Physiological Interactions Between the Herbicide Pretilachlor and the Safener Fenclorim on Rice

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除草劑 Pretilachlor와 解毒劑 Fenclorim의 水稻에 대한 生理的 相互作用

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ABSTRACT

The incividual and combined effects of the chloroacetanilide herbicide pretilachlor and of the safener fenclorim on the growth and selected physiological processes of rice (*Oryza sativa* L., var 'Lemont') were evaluated under greenhouse and laboratory conditions. Fenclorim applied at rates ranging from 50 to 300 g a.i./ha antagonized the injurious effects caused by 150 to 900 g a.i./ha of pretilachlor on 15-day old wet-sown rice grown under greenhouse conditions. When used rates of 150 g/ha or higher, fenclorim reversed completely the effects of all doses of pretilachlor on rice. When the two compounds were given simultaneously, fenclorim enhanced the uptake of [^{14}C]pretilachlor into rice leaf mesophyll protoplasts measured for 1 hr, indicating that competition for uptake at the protoplast level is not involved in the protective action of this safener.

The safener-induced stimulation of pretilachlor uptake was particu-larly evident when fenclorim was used at concentrations of 10, 20 and 40 uM. Following 4 hr of incubation, individual treatments with pretilachlor inhibited the *in vitro* incorporation of radiolabeled precursors into proteins, DNA, and lipids of rice leaf protoplasts only when used at the high concentration of 100 μM. Individual treatments with high concentrations (10 or 100 μM) of the safener fenclorim inhibited the incorporation of radiolabeled precursors into proteins and lipids of rice protoplasts, but had no DNA synthesis. The combined effects of pretilachlor and fenclorim on the incorporation of radiolabeled precursors into these macromolecules of isolated rice mesophyll protoplasts appeared to be additive or silightly synergistic rather than antagonistic. Fenclorim at 1 μM antagonized the effects of pretilachlor on total lipids of rice leaf protoplasts. In addition, individual and combined treat-ments with pretilachlor and fenclorim influenced the incoroporation of[^{14}C]acetate into polar lipids, triglycerides and steryl esters of rice leaf protoplas causing a redistribution of carbon in these lipid fractions. However, these effects were not large enough to explain the herbicidal activity of pretilachlor or to account for the protective action of the safener fenclorim. Overall, the uesults of the present study idnicate that the safener fenclorim does not seem to protect rice against pretilachlor injury by antagonizing its effects on protein, DNA, or lipid syntheses.

INTRODUCTION

The recent development of the safener fenclorim (Fig. 1) has increased the marigin of crop selectivity of the herbicide pretilachlor and has allowed its use in sensitive crops such as wet-sown rice (1).

The potential interactions between safeners and herbicides in protected plants have been studied extensively and several theories have been proposed (2, 3).

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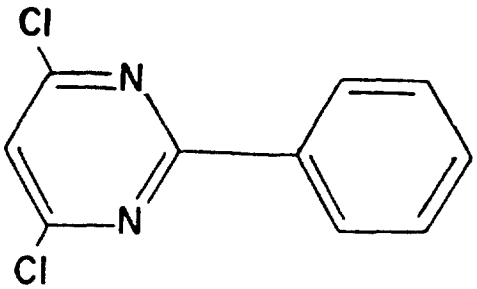

Fig. 1. Chemical structure of the rice safener fenclorim.

It is presently accepted that safeners may act either as bioregulators influencing the amount of a given herbicide that reaches its target site in an active form or as antagonists of herbicides at a common target site (4).

Pretilachlor and other chloroacetanilide herbicides have been reported to inhibit the early development of susceptible plants, but their exact mechanism of action is unknown (5). Protein, nucleic acid, lipid, terpenoid, and gibberellic acid syntheses have all been reported as potential target sites affected by chloroacetanilide herbicides (5-7).

Reports on the potential interactions between pretilachlor and fenclorim at a common site of rice are limited. Cell suspensions of maize (*Zea mays L.*) *and enzymatically isolated sorghum*[*Sorghum bicolor* (L.) Moench]leaf protoplasts were very useful in studying the potential effects of herbicide safeners on herbicide uptake and action (8-10).

The objectives of the experiments reported in this study were (a) to document the safening effect of fenclorim on rice against pretilachlor injury under greenhouse conditions, (b) to examine the potential influence of fenclorim on ^{14}C-pretilachlor uptake by enzymatically isolated leaf mesophyll protoplasts of rice, and (c) to characterize the interactive effects between pretilachlor and fenclorim on the incorporation of appropriate radiolabeled precursors into pro-teins, nucleic acids, and lipids of rice leaf mesophyll protoplasts.

MATERIALS AND METHODS

Chemicals and reagents. Formulated, analytical grade (95% pure) and radiolabeled ([^{14}C]-uniformly ring labeled, sp. act. 38.5 μCi/μg) pretilachlor as well as pretilachlor as well as formulated and analytical grade fenclorim were provided by CIBA-GEIGY Corporation, Basle, Switzerland. Maceroenzymes and all other reagents used were obtained commercially from various sources.

Interaction of pretilachlor and fenclorim on direct-seeded wet-sown rice. Seeds of 'Lemont' rice (*Oryza sativa L.*)were screened in salted water, washed several times in tap water, and submersed in water for 24 hr. The wet seeds were germinated in the dark for 48 hr at 30℃. After germination, seeds were planted in styrofoam cups containing a mixture of potting medium of Weblite, vermiculite, and sphagnum peat moss in a 2 : 2 : 1 : ratio and a controlled-release fertilizer (14-14-14), and grown under greenhouse conditions. Three days after sowing of the germinated seeds, rice seedlings were treated with formulated pretilachlor and fenclorim applied alone or in combination. Pretilachlor was applied at 0, 150, 300, 450, 600, 750 and 900 g/ha while fenclorim was used at 0, 50, 100, 150, 200, 250, and 300 g/ha. During the first week after sowing, the rice seedlings were growing in moisture-saturated soil. After this period rice seedlings were growing in flooded soil.

At 15 days after treatment, shoot heights and fresh weights of rice seedling were recorded. This experiment was repeated three times and all treatments in each experiments were replicated three times. The obtained data were expressed as percent of control and analyzed for variance following arcsine transformation. Statistically significant interactions for each combination treatment were identified by F-test for a two-by-two comparison of that treatment with the control and the separate level of pretilachlor and fenclorim involved, as described by Nash (11). In addition, the expected responses for the combination treatments were calculated assuming no interactions according to the method of Colby (12). For example, in Table 1, the expected response of rice seedlings to the combined treatment of pretilachlor at 150 g/ha and fenclroim at 50 g/ha was calculated as follows : (66 x105)/100=69. In Tables 1 and 2 the calculated expected responses for each treatment combination

are given in parentheses. These responses were then used for the characterization of the combined effects of pretilachlor and fenclorim as additive, synergistic or antagonistic as previously described (13).

Protoplast isolation leaf mesophyll protoplasts of rice isolated enzymatically following the procedures of Leegood et al. (14) as modified by Zama and Hatzios (10). Protoplasts were isolated from leaves of 15-day old rice seedlings (4-leaf stage) grown under greenhouse conditions as described earlier. An appropriate number of leaves were detached, washed in distilled water, blotted dry and then had their midribs removed. Leaf segments of approximately 0.25 mm were prepared with a sharp razor blade and 5 g of cut leaves were infiltrated with a digestion medium consisting of 2% (w/v) cellulase (Cellulysin, Calbiochem, La Jolla, Calif.), 0.3% (w/v) pectinase (Macerase, Calbiochem), 500mM sorbitol, 1 mM $CaCl_2$, 0.05% (w/v) BSA, and 5 mM MES-KOH buffer, adjusted to pH 5.5. The infiltrated tissue and the enzyme medium were transferred into a large petri-dish, and 60 ml of enzyme medium was added to the dish. The dish was then placed on a reciprocal shaking water bath and incubated for 4 hr at 30℃ under low light.

A layer of parafilm was used to reduce the light at the top of the dish and a slow agitation (30 strokes/min) was performed to improve the digestion of the leaf tissue. After the incubation period the undigested material was removed by filtration through an 80-μm nylon net. This filtrate was centrifuged at 160 g for 5 min and the supernatant was removed by suction. The pellet was resuspended gently in 20 ml of ice-cold medium I containing 0.5 M sorbitol, 1 mM $CaCl_2$, 5 mM MES-KOH buffer (pH 6.0) and centrifuged at 160 g for 5 min. The supernatant was discarded by suction and the pellet was resuspended in 5 ml of ice-cold medium III containing 0.5 M sucrose, 1 mM $CaCl_2$, 5 mM MES-KOH (pH 6.0), 8% (w/v) Dextran T_{40}. At the top of this medium, 2 ml of ice-cold Medium II containing 0.4 M sucrose, 0.1 M sorbitol, 1 mM $CaCl_2$, 5 mM MES-KOH (pH 6.0) and 1 ml of ice-cold medium I were added. The suspension was centrifuged at 250 g for 5 min and the pure protoplast fraction at the interface of the three purification media was collected with a Pasteur pipette. The protoplast preparation was then diluted to the desired volume with ice-cold dilution medium containing 0.4 M sorbitol, 1 mM EDTA, 2 mM KH_2PO_4, and 50 mM MES-KOH (pH 6.0). The protoplasts were used immediately after prparation for the experiments on uptake or incorporation of radiolabeled precursors. The chlorophyll content of the protoplasts was determined by the method of Arnon (15).

Influence of fenclorim on [^{14}C]pretilachlor uptake Uptake of radiolabeled pretilachlor by rice leaf mesophyll protoplasts was studied in the presence or absence of the safener fenclorim according to the procedures of Ezra et al. (8) and Zama and Hatzios (10). Assay mixtures in 25-ml Erlenmeyer flasks con-tained 2 ml of protoplast suspension, 0.05ml of [^{14}C] pretilachlor containing 0.7 μCi of radioactivity and adjusted to 10 μM with nonlabeled pretilachlor, and 0.05 ml of fenclorim. Fenclorim was used at concentrations of 0.1, 1, 10, 20, 40, 60, 80, and 160 μM and added to the assay mixture immediately after the addition of pretilachlor. The assay mixtures were incubated at 30℃ for 1 hr in a shaking water bath at 30 oscillations/min. After incubation the reaction was stopped by the addition of 4 ml of dilution medium containing 100 μM of unlabeled pretilachlor. The protoplasts were then filtered onto Whatman glass-fiber (GF/C) filters and washed three times with incubation medium that had no pretilachlor.

The filters were dried at 70℃ and counted for radioactivity after being placed into scintillation vials with 10 ml of scintillation fluid (Scintiverse E, Fisher Scientific Co.). Nonspecific adsorption on [^{14}]pretilachloronto protoplast surface was estimated by determining the radioactivity of[^{14}C]pretilachlor followed immediately by washing GF/C filters at the beginning addition to the incorporation of [^{14}C]acetate into total lipids, the incorporation of this radiolabeled precursor into polar lipids, free sterols, free fatty acids, triglycerides, and sterol esters of rice protoplasts was determined following the medthods of Yenne and Hatzios (17). Following extraction with a chloroform/methanol mixture (2 : 1 v/v), the sam-

ples were analyzed by thin-layer chromatograghy using 220-μm high-performance silica gel plates (Whatman LHP-KD). Plates were developed using hexanes : diethyl ether : acetic acid (40 : 10 : 0.5v/v/v). The plates were then sprayed with 0.1% 2, 7-dichlorofluoroscin/methanol stain. Silica gel was scraped from areas on the plate correspoding to cochromatographed standards of polar lipids, free sterols, free fatty acids, triglycerides, and sterol esters. The[^{14}C]on the silica gel was quantified using standard scintillation counting methods.

Data presented are the means of two experiments with two replicates per experiment and they are given in the form of histograms including the standard errors of each mean in Figures 2-5.

RESULTS AND DISCUSSION

Safening of direct-seeded wet-sown rice against pretilachlor with fenclorim

Pretilachlor applied alone at any of the examined rates reduced significantly the shoot fresh weight (Table 1) or the shoot height (Table 2) of wet-sown rice grown under greenhouse conditions. The shoot height and fresh weight of rice treated with pretilachlor decreased gradually as the rate of pretilachlor increased, reaching a maximum level of injury (about 70%) caused by 450 g/ha of pretilachlor (Tables 1 and 2). Additional increases in the use rate of pretilachlor did not produce any higher levels of injury to rice seedlings.

Fenclorim applied alone did not cause any significant decreases in the shoot height or fresh weight of wet-sown rice (Table 1 and 2). In contrast, treat-ment with some rates of fenclorim slightly increased the fresh weight of rice seedlings (Table 1).

The responses of wet-sown rice seedlings exposed to combined treatments of pretilachlor and fenclorim show that the safener fenclorim antagonized the injurious effects of the herbicide pretilachlor at all rates examined (Tables 1 and 2). When used at rates of 150 g/ha or higher, fenclorim reversed completely the effects of pretilachlor on rice.

These data demonstrate the excellent potential of fenclorim as a safener of wet-sown rice against pretilachlor injury and confirm similar conclusions made in previously published reports (1, 18, 19).

Effect of fenclorim on the uptake of ^{14}C-pretilachlor into leaf protoplasts of Rice.

Data in Table 3 show that the safener fenclorim, at any concentration examined, did not reduce the uptake of radiolabeled pretilachlor into rice leaf proto-

Table 1. Interaction between pretilachlor and fenclorim on fresh weight of directed-seeded wet-sown rice.

Fenclorim (g, a.i./Ha)	Pretilachlor (g, a.i./Ha)						
	0	150	300	400	600	750	900
	percent (%) of control[1)]						
0	100	66	41	32	29	27	24
50	105	115**	107**	107**	107**	71**	61**
		(69)	(43)	(34)	(30)	(28)	(25)
100	115	117**	110**	110**	102**	78**	51**
		(76)	(47)	(37)	(33)	(31)	(28)
150	122	120**	120**	105**	102**	90**	90**
		(81)	(50)	(39)	(35)	(33)	(29)
200	112	117**	112**	115**	112**	113**	110**
		(74)	(46)	(36)	(32)	(30)	(27)
250	110	115**	115**	112**	112**	100**	102**
		(72)	(45)	(35)	(32)	(30)	(26)
300	105	109**	109**	110**	105**	98**	93**
		(69)	(43)	(34)	(30)	(28)	(25)

[1)] Expected responses for combined treatments with pretilachlor and fenclorim, calculated by the method of Colby (12), are shown in parentheses below each observed response. Asterisks indicate significant interactions at the 1% level of probability as determined by F values for each combination treatment calculated for 2-by-2 comparisons of that treatment with the control and the separate level of pretilachlor and fenclorim involved.

Talbe 2. Interactions between pretilachlor and fenclorim on plant height of direct-seeded wet-sown rice.

Fenclorim (g, a.i./Ha)	Pretilachlor (g, a.i./Ha)						
	0	150	300	400	600	750	900
	Percent (%) of control[1]						
0	100	65	40	28	33	28	26
50	99	93**	84**	87**	88**	73**	65**
		(64)	(40)	(28)	(33)	(28)	(26)
100	101	109**	92**	98**	87**	75**	60**
		(66)	(40)	(28)	(33)	(28)	(26)
150	101	92**	97**	86**	81**	74**	78**
		(66)	(40)	(28)	(33)	(28)	(26)
200	96	100**	89**	95**	89**	95**	90**
		(62)	(38)	(27)	(32)	(279	(25)
250	102	96**	93**	87**	92**	87**	81**
		(66)	(41)	(299	(34)	(29)	(27)
300	99	96**	95**	95**	86**	91**	86**
		(64)	(40)	(28)	(33)	(28)	(26)

[1] Expected responses for combined treatments with pretilachlor and fenclorim, calculated by the method of Colby (12), are shown in parentheses below each observed response. Asterisks indicate significant interactions at the 1% level of probability as determined by F values for each combination treatment calculated for 2-by-2 comparisons of that treatment with the control and the separate level of pretilachlor and fenclorim involved.

Table 3. Influence of fenclorim on the uptake of [^{14}C]pretilachlor into rice leaf protoplasts after 1 hr of incubation.

Fenclorim Concentration (μM)	Uptake of [^{14}C]Pretilachlor[1] (dpm/μg Chl)	Percentage of Control (%)
0	6811±579	100
0.1	7015±233	103
1	7218±195	106
10	10071±600	148
20	10206±138	150
40	8461±636	124
60	7439±222	109
80	7089±203	104
160	7277±601	107

[1] Mean values from three replications ± standard error of each mean.

plasts following a 1-hr incubation period. In contrast, when used at 10, 20, and 40 μM, fenclorim incraesed significantly the uptake of [^{14}C]pretilachlor into rice mesophyll protoplasts.

A time- and concentration-dependent stimulation of the uptake of choroacetanilide herbicides into isolated leaf protoplasts or excised shoots of grain sorghum caused by the oxime ether safeners cyometrinil and oxabetrinil has been previously reported (10, 20, 21). The physiological significance of a safener-induced stimulation of herbicide uptake in protected plants is not known at the present time. It has been suggested that enhanced uptake of [^{14}C]metolachlor in oxabetrinil-treated seedlings of grain sorghum could result from a safener-induced stimulation of herbicide metabolism (21).

These results show that a safener-induced reduction of herbicide uptake does not appear to be a potential mechanism explaining the protective action of fenclorim against pretilachlor injury to rice. However, since mesophyll protoplasts isolated from rice leaves represent an artificial system for the study of herbicide uptake, additional studies on the effects of fenclorim on pretilachlor uptake by rice seedlings under *in vivo* conditions are needed to confirm this conclusion. The results of such studies are discussed in the accompany-

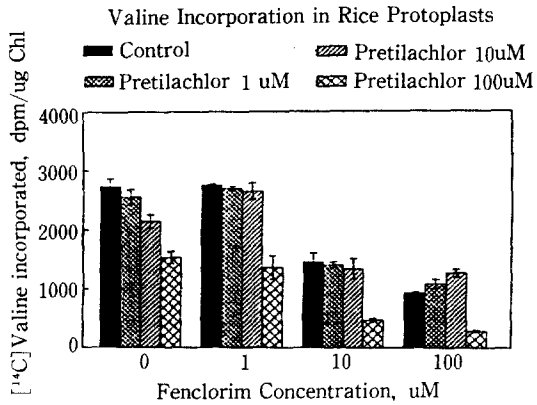

Fig. 2. Interactive effects of pretilachlor and fenclorim on the in vitro incorporation of [^{14}C] valine into rice leaf protoplasts after 4 hr of incubation. Bars on each histogram represent the standard error of each mean.

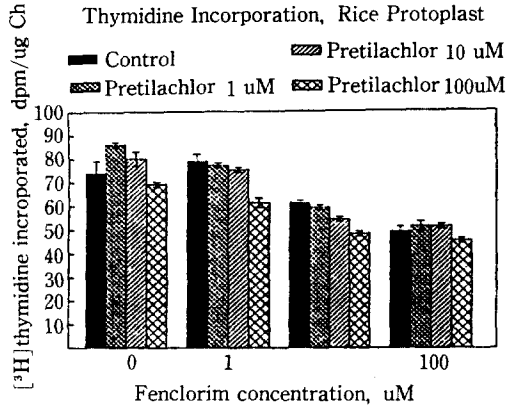

Fig. 3. Interactive affects of pretilachlor and fenclorim on the in vitro incorporation of [^{3}H] thymidine into rice leaf protoplasts after 4 hr of incubation. Bars on each histogram represent the standard error of each mean.

ing paper (22).

Interactive effects of pretilachlor and fenclorim on the incorporation of radiolabeled precursors in rice leaf protoplasts.

Independent and combined effects of pretilachlor and fenclorim on the incorporation of radiolabeled valine, thymidine, and acetate into proteins, nucleic acids, and lipids of isolated rice leaf protoplasts are shown in Figures 2 through 5. The main purpose of these studies was to evaluate the potential involvement of a competitive antagonism of pretilachlor effects by fenclorim in the protective action of this safener.

Data in Fig 2 show that following 4 hr of incubation, individual treatments with 10 and 100 μM of the herbicide pretilachlor or the safener fenclorim inhibited the incorporation of [^{14}C]valine into proteins of rice mesophyll protoplasts. Fenclorim and pretilachlor at 1 μM did not inhibit the incorporation of [^{14}C] valine into protein of isolated rice leaf protoplasts. Inhibition to the *in vitro* incoporation of radiolabeled amino acids into proteins of plants treated with chloroacetanilide herbicides and selected safeners has been reported previously (4, 5).

When used at 1 μM, fenclorim antagonized the inhibitory effect caused by 10 μM of pretilachlor on protein synthesis of rice protoplasts. However, the combined effects of all other concentrations of fenclorim and pretilachlor appeared to be additive or synergistic rather than antagonistic. a strong syn-ergism was particularly evident when pretilachlor at 100 μM was combined with 10 or 100 μM of fenclorim (Fig. 2). These results clearly indicate that within the time limits of this study, a competitive antagonism of pretilachlor effects on protein sythesis by the safener fenclorim is not a likely mechanism of the protective action of this safener.

The effects of pretilachlor and fenclorim on *in vitro* incorporation of [^{3}H]thymidine into deoxyribonucleic

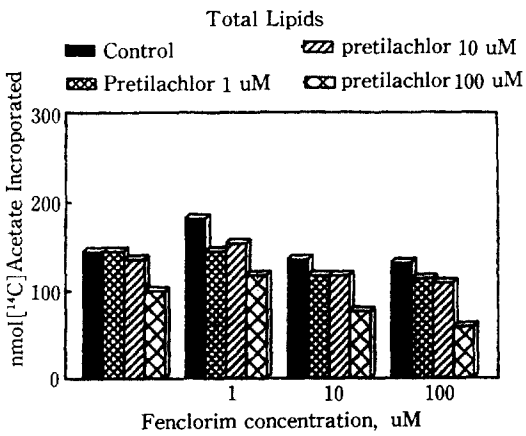

Fig. 4. Interactive effects of pretilachlor and fenclorim on the in vitro incorporation of [^{14}] acetate into rice leaf protoplasts after 4 hr of incubation. Bars on each histogram represent the standard error of each mean.

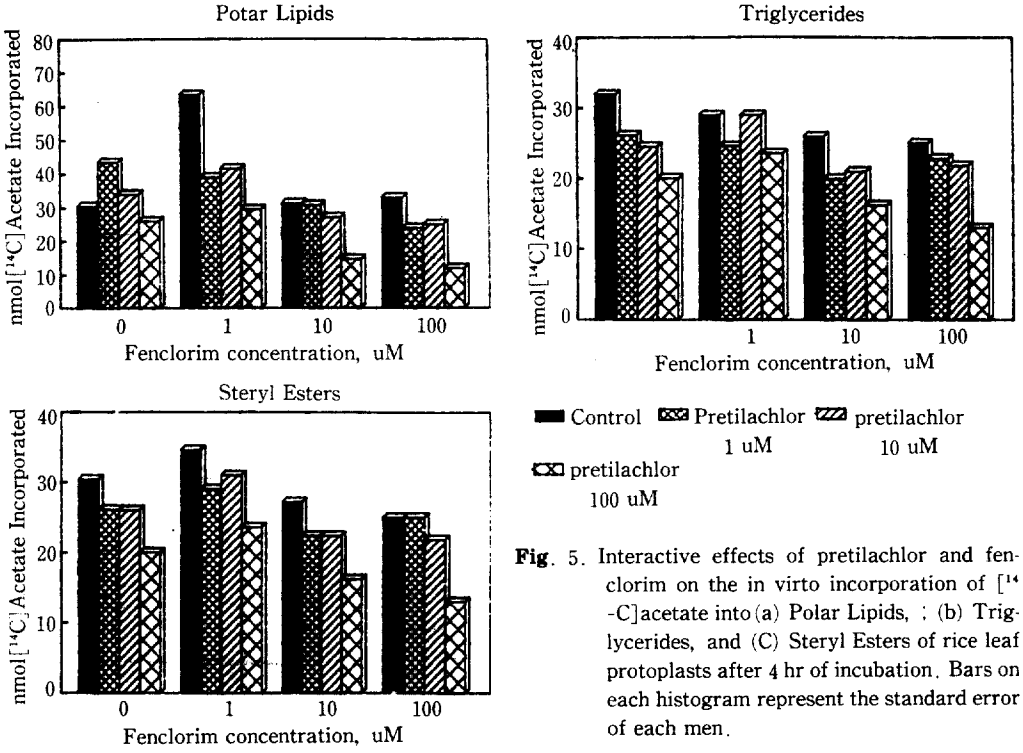

Fig. 5. Interactive effects of pretilachlor and fenclorim on the in virto incorporation of [^{14}-C]acetate into (a) Polar Lipids, ; (b) Triglycerides, and (C) Steryl Esters of rice leaf protoplasts after 4 hr of incubation. Bars on each histogram represent the standard error of each men.

acid (DNA) of rice leaf protoplasts are outlined in Fig. 3. Comparisons between control and pretilachlor -treated mean observed responses, with the help of the standard errors of each mean, show that indivdual treatments with pretilachlor were slightly stmmulatory rather than inhibitory on this process. In contrast, similar comparisons revealed that fen -clorim at 10 and 100 μM reduced significantly the incorporation of [^{3}H]thymidine into rice protoplasts following a 4-hr incubation (Fig. 3). The combined effects of pretilachlor and fenclorim at any concentration examined appeared to be mainly additive or slightly antagonistic. The synthisis of nucleic acids by grain sorghum protoplasts has been reported to be inhibited by high concentrations of the oxime ether safener oxabetrinil (10).

The incorporation of [^{14}C]acetate into total lipids of rice protoplasts was inhibited significantly only by the highest concentration (100 μM) of pretilachlor after a 4-hr incubation (Fig. 4). A strong inhibition of lipid synthesis by chloroacetanilide herbicides has been reported by a number of investigators (5, 6, 23). Fenclorim did not inhibit the *in vitro* incoroporation of [^{14}C]acetate into total lipids of rice protoplasts at any concentration examined (Fig. 4.). Fenclorim at 1 μM antagonized the effect caused by 100 μM of pretilachlor on the incorporation of radiolabeled acetate into total lipids of rice protoplasts (Fig. 4.). In all other cases, the combined effects of fenclorim and pretilachlor on total lipid synthesis of rice protoplasts were generally additive.

With respect to individual lipid classes, pretilachlor of fenclorim at any concentration did not have any effect on the incorporation of [^{14}C]acetate into free sterols and free fatty acids of rice leaf protoplats (data not shown). The lack of any effects of chloroacetanilide herbicides on sterol synthesis has been also reported by Ebert and Ramsteiner (24) in studies with metolachlor and grain sorghum.

Fenclorim at 1 μM and pretilachlor at 1 and 10 μM stimulated the incorporation of [^{14}C]acetate into polar lipids (Fig. 5a). At all concentrations examined, fenclorim was slightly inhibitory of the incorporation of radiolabeled acetate into triglycer-

ides and steryl esters of rice leaf protoplasts (Fig. 5b and 5c). An exeption to that was the stimulation of the incorporation of [^{14}C]acetate into steryl esters of rice protoplasts observed in the presence of 1 μM of fenclorim (Fig. 5c). A significant inhibitory effect on the incorporation of [^{14}C]acetate into triglyceyides of grain sorghum has been also reported with the safener flurazole (25), which protects sorghum against injury caused by the herbicide alachlor.

Individually applied pretilachlor inhibited the incorporation of radiolabeled acetate into triglycerides and steryl esters of rice protoplasts at all concentrations examined following a 4-hr incubation period (Fig. 5b and 5c). In general, the combined effects of fenclorim and pretilachlor on the incorporation of[^{14}C]acetate into polar lipids, triglycerides and steryl esters were additive(Fig. 5a, 5b, 5c).

These results indicate that selected concentrations of the chloroacetanilide pritilachlor and the safener fenclorim can influence lipid metabolism causing a redistribution of carbon in the lipid fractions of rice leaf protoplasts. However, again these effects were not of sufficient magnitude to explain the herbicidal activity of pretilachlor or to account for the safening activity of fenclorim on rice.

Taken as whole the results of the present study indicate that the safener fenclorim does not seem to protect rice against injury from the chloroacetanilide herbicide pretilachlor by either reducing its uptake into rice protoplasts or by antagonizing its effects on protein, DNA, or lipid syntheses of rice protoplasts. A fenclorimmediated enhancement of trhe metabolic detoxification of protective action of this safener. Experimental results supporting this mechanism are presented in the second report of our investigations on the interactions between the herbicide pretilachlor and the safener fenclorim on rice(21).

ACKNOWLEDGEMENTS

This work was supported in part by a research grant from CIBA-GEIGY Corporation, Greensboro, NC. Additional support for these studies was provided by the Korean Science and Engineering Foundation and the Virginia Agricultural Experiment Station. Gratitude is also expressed to CIBA-GEIGY Corporation, Basle, Switzerland for providing the formulated, analytical, and radiolabeled samples of the herbicide pretilachlor and the safener fenclorim. Thanks are also due to Dr. Roy Smith of the USDA, ARS, in Stuttgart, Arkansas for providing seeds of the rice cultivars used in this study.

摘 要

벼의 生育과 生理的 相互作用에 끼치는 chloroacetanilide系 除草劑 pretilachlor와 解毒劑 fenclorim의 單獨 또는 組合處理의 影響을 溫室과 室內條件下에서 檢討하였다. Fenclorim 50-300g a.i./ha과 pretilachlor 150-900g a.i./ha을 벼 湛水直播後 3일後에 組合處理하여 生育시킨 結果 fenclorim은 15일後의 jpretilachlor의 벼에 대한 藥害에 抵抗的으로 作用하였고, fenclorim 150g ai/ha 以上을 處理하였을 때 pretilachlor 全試驗藥量에서의 水稻에 대한 藥害가 輕減되었다. 이 두 和合物을 同時에 處理하였을 때 fenclorim은 1時間 동안 測定된 水稻 葉肉 原型質體의 [^{14}C]pretilachlor의 吸收를 增大시켰고, pretilachlor 吸收의 解毒劑 誘起 刺戟은 fenclorim 10, 20 및 40 uM에서 明白하게 일어났다. Pretilachlor 100 uM의 높은 濃度를 處理한 水稻 葉肉 原型質體에서 放射能標識 前驅物質의 蛋白質, DNA, 指質로의 試驗管內 incorporation을 抑制하였다. Fenclorim 10 uM 또는 100 uM을 處理한 水稻 原刑質體에서도 前鷗物質의 蛋白質과 脂質로의 incorporation을 抑制하였으나 DNA合成은 抑制되지 않았다. Pretilachlor와 fenclorim의 混合處理는 이들 高分子物質의 合成에 拮抗作用하기보다는 附加 또는 協力作用하는 것으로 나타났다. Fenclorim 1 uM을 處理한 原型質體의 總 脂質含量에 끼치는 pretilachlor의 影響에 拮抗的로 作用하였다. Pretilachlor와 fenclorim의 單獨 또는 組合處理로 原型質體에서의 [^{14}C]acetate와 極性脂質, triglyceride와 steryl ester의 incorporation에 影響을 끼쳤으나 이 影響이 pretilachlor의 活性 또는 fenclorim의 保護作用을 說明하기에는 충분치 않았다. 結局 本 研究結果는 解毒劑 fenclorim이 蛋白質, DNA 및 脂質合成에 拮抗作用을 하여 除草劑 pretilachlor의 水稻에 대한 藥害를 保護한다고 할 수 없음을 示唆한다.

LETERATURE CITED

1. Rufener, J. and M. Quadranti. 1983. Early weed control in wet sown-rice : The role of the safener CGA-123407. Proc. Int. Congr. Plant Prot. 9, 332.
2. Pallos, F.M. and J.E. Casida. 1978. Chemistry and Action of Herbicde Antidotes. p. 171, Academic Press, New York.
3. Hatzios, K.K. and R.E. Hoagland, 1989. Crop Safeners for Herbicdes : Development, Uses, and Mechanisms of Action. p. 400, Academic Press, San Diego.
4. Hatzios. K.K. 1989. Mechanisms of Action of Herbicide Safeners : An overview, *in* Crop Safeners for Herbicides : Development, Uses, and Mechanisms of Action (K.K. Hatzions and R.E. Hoagland, Eds.). pp. 65-101, Academic Press, San Diego.
5. LeBaron, H.M. J.E. McFarland, B.J. Simoneaux, and E. Ebert. 1988. Metolachlor, *in* Herbicides : Chemistry, Degradation, and Mode of Action(P.C. Kearney and D.D. Kaufman, Eds.). Vol. 3, pp. 335-381, Dekker, New York.
6. Fuerst, E.P. 1987. Understanding the mode of action of chloroacetanilide and thiocarbamate herbicides. Weed Technol. 1 : 270.
7. Wilkinson, R.E. 1989. Terpenoid biosynthesis as a site of action for herbicide safeners, *in* "Crop Safeners for Herbicides : Development, Uses, and Mechanisms of Action"(K.K. Hatzios and R.E. Hoagland, Eds.). pp. 221-240, Academic Press, San Diego.
8. Ezra, G. E. Krochmal, and J. Gressel. 1982. Competition between a thiocarbamate herbicide and herbicide protectants at the level of uptake into maize cells in culture. Pestic. Biochem. Physiol. 18 : 107.
9. Ezra, G. J. Gressel, and H.M. Flowers. 1983. Effects of the herbicide EPTC and the protectant DDCA on incorporation and distribution of [2-^{14}C]acetate into major lipid fractions of maize cell suspension cultures. Pestic. Biochem. Physiol. 19 : 225.
10. Zama, P. and K.K. Hatziosm. 1987. Interactions between the herbicide metolachlor and the safener CGA-92194 at the levels of uptake and macromolecular synthesis in sorghum leaf protoplasts. Pestic. Biochem. Physiol. 27 : 86.
11. Nash, R.G., 1981. Phytotoxic interaction stuides-Techmiques for evaluation and presentation of results. Weed Sci. 29 : 147.
12. Colby, S.R., 1967. Calculating synergistic and antagonistic responses of herbicide combinations. Weeds 15 : 20.
13. Hatzios, K.K. and D. Penner. 1985. Interactions of herbicides with other agrochemicals in higher plants. Rev. Weed Sci. 1 : 1/
14 Leegood, R.C. G.E. Edwards and D.A. Walker. 1982. Chloroplasts and protoplasts, *in* Techniques in Bioproductivity and Photosynthesis (J. Coombs and D.O. Hall, Eds.). p. 94, Pergamon, Oxford.
15. Arnon, D.I. 1949. Copper enzymes in isolated chloroplasts. Polyphenoloxidase in *Beta vulgaris* L., Plant Physiol. 24 : 1.
16. Ahsotn, F.M., O.T. Devilliers, R.K. Glenn, and W.B. Duke. 1977. Localization of metabolic sites of action of herbicides. Pestic. Biochem. Physiol. 7 : 122.
17. Yenne, S.P. and K.K. Hatzios. 1989. Influence of oxime ether safeners and metolachlor on acetate incorporation into lipids and on acetyl-CoA carboxylase of grain sorghum. Pestic. Biochem. Physiol. 35 : 146.
18. Chirst, R.A. 1985. Effect of CGA 123407 as a safener for pretilachlor in rice (*Oryza sativa* L.). Recordings of elongation of single rice leaves. Weed Res. 25 : 193.
19. Ebert, E. and H. Gerber. 1989. Differential effects of oxabetrinil and fenclorim against metolachlor and pretilachlor injury on various grasses, *in* Crop Safeners for Herbicides : Development, Uses, and Mechanisms of Action (K. Hatzios and R.E. Hoagland, Eds.). pp. 177-193, Academic Press, San Diego.

20. Ketchersid, M.L., D.M. Vietor, and M. G. Merkle. 1982. CGA-43089 effects on metolachlor uptake and membrane permeability in grain sorghum (*Sorghum bicolor*). J. Plant Growth Regul. 1 : 285.
21. Fuerst, E.P. and J.W. Gronwald. 1986. Induction of rapid metabolism of metolachlor in sorghum (*Sorghum bicolor*) shoots by CGA-92194 and other antidotes. Weed Sci. 34 : 354.
22. Han, S. K.K. Hatzios. 1990. Uptake, translocation, and metabolism of pretilachlor in fenclorim -safened and unsafened rice. Pestic. Biochem. Physiol. (submitted for pubication).
23. Weisshaar, H. and P. Boger. 1987. Primary effects of chloroacetanilides. Pestic. Biochem. Physiol. 28 : 286.
24. Ebert, E. and K. Ramsteiner. 1984. Influence of metolachlor and the metolachlor protectant CGA -43089 on the biosynthesis of epicuticular waxes of the primary leaves of *Sorghum bicolor* Moench. Weed Res. 24 : 383. 24 : 383.
25. Warmund, M.R., H.D. Kerr, and E.J. Peters. 1985. Lipid metabolism in grain sorghum (*Sorghum bicolor*) treated with alachlor plus flurazole. Weed Sci. 33 : 25.