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Uptake of Butachlor by Rice Seedlings and Its Phytotoxic Action to the Physiological Activities [1)]

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水稻苗의 Butachlor 吸收 및 藥害發生 特性에 관한 生理的 硏究

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ABSTRACT

To clarify the mode of uptake of butachlor (2-chloro-2', 6'-diethyl-N-(butoxymethyl) acetanilide) by rice seedlings, its phytotoxic action to growth and physiological activities, studies were conducted with rice seedlings, at the 6th or 7th leaf-stage, which were treated with nutrient solution containing butachlor 0, 1.8, 3.6, 7.2, 10.8 or 14.4 ppm for 1, 2 or 4 days, in other case, the solutions were thereafter renewed with the untreated nutrient solution for further growth.

Uptake of butachlor by rice seedlings increased linearly with increase of its concentration and duration of uptake. Butachlor inhibited root growth more than shoot growth, furthermore, the inhibitory effect on the shoot growth was greater in height than in weight or leafing rate. After 4 day-treatment, the rates of shoot growth in weight were delayed for 4 days.

Butachlor inhibited water uptake rapidly and linearly with increase of its external concentration. The reduced uptake of water was followed by slow increase in the stomatal resistance of leaves. Upon completion of butachlor treatment, rate of water uptake was recovered rapidly, but the stomatal resistance with lag in time.

Butachlor did not affect the uptake of cation such as ammonium, potassium and calcium, but inhibited substantially uptake of nitrate in proportion to its concentration. Especially, butachlor did not affect synthesis and degradation of nitrate reductase. In addition, butachlor has shown much greater binding to the lipidic substances from rice roots than the proteinous material. The primary mechanism of phytotoxic action of butachlor does not seem to be its effect on the protein synthesis, but great affinity to membranes. The inhibition of water uptake, and its subsequent closure of stomates is thought very important for reduced growth under mild phytotoxicity.

* *Key words:* butachlor, rice seedling, phytotoxicity, uptake, protein synthesis, water uptake, stomatal resistance, liposome, nitrate reductase.

INTRODUCTION

Butachlor (2-chloro-2', 6'-diethyl-N-(butoxymethyl) acetanilide) is a selective herbicide controlling most annual grasses and certain broad–leaf weeds. It has been widely used to control weeds of rice and of winter barley during the past 6 years in Korea.

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It has been generally safe in transplanted rice when properly used as instructions on its label. However, its safety is much influenced by age of rice seedlings. It is not recommended for weed cotntrol of wet-nusery rice or directly seeded rice under flooded condition. At higher rates, temporary stunting and anomalies of rice growth may occur even in the usual hand-transplanted rice. Particularly, recent shift in cultural method from hand-transplanting to machine-transplanting renders it much more liable to phytotoxicity than in past years. The rice seedlings for machine-transplanting are usually not only 20 to 10 days younger than the seedling for hand-transplanting, but also small and less adapted physiologically to adverse environmental conditions. Moreover, its safety may be influenced by environmental factors such as soil condition, water depth, pH, and temperature, and by physiological status of rice plants. Also, uncautious overdose by farmers may be often practiced.

The visible symptoms of its phytotoxicity have been generally known as stunted rice plants with dark-green leaves. As the case with other acetanilides such as CDAA, alachlor and propachlor (Jaworski [12], Mann *et al* [15], Duke *et al* [8, 9]), its mechanisms of phytotoxicity has been conjectured as inhibition of protein synthesis. However, no information on the mechanism of butachlor is available.

Since Baird and Upchurch[2] reported on the characteristics of butachlor as herbicide for rice, some informations have been accumulated: Baird[1] studied the influence of soil moisture, pH, temperature, and organic matter on the detoxification rate of butachlor in soil. Beestman and Deming[4] reported that the major avenue of dissipation of acetanilide herbicides such as propachlor, alachlor and butachlor from soil was by microbial decomposition, and volatilization rate of butachlor was very low among the acetanilides. Cheng *et al*[7] studied the degradation and dissipation of herbicide butachlor in paddy fields. However, the above reports have not mentioned about its mode of action and its physiological aspects of phytotoxicity in rice plants.

The present study was done to clarify the effect of butachlor concentration and time factors on the uptake of butachlor by rice seedlings in relation to its phytotoxicity. The effect of butachlor on the uptake of water and mineral nutrients, stomatal resistance and protein synthesis in intact seedlings were also examined together with the affinity of butachlor to membrane components such as protein and lipid.

MATERIALS AND METHODS

Preparation of plant materials:

Rice seeds of cultivar "Mansok" were sown in 40 x 50 x 7cm plastic vats filled with silty loam soils. The soils were fertilized with urea at a level of 12Kg/10a nitrogen, with fused phosphate at a level of 14Kg/10a phosphate, and with potassium chloride at a level of 12Kg/10a potassium. Seeds were germinated and grown to 5th or 6th leaf-stage under outdoor condition. At the very stages, roots were trimmed off at 1cm below the base of the shoot. They were transferred to 1/4 strength nutrient solution in one liter glass beaker wrapped with aluminum foils (ten seedlings per beaker) in a growth chamber, and allowed to develop new, uniform root system. Then the seedlings were subjected to treatments. They were supported with spongy scrap on styrofoam lid.

The nutrient solution used in these experiments was basically the same as that reported by IRRI (1972)[25] The compostion was as follows: 1.5mM $NaNO_3$, 1.5mM $(NH_4)_2SO_4$, 0.3mM $Na_2HPO_4 \cdot 12H_2O$, 1.0mM K_2SO_4, 1.0mM $CaCl_2$, 1.6mM $MgSO_4 \cdot 7H_2O$, 0.36mM Fe-EDTA, 0.09mM $MnSO_4 \cdot 5H_2O$. 0.018mM H_2BO_3, 0.2uM $ZnSO_4 . 7H_2O$, 0.2uM $CuSO_4 . 5H_2O$, 0.5uM $Na_2MoO_4 . 2H_2O$. Solutions were made with reagent grade chemicals dissolved in distilled water.

Condition of the growth chamber (Percival MB-60B, Boone, Iowa, USA) during experiments was set at 28°/24°C day/night temperature, 14-hour day-length with 300 $uE \cdot m^{-2} \cdot hr^{-1}$ light intensity, and 56% relative humidity. Continuous forced aeration to the nutrient solution was applied throughout the experiments.

Contents of treatments:

Experiment 1. Uptake of butachlor by rice seedlings and its effects on uptake of water and nutrients, and growth responses

Rice seedlings with well developed new root systems were allowed to grow in half strength solution containing butachlor at concentrations 0, 1.8, 3.6, 7.2 or 14.4 ppm for 1,2 or 4 days, and then they were transferred to fresh half strength nutrient solutions without butachlor and grown for 2 or 6 days in order to observe the responses in uptake of nutrients and water, changes in diffusive resistance of stomates and growth during and after butachlor treatments. Rice seedlings were sampled to determine fresh weight of shoots and roots, shoot length, and leaf number. Amount of water uptake was measured every 2 days by measuring the water volume in the adaxial and abaxial side of the first and second leaves from the top. Aliquots of the treatment solutions were collected and stored under refrigeration prior to analysis. The butachlor used was commercial E.C. containing A.I. of 58.8%.

Experiment 2. Effect of butachlor on the induction and degradation of nitrate reductase

As a method of determination of butachlor effect on the protein synthesis, the effect of butachlor on the induction and degradation of nitrate reductase was studied. Rice seedlings were grown in nutrient solutions containing ammonium as sole source of nitrogen for six days. Thereafter the plants had negligible level of nitrate reductase and they were treated as follows : One set of seedlings was grown with nutrient solution containing nitrate ion only or nitrate ion with butachlor for three days, followed by ammonium ion only or ammonium with butachlor for two days to each of them. Another set of seedlings were treated with ammonium ion with butachlor for three days and then nitrate ion without butachlor for two days. The other compositions of nutrient solution except nitrogen source were same as in the previous experiment. The concentrations of butachlor, ammonium ion, and nitrate ion were 10.6 ppm, 3.0mM, and 3.0mM, respectively. Number of rice seedlings used in this experiment was 12 plants at 7th leaf-stage per treatment. Assays were performed on leaf blades and roots.

Experiment 3. Extent of adsorption of butachlor onto protein and liposomes of lecithin or root lipids

Using egg albumin and liposomes made of soybean lecithin, reagent chemical, or rice root lipids, the extent of adsorption of butachlor to protein and lipids was studied. Twenty mg of egg albumin was completely dissolved in 5ml of water, and 10ml of butachlor at 10.8ppm was added to this solution.

After good shaking egg albumin was precipitated with trichloroacetic acid at final concentration of 5% (W/V). The precipitate was dissolved with deionized water, and pelleted by centrifugation at 3,000 rpm for 15 minutes with bench top centirfuge. This was repeated three times. Then 10ml of hexane was added to extract butachlor adsorbed to egg albumin for analysis of butachlor.

To attain the root lipids, rice root tissues were frozen and thawed, which were washed out with running tap water for three minutes. They were ground and washed out three times with deionized water. Root lipids were extracted from the prepared 10g of root tissues using the solvent system which was composed of chloroform : methanol : water (2:2:1.8) (V/V).

The preparation of liposomes was done as follows: 20mg of lecithin or root lipids was added to 5ml of Tris-HCl buffer (2mM, pH 7.4) inclusive of histidine at 2mM and EDTA at 0.01mM. This was sonicated at 21°C for 30 minutes and checked for liposome formation with microscope (x400). Liposomes of lecithin or root lipids were centrifugated at 18,000 x g for two hours. The sedimented liposomes were collected. Ten ml of butachlor at 10.8 ppm was added to these liposomes and they were shaken well. Then liposomes were pelleted by centrifugation at 18.000 x g for two hours. They were washed out three times with deionized water. Then two ml of water was added to the sediments and were shaken well. Ten ml of hexane was added to this solution for the extraction of butachlor.

Analyses

Analyses for NO_3^-, NH_4^+, phosphate, K^+, Ca^{++}, and butachlor were conducted on aliquots collected during experiments. Nitrate, ammonium, potassium, and calcium were analyzed with Orion 901 ion analyzer (Orion Research Co, Cambridge, USA), and nitrate ion electrode with double junction reference electrode, ammonium ion electrode, or potassium or calcium ion electrode with single junction reference electrode. Phosphate determination was made using the vanadate and molybdate method. Potassium and calcium were also analyzed few times by atomic absorption spectrophotometry to check the reliability of analysis by ion electrodes.

The measurement of diffusive resistance was carried out with LI-60 Diffusive Resistance Meter (Lambda Co., Lincoln, Nebraska, USA). ECD gas chromatograph was performed for butachlor determination with Shimadzu Gas Chromatograph Model GC-5A type (Japan). The glass column (2 x 3mm I.D.) with 5% OV-17 coated on 60/80 mesh Chromosorb W was employed. Operating temperatures were as follows : injection port, 275°C; column, 250°C; detector, 290°C. Nitrogen gas at a pressure of 3 Kg/cm^2 was used as a carrier gas.

Activity of nitrate reductase (NR) was assayed by the method done by Schrader *et al*[17] using FMN and Na-dithionite as electron donor system.

Calculation and experimental design

Calculation for uptake of nutrients and butachlor as n moles or u moles/g fresh weight was based on the concentration of ions and butachlor and the volumes of the treatment solutions. Analyses on nutrient uptake, butachlor and NR were conducted with three replications within experimental unit. Experiment 1 and 2 were conducted by completely randomized design with two replication and the Experiment 3 with 3 replications.

RESULTS

1. Uptake of butachlor by rice seedlings

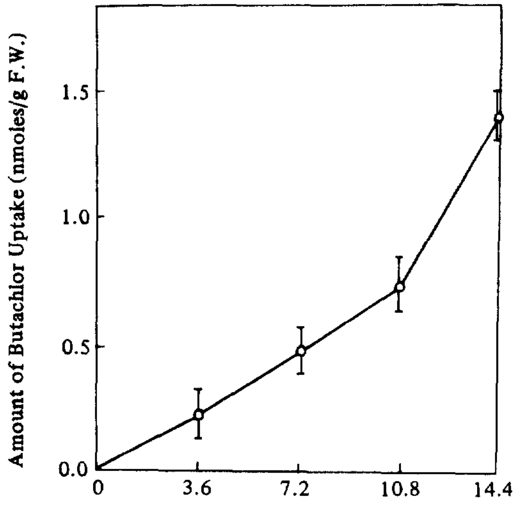

Fig. 1. Effect of butachlor concentration in the solution on its uptake by rice seedlings during 24 hour-period of initial exposure. LSD values are indicated by vertical bars at 5% level.

Figure 1 shows the effect of butachlor concentration in the nutrient solution on the uptake of butachlor by rice seedlings at the 6th leaf-stage during initial 24 hours period. Uptake of butachlor by rice seedlings increased linearly with increase in concentration of butachlor up to 10.8 ppm, but it increased more at 14.4 ppm. The amount of butachlor uptake for four days is shown in the figure 2. The rate of butachlor uptake was almost constant during 4 days when the seedlings were treated with butachlor at 3.6 ppm. In the treatment with butachlor at 7.2 ppm the rate of butachlor uptake was almost constant during initial two days, but slightly decreased afterwards.

Considering the characteristics of butachlor uptake obtained in the present study, the uptake of butachlor by transplanted rice may be regarded practically as being dependent linearly on the concentration of butachlor in the environment and duration of uptake.

2. Effect of butachlor on the growth of rice seedlings

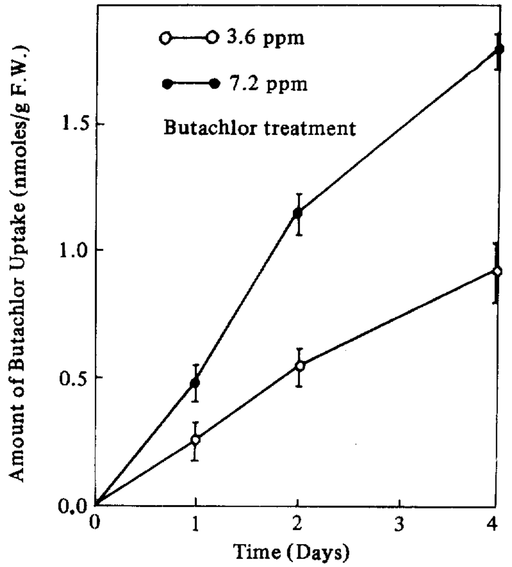

Fig. 2. Cummulative uptake of butachlor by rice seedlings for four days. LSD values are indicated by vertical bars at 5% level.

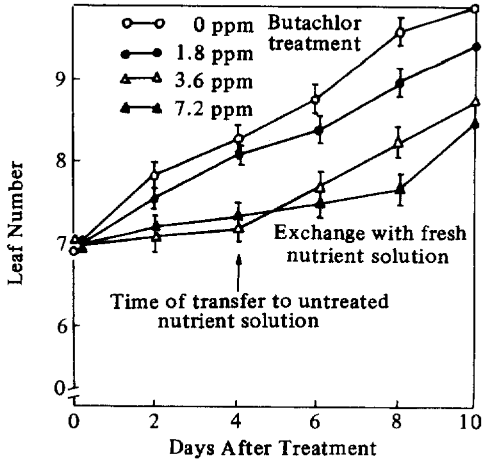

Fig. 3. Effect of butachlor on leafing of rice seedlings during and after treatment with its different concentrations. LSD values are indicated by vertical bars at 5% level.

Rice seedlings were grown for four days in a nutrient solution with butachlor and subsequently grown in the butachlor-free nutrient solution to study the effect of butachlor on the growth of rice.

Figure 3 shows the effect of butachlor on leafing of the rice seedlings during and after treatment of butachlor at the concentrations of 0, 1.8, 3.6, or 7.2 ppm.

During the treatment development of rice leaves was inhibited by butachlor very slightly at 1.8 ppm. Very little growth of leaves was observed at higher concentrations. After transfer of seedlings to nutrient solution without butachlor, leafing speed for the seedlings treated with 1.8 ppm butachlor remained the same as during treatment. The seedlings treated with 3.6 ppm recovered immediately up to almost the speed of control plants, and seedlings treated with 7.2 ppm started to recover 4 days later than in the case of 3.6 ppm.

The height of shoot was reduced more as the concentration of butachlor increased during butachlor treatments. (Fig. 4). The inhibitory effect on shoot length was immediate at higher concentrations and lasted for four days after treatment.

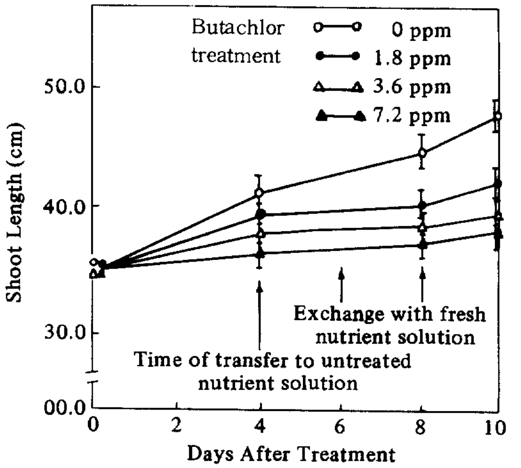

Fig. 4. Effect of butachlor on shoot length of rice seedlings during and after treatment with its different concentrations. LSD values are indicated by vertical bars at 5% level.

As shown in Figure 5, rate of increase in shoot weight was suppressed linearly with increase of butachlor concentration during butachlor uptake similarly to height of shoot. However, growth rate in shoot weight was recovered almost immediately upon transfer of seedlings to nutrient solution without butachlor.

As the concentration of butachlor increased, the

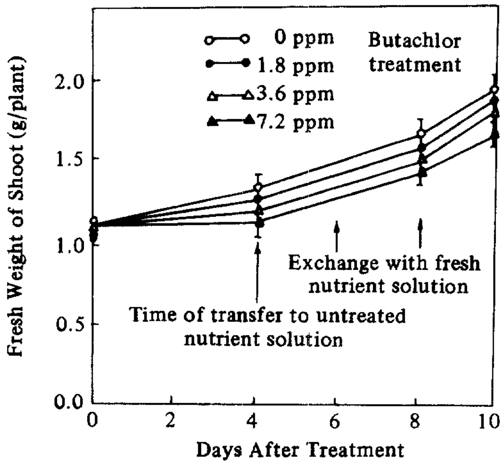

Fig. 5. Effect of butachlor on fresh weight of shoot of rice seedlings during and after treatment with its different concentrations. LSD values are indicated by vertical bars at 5% level. 5% level.

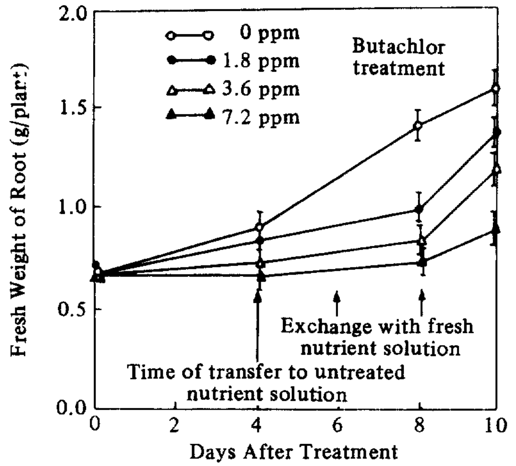

Fig. 6. Effect of butachlor on fresh weight of root of rice seedlings during and after treatment with its different concentrations. LSD values are indicated by vertical bars at 5% level. 5% level.

inhibition of root growth become greater during treatment. The extent of inhibition was somewhat more severe at above 3.6 ppm. The inhibitory effect lasted for four days longer even after the transfer to the nutrient solution without butachlor and then the seedlings began to recover at the normal rate of root growth (Fig. 6).

The visible phytotoxicity in growth of rice seedlings began to appear severely hindered from 2 days after treatment in the nutrient solution with butachlor. Visible symptoms of phytotoxicity was twisted, somewhat wilted, and dark-green leaves, and stunting of plant appearance. These were not so easily discernible at 1.8 ppm, but clearly noticeable at 3.6 ppm and significant at 7.2 ppm (Photo. 1). And, butachlor taken up by rice seedlings inhibited more growth of the second leaf than the first leaf growing from apex. Protrusion of newly growing leaf (first leaf) was hindered, because of inhibited growth of leaf collar and sheath of the second leaf. So, rice plants appeared stunt in spite of comparatively normal rate of shoot growth in weight as noted in Figure 5.

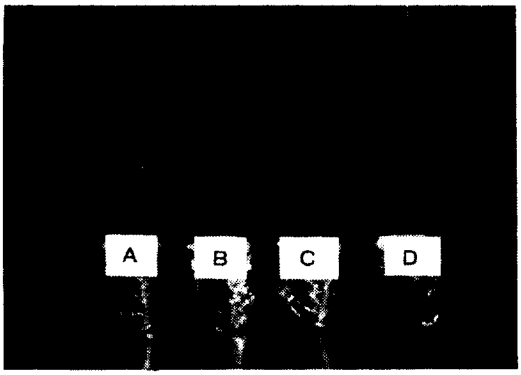

Photo. 1. Appearance of rice seedlings treated with butachlor containing nutrient solutions for 4 days. Butachlor concentrations were A: control, B: 1.8 ppm, C: 3.6 ppm, D: 7.2 ppm.

A inhibition of butachlor on the root growth was noticed greater than on shoot growth. In shoot growth, the growth rate of plant height was more inhibited than that of shoot weight or leafing. The recovery of growth rate may indicate that the recovery is the result of detoxification of uptaken butachlor in the shoots and roots of rice seedlings.

3. Effect of butachlor on the uptake of water by roots and stomatal resistance of leaves

Figure 7 shows the effect of butachlor on the uptake of water by rice seedlings. It is indicated that butachlor inhibited significantly the uptake of water

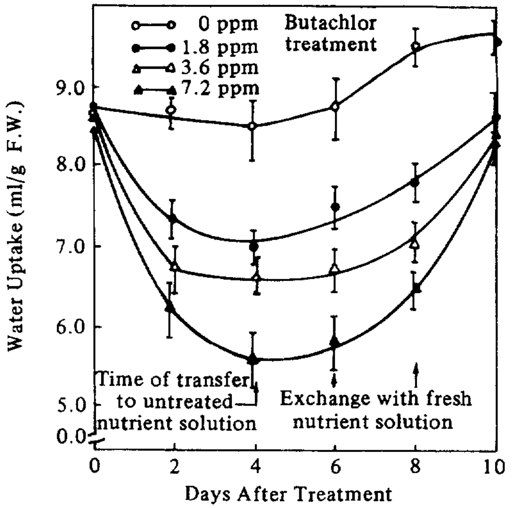

Fig. 7. Effect of butachlor on water uptake per two days by rice seedling during and after treatment with its different concentrations. LSD values are indicated by vertical bars at 5% level.

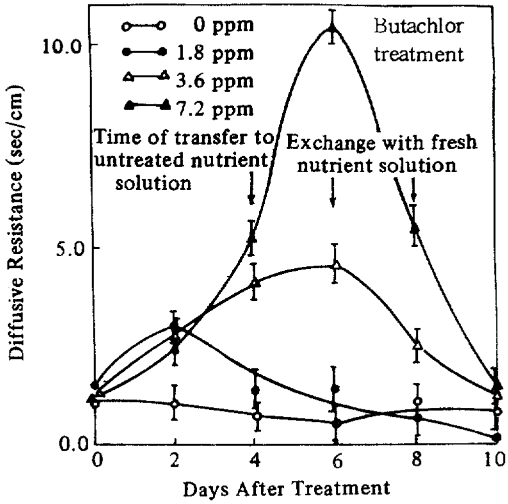

Fig. 8. Effect of butachlor on diffusive resistance of rice seedlings during and after treatment with its different concentrations. LSD values are indicated by vertical bars at 5% level. level.

by roots. The uptake of water was reduced by 18, 36, 42% during initial two days by butachlor at concentration of 1.8, 3.6 and 7.2 ppm, respectively. The uptake of water was reduced more in the highest concentration of butachlor (7.2 ppm) until the end of the fourth day of treatment. As the seedlings were supplied with new nutrient solution without butachlor, uptake of water began to recover rapidly.

Figure 8 shows the changes in diffusive resistance (stomatal resistance) of leaves during the period of presence and absence of butachlor in nutrient solution. Butachlor at 1.8 ppm did not affect stomatal resistance significantly. However, higher concentration of butachlor increased continuously the magnitude of stomatal resistance until two days after treatment. Thereafter, stomatal resistance decreased rapidly to the level of control plants.

Comparing the changes in water uptake by roots with that of stomatal resistance, it is indicated that inhibition of water uptake occurred at least 2 days earilier than the increase of stomatal resistance.

4. Effect of butachlor on the uptake of N, P, K, and Ca by rice seedlings

The effect of butachlor on the uptake of nitrate, ammonium, phosphate, potassium, and calcium ions during two days treatments are shown in Table 1. Butachlor at concentration above 3.6 ppm inhibited

Table 1. Uptake of nutrients and butachlor as affected by butachlor concentration during 2 days[1)]

Concentration	NO_3^- Index (%)	NH_4^+	Total N	Phosphate (%)	K^+	Ca^{++}	Uptake of butachlor (umoles/g F.W.)
0 ppm	35.0 (100)	69.5	104.5	3.4 (100)	30.9	0.0	0.000
1.8 ppm	32.3 (92)	69.5	101.8	3.3 (98)	29.3	1.6	0.021
3.6 ppm	27.6 (79)	82.0	109.6	3.3 (98)	31.3	1.0	0.046
7.2 ppm	18.9 (54)	81.7	100.6	2.3 (68)	27.4	0.0	0.085
LSD .05	4.0	N.S.*	N.S.*	0.6	N.S.*	N.S.*	0.010

1) Rice seedlings were at 7th leaf-stage. *N.S. means insignificant difference among treatment means.

Table 2. Effect of the butachlor uptaken for 2 days on the nutrient uptake in the absence of external butachlor.[1)]

Concentration	Nutrient (umoles/g F.W.)							
	NO_3^-	Index (%)	NH_4^+	Total N	Phosphate	Index (%)	K^+	Ca^{++}
0 ppm	28.0	(100)	101.0	129.0	8.0	(100)	14.3	1.5
3.6 ppm	25.1	(90)	96.3	121.4	6.5	(81)	10.0	1.3
7.2 ppm	23.4	(84)	92.6	116.0	6.0	(75)	12.1	0.1
LSD .05	1.5		N.S*	N.S.*	0.8		N.S.*	N.S.*

1)Rice seedlings were at 6th leaf-stage. * N.S. means insignificant difference among treatment means.

substantially uptake of nitrate. The uptake of nitrate ions by rice seedlings was decreased by 18, 21 and 46% compared to control at 1.8, 3.6, and 7.2 ppm of butachlor, respectively. The degree of inhibition on nitrate uptake was almost proportional to the amount of butachlor uptake during the treatment. Uptake of phosphate was inhibited substantially only at 7.2 ppm. On the other hand no significant influence was noticed on the uptake of ammonium, potassium, and calcium ions, and total nitrogen.

Table 2 shows the effect of butachlor on the recovery of the uptake of nutrients in the solution without butachlor after 2-day treatment. Butachlor seems to affect absorption of nitrate and phosphate ions by roots. Again, uptake of ammonium, potassium and calcium ions were not affected significantly. No significant difference was recognized among treatments in uptake of total nitrogen.

5. Effect of butachlor on induction and degradation of nitrate reductase in rice seedlings.

The results described in the previous section show the inhibitory effect of butachlor on nitrate uptake. The mechanism of phytotoxic action of alachlor, which is very similar to butachlor in chemical structure as well as in visible symptoms of herbicidal action to the susceptible plant species, has been proposed to be inhibition of protein synthesis. Nitrate reductase (NR) is an inducible enzyme and it is known to be under very rapid turnover system with short half life *in vivo*.[5)] Test for the effect of butachlor on the induction and degradation of the NR *in vivo* would be very much worthy in assessment of the effect of butachlor on protein synthesis and on nitrogen assimilatory processes.

The effect of butachlor on the induction and degradation of NR in rice seedlings is shown in Figure 9 as rice seedlings were manipulated to grow with presence or absence of nitrate and butachlor in the nutrient solution. All the seedlings tested were grown only with ammonium ion for 6 days in order to minimize the presence of NR in the plant. As a result, the activity of NR in shoots and roots was at a trace level at the beginning of treatments. When these seedlings were supplied with nitrate at 3.0mM, NR was induced very rapidly to a level of 26 u moles nitrate conversion into nitrite/g fresh weight of shoot per hour *in vitro*. Butachlor tended to reduce slightly

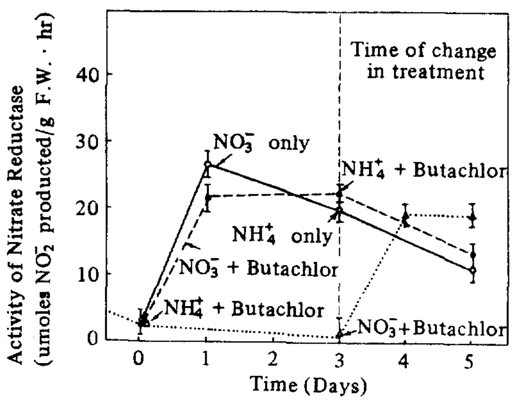

Fig. 9. Effect of butachlor on induction and degradation of nitrate reductase in leaf blades of rice seedlings. The concentrations of butachlor, NO_3^-, and NH_4^+ were 10.8 ppm, 3.0 mM, and 3.0mM, respectively. The rice seedlings were at 7th leaf-stage. LSD values are indicated by vertical bars at 5% level. Activity of nitrate reductase of roots was insignificant in all treatments.

the induction of NR only during the first day, but the level of NR in the shoots became similar to the control plants afterwards. Less induction of NR in the butachlor treated plants than the untreated during the 1st day could be ascribed to inhibition of nitrate uptake by butachlor which resulted in lower concentration of nitrate in the inducer pool. The rice seedlings taken up butachlor for three days in the nutrient solution containing ammonium only could also synthesize NR to a level of 20 u moles reduction of nitrate/g. fresh weight per hour *in vitro* as nitrate was supplied for one day. On the other hand, the degradation of NR upon removal of nitrate in the solution proceeded at the same rate in both plants treated with and without butachlor. These results suggest that butachlor does not inhibit NR synthesis including the transcription of m-RNA or the enzyme synthesis at polysomes.

6. Adsorption of butachlor onto egg albumin, and liposomes of lecithin or root lipids of rice seedlings

Butachlor seemed not to affect protein synthesis. Butachlor has limited solubility in water, but is highly soluble in non-polar organic solvent. Butachlor inhibited water uptake immediately and proportionally to its concentration and this was most prominent among its inhibitory actions to the physiological processes in the present study. Certain herbicides bind to cellular membranes and to proteins. Butachlor is a rather small moleculr and non-polar in nature. It may bind to membraneous structure. And, if it binds to plasmalemma to a certain extent, then water uptake may be assumed to be reduced. To test the property of butachlor to bind to soluble protein and membranes, egg albumin and liposomes which was prepared with soybean lecithin or rice root lipids were used.

Table 3 shows the extent of adsorption of butachlor onto egg albumin, and liposomes. Butachlor was adsorbed much more to lipids than egg albumin, and to be liposomes of root lipid than to the lecithin-liposomes on the lipid weight basis. The amount of butachlor adsorbed onto the liposomes of root lipids was nine times greater than that of egg albumin.

Table 3. Adsorption of butachlor onto egg albumin, and liposomes of lecithin or root lipid of rice seedlings.[1]

Materials	Amount of butachlor adsorbed (umoles/g material)
Egg albumin	0.53 ± 0.02
Liposomes of lecithin	2.36 ± 0.08
Liposomes of root lipids	4.81 ± 0.11

[1] Each value is an average of three replications.

DISCUSSION

Butachlor has been a major herbicide for rice and used very extensively.

Practically, granular formulation of butachlor containing 6% of active ingredient, which is a clay impregnated type without surfactant, is mostly used at the rate of 3 Kg/10a for transplanted rice in Korea. And the following condition may be supposed to visualize the regime of butachlor concentration in the rice field in actual use. When 6% G formulation of butachlor is applied at the rate of 3 Kg/10a to the rice field irrigated 5 cm deep, the maximum concentration of butachlor in the water would be 3.6 ppm, under the assumption that the active ingredient of 6% G dissolves completely into the irrigated water without dissipation, adsorption to soil particles or other losses. In reality of field situation, butachlor is not dissolved immediately and dissolution of granular formulation may preceed dynamically with solvation, adsorption, seepage and other processes. Based on the above situation, the concentrations of butachlor used in this experiment may be related to butachlor concentration in field for interpretation of phytotoxicity data.

Uptake of butachlor by rice seedlings tend to increase linearly with its concentration and duration of uptake. This result is much similar to the report of Schreiber *et al*[18], who observed that bromacil uptake into root of wheat plants increased proportionally with time at low concentrations. On the contrary, there are few studies different from this result. Smith *et al*[21] discussed parabolic uptake curve of ^{14}C-labeled N, N-diallyl-2-chloroacetamide in corn, soybean and oat seedlings, and sigmoidal in cucumber seedlings. Upadhyaya[22] observed a parabolic uptake of ^{14}C oryzalin by corn root segments.

Butachlor inhibited severely the root and shoot growth. Eshel *et al*[10] and Luanne *et al*[13] indicated that alachlor and metolachlor inhibited root growth more than shoot growth. The degree of inhibition on growth appears rather linearly dependent upon butachlor concentration in its uptake environment and duration of uptake. Putting this result and Cheng's[7] together, who reported that even 0.5 ppm of butachlor could cause conspicuous phytotoxic symptoms of rice plants when they were grown in the treated nutrient solution for 20 days, the absolute amount of butachlor absorbed by rice seedlings may be a major determinant for the extent of phytotoxicity.

The relatively rapid recovery of growth rate after transfer of seedlings to nutrient solution without butachlor appears indicative of comparatively rapid detoxification of uptaken butachlor in the shoots and roots of rice seedlings. Hence, the ratio of inactivation of the cummulative amount of uptake is considered important, and it may vary greatly with seedling age.

Butachlor increased stomatal resistance much more slowly than inhibition of water uptake, indicating that the reduced water uptake was a cause to increase stomatal resistance. It led to somewhat wilted appearance of rice seedlings. Yoshida[25] reported reduction of water uptake by PCP in rice and barnyardgrass. Schreiber *et al*[18], Shabir Ahmed *et al*[19] and Shaner[20] observed inhibition of transpiration by bromacil, diuron or glyphosate, respectively.

The degree of inhibition of water uptake and transpiration varies with plant species and herbicides used. But the effect of butachlor on water uptake might be regarded as an important property of phytotoxic action, since decreased water uptake caused closure of stomates. It, thereby, must have reduced photosynthesis, too.

Butachlor did not inhibit the uptake of cations such as ammonium, potassium and calcium, but inhibited substantially the uptake of nitrate proportionately to its concentration, and uptake of phosphate only at 7.2 ppm. Freed[11], Berg *et al*[6] and Nashed *et al*[16] found inhibition of nitrate uptake by monuron and linuron in soybean, corn, crabgrass, Russian pigweed and Canada thistle. Yoshida[25] reported effect of PCP on ammonium, phosphste, calcium and silicate in rice and barnyardgrass. Balke[3] reported inhibition of uptake of potassium and chloride by alachlor in corn root segments. The present results are not interpretable, but the effect on nutrient uptake appears different between the kinds of herbicides and plant species.

Synthesis and degradation of nitrate reductase in rice seedlings were not affected by butachlor. The steps of nitrate reductase induction in the present study comprise derepression of gene, synthesis of m-RNA, role of m-RNA and t-RNA at polyribosomes. No effect of butachlor on induction of nitrate reductase is indicative of no effect on all of the processes for nitrate reductase synthesis. Herbicide effect on the protein synthesis may differ with kinds of proteins. However, butachlor does not appear to inhibit protein synthesis as its specific mechanism of herbicidal action. On the contrary, butachlor inhibited water uptake immediately and proportionally to its concentration, which was most prominent among its inhibitory actions in the physiological process. Butachlor was adsorbed much more onto the liposomes than onto egg albumin. The liposomes made of root lipids adsorbed much more than the lecithin-liposomes. These results appears to suggest that greater possibility of butachlor binding to root membranes of rice plants, and thereby alteration of permeability of membrane to water.

CONCLUSION

Uptake of butachlor by rice seedlings was linearly increased by its concentration in environment and duration of uptake, and the phytotoxicity of butachlor appears to depend on the amount of its uptake by rice plants. Although the mechanism of phytotoxicity has not been completely elucidated, the principal mechanism of phytotoxic action of butachlor is thought not to be its effect on the protein synthesis, but its greater affinity to the lipidic components of plant cells. The inhibition of water uptake and its subsequent closure of stomates seems to be very important for reduced growth under mild phytotoxicity.

摘 要

水稻苗의 Butachlor(2-chloro-2′, 6′-diethyl-N-(butoxymethyl) acetanilide) 吸收特性과 藥害發生機構를 밝히어 Butachlor의 安全使用에 도움을 주고자 試圖되었다. 水稻品種 '萬石'(水原 264號)을 使用하여 第 6, 7葉期까지 水耕栽培한 후 Butachlor를 0, 1.8, 3.6, 7.2, 10.8 또는 14.4ppm 되도록 處理한 水耕液으로 1, 2, 4日間 生育시킨 경우와 Butachlor 處理 後 正常水耕液으로 交替하고 6日間 生長시킬 경우 藥害의 進展 또는 恢復過程中에 일어나는 水稻의 Butachlor 吸收에 따른 生長反應, 水分吸收, 養分吸收, 氣孔의 開閉, 窒酸還元酵素(Nitrate reductase)의 生合成 및 分解作用의 變化를 檢定했고, Butachlor의 細胞構成物質들에 대한 吸着特性을 比較하였으며 그 結果는 다음과 같이 要約된다.

1. 水稻苗의 Butachlor의 吸收는 處理濃度 및 期間에 比例하여 거의 直線的으로 增加하였다.

2. Butachlor는 地上部 生育보다는 뿌리生育을, 地上部 生體重 및 出葉보다는 草長의 生育을 더욱 沮害하였으며, 處理終了 後 地上部 生體重과 出葉은 早速히 恢復되었으나 草長 및 뿌리生體重 生長은 4日以後 恢復勢를 보였다.

3. Butachlor는 뿌리의 水分吸收를 處理濃度에 比例하여 곧 沮害하였고, 그 結果 葉面 氣孔의 抵抗을 增加시켰으며, 處理終了 後에는 水分吸收力은 곧 恢復되었으나 氣孔의 開度는 서서히 恢復되었다.

4. Butachlor는 處理 前·後 水稻苗의 NH_4^+, K^+ 및 Ca^{++} 같은 陽이온의 吸收에는 影響하지 않았으나 NO_3^-의 吸收를 뚜렷이 沮害했고, 7.2 ppm의 高濃度에서는 燐酸의 吸收도 沮害했다.

5. Butachlor는 Nitrate Reductase의 生合成 또는 分解에는 影響하지 않았으며, 生體物質들 중 蛋白質보다는 脂質, 특히 벼뿌리 構成脂質에 많이 吸着되었다.

6. Butachlor에 의한 藥害發生은 蛋白質合成의 沮害에 의한 것이 아니고 細胞들을 構成하고 있는 脂質成分들에 대한 吸着에 의한 것으로 推論되며, 比較的 低濃度 處理時 發生하는 生長沮害는 Butachlor의 水分吸收沮害 및 그에 따른 氣孔의 閉鎖와 光合成 沮害에 크게 起因하는 것으로 생각된다.

REFERENCES

1. Baird, D.D.(1971) Dissertation, The University of Tennessee, knoxville. Recited from Herbicides, Marcel Dekker, Vol. 1: 349-376.
2. Baird, D.D. and R.P. Upchurch(1970) Proc. South. Weed Sci. Soc., 23, 101. Recited from Herbicides, Marcel Dekker, Vol. 1, 349-376.
3. Balke, N.E.(1979) Inhibition of ion absorption by acetanilide herbicides. Abstr. Weed Sci. Soc. Am. P. 93. Recited from Herbicides, Marcel Dekker, Vol. 1: 349-376.
4. Beestman, G.B. and J.M. Deming(1974). Dissipation of acetanilide herbicides from soils. Agron. Jour. 66: 88-92.
5. Beevers, L. and R.H. Hageman(1969). Nitrate reduction in higher plants. Annu. Rev. Plant Physiol. 20: 495-522.
6. Berg, R.T. and L.W. McElroy(1953) Effect of 2,4-D on the nitrate content of forage crops and weeds. Canadian J. Agr. Sci. 33:354-358.
7. Cheng, Y.L. and J.S. Cheng(1979). Degradation and dissipation of herbicide butachlor in paddy fields. J. Pesticide Sci. 4: 431-438.
8. Duke, W.B., F.W.(1968) Proc. Northeast. Weed Control Cont. 22, 504. Recited from Herbicides, Marcel Dekker. Vol. 1: 349-376.
9. Duke, W.B., F.W. Slife, J.B. Hanson, and H.S. Bulter(1975). An vestigation of the mechanism of action of propachlor. Weed Sci. 23: 142-147.
10. Eshel, Y.(1969). Phytotoxicity, leachability, and

site of uptake of 2-chloro-2′, 6′-diethyl-N-(methoxymethyl) acetanilide. Weed Sci. 17: 441.

11. Freed, V.H.(1953). Herbicide mechanisms-Mode of action other than aryl oxylalkyl acids. J. Agr. Food Chem. 1: 47-51.
12. Jaworski, E.G.(1975) Chloroacetanilides In; P.C. Kearney and D.D. Kaufman, Herbidices, Marcel Dekker,Vol. 1: 349-376.
13. Luanne, M.D. and F.D. Hess(1980). Analysis of the growth inhibitory characteristics of alachlor and metolachlor. Weed Sci. 18: 168-175.
14. Luanne, M.D., J.T. Reeves, B.A. Larkings, and F.D. Hess(1980) Use of an *in vitro* protein synthesizing system to test the mode of action of chloroacetanilides. Weed Sci. 18: 190-196.
15. Mann, J.D., L.S. Jordan, and B.E. Day(1965). A survey of herbicides for their effect on protein synthesis. Plant Physiol. 40: 840-843.
16. Nashed, R.B. and R.D. Ilnicki(1967). The effect of linuron on ion uptake in corn, soybean, and crabgrass. Weed Sci. 188-192.
17. Schrader, L.E., G.L. Kitenour, G.L. Eilrich, and R.H. Hageman(1968) Some characteristics of nitrate reductase from higher plants. Plant Physiol. 43: 930-940.
18. Schreiber, J.D., V.V. Volk, and L. Boersma (1975). Soil water potential and bromacil uptake by wheat. Weed Sci. 23: 127-130.
19. Shabir Ahmed and R.A. Fletcher(1980) Reduced transpiration and increased water deficiency by diuron in corn (*Zea mays*). Weed Sci. 18: 180-185.
20. Shaner D.L.(1978) Effects of glyphosate on transpiration. Weed Sci. 26: 513-516.
21. Smith, G.R., C.A. Porter, and E.G. Joworski (1966). American Chemical Society, 152nd Meeting, New York. Recited from Herbicides, Marcel Dekker, Vol. 1: 349-376.
22. Upadhyaya, M.K. and L.D. Nooden(1980). Mode of dinitroaniline herbicide action. Plant Physiol. 66, 1048-1052.
23. Veldstra, H. and Booij, H.L.(1977). Biochim. biophys. Acta, 3, 278-312. Recited from Herbicides, Physiology, Biochemistry, Ecology. Academ. Press. Vol. 2.
24. Wort, D.J.(1964). Effects of herbicides on plant compostion and metabolism. In; L.J. Audus(ed.) The Physiology and Biochemistry of Herbicides. Academic Press, London and New York, pp.291-334.
25. Yoshida and Takaheashi(1970). Effect of PCP on nutrient uptake in rice and barnyard grass. Jour. Soil Sci. Japan 38: 342-344.
26. Yoshida, S., B.A. Forno, J.H. Cock, and A.G. kwanchai(1972) Laboratory mannual for physiological studies of rice. 2nd Ed. IRRI p.13.