets topkill and no sprouting. The variable response appeared to be due to inherent physiological

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differences and was not related to vigour as measured by rapidity of growth.

Yamasue et al. (1981) obtained significant differences in percent reduction in plant height by nitrofen and thiobencarb, and in the necrotic length of the primary leaf by propanil for 25 *Echinochloa phylapogon* (Stapf) Koss. ecotypes. There was no correlation between seed weight of the ecotypes and their susceptibility to nitrofen and propanil. However, there was a significant negative correlation for thiobencarb.

In a previous study (Chun and Moody, 1987), twelve ecotypes of *E. colona* were characterized based on the latitude and habitat at the collection sites using several different parameters of growth. The present study was conducted to determine if *E. colona* ecotypes vary in susceptibility to butachlor, thiobencarb, and propanil.

MATERIALS AND METHODS

E. colona seeds were obtained from mature plants of different ecotypes grown in the Agronomy greenhouse at the International Rice Research Institute (IRRI), Los Banos, Philippines. The sites in which the seeds of the different ecotypes were originally collected have been described previously (Chun and Moody, 1987).

Response to Preemergence Herbicides

To determine the response of seeds of the different ecotypes to butachlor and thiobencarb, thirty seeds of each ecotype were placed on a Whatman No. 1 filter paper in a 250 ml Erlenmeyer flask containing 3 ml of herbicide solution. Herbicide solutions having concentrations of 1 and 3 ppm were prepared using distilled water. After closing the flasks with a rubber stopper, they were placed in the greenhouse. A completely randomized design with four replications was used. The number of germinated seeds was counted 10 days later and plant height was measured. Seeds were considered germinated if the plant height was 5 mm.

To determine seedling response of the ecotypes to the herbicides, herbicide solutions (1 and 3 ppm) were prepared using a nutrient solution (Yoshida et al., 1976). Roots of 10-day-old seedlings of the twelve ecotypes grown in soil in the greenhouse were rinsed with distilled water and transplanted into vermiculite which had been treated with herbicide-nutrient solution in a plasic tray (68x40x20cm). Plant heights were measured before the seedlings were transplanted and 10 days after transplanting (DAT). The increase in plant height was obtained by subtracting the height before transplanting from the height 10 DAT. The response of each ecotype to herbicides is presented as a percent of the untreated check.

Response to Postemergence Herbicide

Whole leaf blades of the second leaf of 10-day-old seedlings and the third leaf of 20-day-old seedlings of each ecotype were dipped into a 250-ppm propanil solution for 5 seconds. The seedlings were grown in herbicde-free nutrient solution as in the above experiment for 48 hrs. The seedlings were then harvested to measure the necrotic length in the herbicide-treated leaf.

RESULTS AND DISCUSSION

Effect of herbicides when applied to seeds

Germination of some ecotypes was significantly reduced by butachlor and thiobencarb (Table 1). At 1 and 3 ppm, the highest germination inhibition by butachlor occurred with the Batangas ecotype. Butachlor also slightly inhibited the germination of the Pangasinan and Camarines Sur ecotypes. Thiobencarb caused a significant germination inhibition of only the Batangas ecotype when treated with 3 ppm of herbicide solution.

None or only slight ingibition of germination of most of *E. colona* ecotypes by these hersbicides is not an unexpected result. Although both acetanilide and thiocarbamate herbicides are readily absorbed by seeds, the herbicides would not affect the germination *per se* (Takematsu et al., 1971 ; Nakamura et al., 1974). Since the shoot is the site of uptake or the site of action or both for acetanilide (Chandler et al., 1974) and thiocarbamate herbicides (Dawson, 1963 ;

Table 1. Inhibition of germination of *Echinochloa colonan* ecotypes as affected by butachlor and thiobencarb[1].

Ecotype	Inhibition of germination (% of untreated check)			
	Butachlor (ppm)		Thiobencarb (ppm)	
	1	3	1	3
Cagayan	0 c	4.2 bc	4.4 a	3.3 b
Pangasinan	15.1 b	13.8 ab	0 a	3.3 b
Nueva Ecija	0 c	0 c	0 a	0 b
IRRI (green)	1.8 c	7.3 bc	0 a	0.9 b
IRRI (red)	0 c	5.8 c	1.0 a	0 b
Batangas	36.7 a	21.9 a	3.3 a	12.7 a
Camarines Sur	11.8 bc	11.7 ab	2.6 a	0 b
Iloilo	3.4 c	4.2 bc	3.3 a	0 b
Leyte	2.8 c	2.6 bc	4.2 a	0 b
Bukidnon	3.9 bc	1.8 bc	0 a	0 b
Zamboanga del Sur	8.5 bc	0 c	0.9 a	0 b
South Cotabato	5.2 bc	5.2 bc	0.8 a	0.9 b

[1] In a column, means followed by a common letter are not significantly different at the 5% level by Duncan's multiple range test.

Burt, 1976), the phytotoxic effect would be manifested in the shoot regions after germination has occurred. However, in our experiment germination of some of the ecotypes was inhibited by the herbicides used. This difference may be attributed to variations in time of germination among the ecotypes which are related with the dormancy characteristics. Wide variation in seed germination of the ecotypes was previousely observed by Chun and Moody (1987).

Variations in plant height of the germinating seeds as affected by butachlor were more pronounced than was the inhibition of germination (Fig. 1). The ecotypes showed greater variations in response to 1 ppm butachlor than to the 3 ppm concentration. The ecotypes most affected by 1 ppm of the herbicide were those from Cagayan, Nueva Ecija, Batangas, Camarines Sur, and South Cotabato. At 3 ppm, severe inhibition of shoot growth of all the ecotypes except for Bukidnon ecotype was observed. The South Cotabato ecotype was the most susceptible of the ecotypes to 3 ppm butachlor. No difference in the response of the shoot growth of the Bukidnon ecotype seems to indicate some tolerance to butachlor. With thiobencarb, the Pangasinan, Iloilo, and Bukidnon ecotypes were least affected by 1 ppm, while the most susceptible ecotype at both herbicide concentrations was the IRRI (green) ecotype (Fig. 2). On the other hand, the Pangasinan, IRRI (red) and Iloilo ecotypes were most affected by the increase in herbicide concentration for both herbicides.

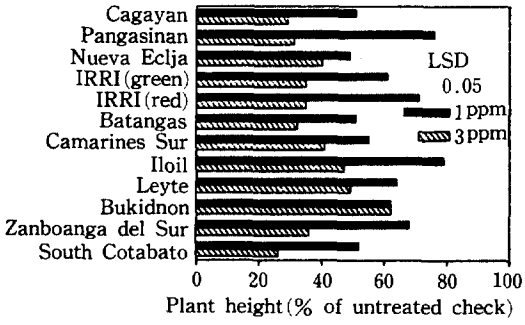

Fig. 1. Plant height of *Echinochloa colona* ecotypes as affected by butachlor applied to the seed.

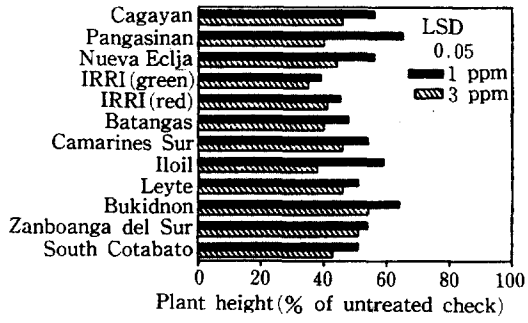

Fig. 2. Plant height of *Echinochloa colona* ecotypes as affected by thiobencarb applied to the seed.

The susceptibility of the *E. colona* ecotypes to the herbicides was not associated with the seed weitht and the days required to heading. Correlation coefficients between shoot lenght and seed weight and days to heading were 0.443 and -1.176($p>0.05$) for butachlor and 0.295 and -0.371 ($p>0.05$) for thiobencarb, respectively, at 1 ppm concentration. Thus, differences in seed size and maturity among the ecotypes may not explain variations in herbicide response.

Response of seedlings to preemergence herbicides

The herbicides when applied to seedlings had a greater effect than when applied to seeds. Plant height was greatly reduced in all ecotypes by both herbicides. There were marked variations among ecotypes in plant height as determined as a percent of the untreated check at 1 ppm, but the differences were not as pronounced at 3 ppm(Table 2). The least reduction in plant height was observed with the Nueva Ecija ecotype at 1 ppm butachlor, whereas this ecotype was the most susceptible to thiobencarb at both herbicide concentrations. However, the Cagayan ecotype which was most affected by butachlor at 1 ppm was one of the least affected by thiobencarb. The Camarines Sur. Leyte, and South Cotabato ecotyes showed similar responses to both herbicides at 1 ppm.

Little variation in plant height was observed between the two herbicide concentrations for the Cagayan ecotype treated with butachlor and the Pangasinan, Nueva Ecija, IRRI(green), IRRI (red), Batangas, Iloilo and Zamboanga del Sur ecotypes treated with thiobencarb. On the other hand, most ecotypes except for the Cagayan ecotype treated with butachlor showed a marked decrease in plant height with increasing the application concentration. The Camarines Sur and South Cotabato ecotypes treated with thiobencarb also responded similarly to the increased concentration.

Response to increase in the herbicide concentration suggested a relative sensitivity to the applied herbiciides among the ecotypes. For example, the Nueva Ecija ecotype treated with thiobencarb resulted in 5.5 and 4.4% increase in plant height as percent of the untreated check, respectively, at 1 and 3 ppm. The ecotype would be killed by 1 ppm, but increase to 3 ppm would have no effect. However, the Camarines Sur ecotype was affected by increase in the herbicide concentration. This indicated that 1 ppm would be insufficient to cause to kill and 3 ppm would be required to control the ecotype. Therefore, the Camarines Sur ecotype was more tolerant to the herbicide than the Nueva Ecija ecotype. The differences among *E. colona* ecotypes in response to herbi-

Table 2. Increase in plant height of *Echinochloa colona* ecotypes as affected by butachlor and thiobencarb[1].

Ecotype	Increase in plant height (% of untreated check)			
	Butachlor (ppm)		Thiobencarb (ppm)	
	1	3	1	3
Cagayan	10.2 d	7.3 a	16.1 abcd	12.0 abc
Pangasinan	22.7 bc	5.2 ab	9.5 cdef	6.5 abc
Nueva Ecija	36.1 a	7.3 a	5.5 f	4.4 bc
IRRI (green)	10.6 d	3.7 ab	6.5 ef	4.5 c
IRRI (red)	14.0 cd	1.8 b	9.4 def	10.3 abc
Batangas	19.2 bcd	8.0 a	7.5 ef	8.0 abc
Camarines Sur	25.2 abc	6.8 a	24.9 a	8.6 abc
Iloilo	15.8 cd	5.2 ab	13.2 bcde	15.0 a
Leyte	27.6 ab	10.5 a	20.5 ab	13.5 ab
Bukidnon	24.6 abc	8.0 a	14.5 bcde	7.4 abc
Zamboanga del Sur	20.6 bc	5.9 ab	16.9 abc	12.8 abc
South Cotabato	29.3 ab	6.7 a	18.9 ab	6.6 abc

[1] In a column, means followed by a common letter are not significantly different at the 5% level by Duncan's multiple range test.

cides suggest that butachlor or thiobencarb application at a given rate may control only susceptible ecotypes while the more tolerant ecotypes survive and infest.

The response of *E. colona* ecotypes to butachlor seems to be independent of their response to thiobencarb. This indicates that if the continuous use of butachlor results in a more butachlor tolerant ecotype population than at present, the population may not necessarily become more thiobencarb tolerant. Alternation of herbicides may reduce the possibility of an increase in the proportion of ecotypes tolerant butachlor or thiobencarb.

These results clearly suggest that there are intraspecific differences in susceptibility to the herbicides. The differential response of the ecotypes is manifested by the types of herbicides used and their concentration. However, the differential sensitivity cannot be explained by physiological maturity of the ecotypes. Correlation coefficients between days to heading of the ecotypes and increase in the plant height after application of the herbicides were 0.171($p>0.05$) for butachlor and 0.317 ($p>0.05$) for thiobencarb at 1 ppm.

Response of seedlings to postemergence herbicide

In this experiment *E. colona* tolerance to propanil increased with the plant age. All the ecotypes except for the Iloilo and South Cotabato ecotypes had less necrotic lenght on third leaf than the second (Table 3). However, differential susceptibility among the ecotypes was greater with the older seedlings than with the younger seedlings. There was little difference in the necrotic lengths of the second leaves of 10-day-old seedlings among ecotypes except that the Camarines Sur ecotype had a significantly greater necrotic length than the Batangas, Leyte, Zamboanga del Sul and South Cotabato ecotypes. The IRRI (red) ecotype also had a longer necrotic length than the South Cotabato ecotype. When the third leaf of the 20-day-old seedlings was used, the necrotic lengths of leaves of the Camarines Sur, Iloilo, IRRI (red) and South Cotabato ecotypes were significantly longer than those of the other ecotypes. Necrosis of the tip of the third leaf was not observed with the Cagayan and Batangas ecotypes.

With old seedling the intraspecific variation in susceptiblity of the ecotypes to the herbicide may be more pronounced, provided that the ecotypes possess different ecophysiological susceptibilities to the herbicide. A possible reason for the difference could be differential penetration of propanil caused by morphological and physiological modification as reported for *Agropyron repens* L. (Hadad and Sagar, 1968) and *Tamarix pentandra* Pall. (Wilkinson, 1980) ecotypes with several postemergence herbicides.

Table 3. Necrotic length of leaves of *Echinochloa colona* ecotypes as affected by propanil[1)]

Ecotype	Necrotic length (mm)	
	Second leaf	Third leaf
Cagayan	25.0 abc	0 b
Pangasinan	23.5 abc	7.3 b
Nueva Ecija	22.5 abc	2.5 b
IRRI (green)	26.0 abc	8.3 b
IRRI (red)	29.0 abc	21.5 a
Batangas	20.8 bc	0 b
Camarines Sur	31.0 a	28.0 a
Iloilo	23.5 abc	23.5 a
Leyte	20.3 bc	6.8 b
Bukidnon	23.8 abc	2.5 b
Zamboanga del Sur	20.5 bc	5.8 b
South Cotabato	18.8 c	24.3 a

[1)]In a column, means followed by a common letter are not significantly different at the 5% level by Duncan's multiple range test.

摘 要

Echinochloa colona (*L.*) *Link* 生態型 12種의 除草劑에 대한 種內 耐性差異를 生育 段階別 檢討하였다. 發芽 및 發芽後 生育 段階에서 Batangas 生態型은 butachlor와 thiobencarb에 가장 큰 感受性을 나타낸 반면, Bukidnon 生態型은 가장 큰 耐性을 보였다. 그러나 生態型들의 感受性 差異는 生態型의 種子 무게 및 熟期와는 相關性이 없었다. 生態型의 2葉期에 除草劑가 處理되었을 때, Nueva Ecija 生態型은 butachlor 1 ppm에 가장 큰 耐性을 보였으나, thiobencarb 1 ppm에 대하여서는 가 큰 感受性을 나타내었다. 그러나 Cagayan 生態型은 正反對의 傾向을 보였다. 播種後 20日된 幼苗의 第3葉에 처리된 propanil에 의한 necrosis 程度로 調査한 生態型들의

感受性 差異는 Camarines Sur, Iloilo 및 IRRI (red) 生態型에서 顯著하였지만, Cagayan 및 Batangas 生態型에서는 necrosis가 전혀 나타나지 않았다. 이러한 反應差異는 處理되는 除草劑의 種類와 處理濃度 및 生態型들의 生育段階에 따라 달랐다.

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