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Original Article

Effect and Metabolism of the Chloroacetamide Herbicide Metazachlor: Comparison of Plant Cell Suspension Cultures and Seedlings*

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Biological activity and fate of metazachlor were compared in cell suspension cultures and seedlings from rice and spinach. Growth of cell cultures was not (rice) or only slightly (spinach) inhibited by 200 μM and 100 μM metazachlor, respectively. In contrast, seedling growth was halved with 50 nM metazachlor (rice) and 35% less with 100 μM (spinach). In rice seedlings, 0.1 μM metazachlor significantly decreased fatty acid desaturation, while 100–200 μM had little or no effect in the other 3 systems. Cell suspension cultures were more tolerant to metazachlor than the corresponding plant systems. Uptake could not explain tolerance since it was lowest in the most sensitive system (rice seedlings). All systems were able to metabolize metazachlor. After 72 hr spinach cell culture and seedlings fed with 200 μM metazachlor still contained 3.3 and 48 μM parent herbicide, respectively. Rice seedlings incubated with 0.1 μM herbicide contained only 18 nM of parent metazachlor after 24 hr. This low but still very phytotoxic concentration of parent metazachlor in rice seedlings compared with the high and yet non-toxic concentration found in spinach suggests that metabolism *via* glutathione conjugation might not be the only protection mechanism in tolerant plants.

INTRODUCTION

Metazachlor [2-chloro-*N*-(2,6-dimethylphenyl)-*N*-(1*H*-pyrazol-1-ylmethyl)acetamide] is a preemergence chloroacetamide herbicide used to control grass and dicotyledonous weeds in rape, soybean, potatoes, and other crops. After almost 40 years, researchers have not yet succeeded in finding the primary mode of action of the chloroacetamides. Numerous effects have been reported including inhibition of protein, lignin and gibberellic acid synthesis, cell division, membrane permeability and ion transport (for review see Refs. 1, 2)). An influence on lipid biosynthesis was also reviewed,[3] and demonstrated by Weisshaar & Böger,[4] who reported inhibition of uptake and incorporation of acetate into acyl lipids of the green alga *Scenedesmus acutus* by alachlor and metazachlor. These effects were observed with different plant systems and required micro to millimolar concentrations of the herbicides, which led to speculate on a multisite action for chloroacetamides.

However, chloroacetamides were found phytotoxic even at submicromolar concentrations. In maize leaves a concentration of 0.7–1 μM parent metazachlor was sufficient to cause plant death.[5] The growth of duckweed was 50% inhibited by an alachlor concentration of 38 nM.[6] After a few hours of incubation ^{14}C-oleic acid incorporation into a precipitate obtained after saponification of *Scenedesmus* cells

* Part of this work was presented at the meeting of the Weed Science Society of America 1993 in Denver, Colorado.
This paper is dedicated to Prof. Hartmut K. Lichtenthaler on the occasion of his 60th birthday.

was 50% reduced by approximately 30 nM metazachlor.[7] Stereospecificity was also demonstrated for this effect since the *S* isomer of a chloroacetamide with an asymetric C-atom was as active as metazachlor while the *R* isomer was more than 100 times less effective (Couderchet *et al.*, unpubl. results). This indicates *one* special site of action which is sensitive to probably even smaller concentrations. Fatty acid desaturation was suggested to be related to the primary mode of action, since 5 μM alachlor or metazachlor drastically decreased the proportion of polyunsaturated fatty acids in *Scenedesmus acutus*.[7,8]

The unicellular green alga *Scenedesmus acutus* offers many advantages to study herbicidal mode of action, *i.e.*, well-defined culture conditions, high metabolic activity in the exponential growth phase, easy addition and removal of cell material, metabolites and herbicides to the liquid culture and good data reproducibility. However, this alga is taxonomically away from weeds and crops, and the results may be difficult to generalize. Therefore a new assay system was investigated. Cell suspension cultures of higher plants present similar technical advantages as microalgae,[9] and heterotrophic cell suspension cultures were found to be good models of root and shoot meristems and helpful to test for herbicidal activity.[6]

The objectives of the studies reported in this paper were to produce a new assay system that would include similar advantages as the algae and could be easily applied to higher plants to study the mode of action of chloroacetamides. Therefore, the effect of metazachlor on cell suspension cultures was compared to the corresponding whole plant seedling system with respect to growth and fatty acid composition. Moreover, the ability to metabolize metazachlor was investigated in both systems.

MATERIALS AND METHODS

1. *Plant Material and Growth Conditions*

The mixotrophic cell suspension culture of *Spinacia oleracea* (var. Atlanta) was developed in this laboratory from calli grown in petri dishes on agar in the medium described below supplemented with 9 g/*l* Difco agar. The calli were suspended in a liquid medium based on Murashige & Skoog[10] supplemented with 30 g/*l* sucrose, 10 mg/*l* indole-3-acetic acid and 0.08 mg/*l* kinetin, adjusted to pH 5.8 with NaOH. The heterotrophic indica rice cell suspension culture (*Oryza sativa*, var. Chinsurah Boro II)[11] was a generous gift of Prof. Dr. I. Potrykus, ETH Zürich, Switzerland. It was cultured in a medium containing per liter: sucrose (20 g), KCl (2.95 g), $MgSO_4 \cdot 7H_2O$ (250 mg), $CaCl_2 \cdot 2H_2O$ (150 mg), $NaH_2PO_4 \cdot H_2O$ (150 mg), KI (0.75 mg), H_3BO_4 (3 mg), $MnSO_4 \cdot H_2O$ (10 mg), $ZnSO_4 \cdot 7H_2O$ (2 mg), $Na_2MoO_4 \cdot 2H_2O$ (0.25 mg), $CuSO_4 \cdot 5H_2O$ (0.025 mg), $CoCl_2 \cdot 6H_2O$ (0.025 mg), Na_2EDTA (37.3 mg), $FeSO_4 \cdot 7H_2O$ (27.8 mg), nicotinic acid (1 mg), pyridoxine hydrochloride (1 mg), thiamine hydrochloride (10 mg), myo-inositol (100 mg), 1-glutamine (876 mg), aspartic acid (266 mg), arginine (174 mg), glycine (7.5 mg), 2.4-dichlorophenoxyacetic acid (1 mg), kinetin (0.2 mg), gibberellic acid GA_3 0.1 mg; pH 5.6 (S.K., Datta, personal communication). Cell suspensions were grown in 250-ml Erlenmeyer flasks containing 100 ml of liquid medium, placed on a rotary shaker at 115 rpm and subcultured every ten days. Temperature was constant at 24°C and light intensity was 100 μEinstein (μE)/m^2/s^1 (spinach) and 15 μE/m^2/s^1 (rice) during the 12 hr photoperiod. Cell fresh weight of 2.5 ml cell suspension was determined by removal of the medium by suction under slight vacuum. The resulting cells were weighed. Growth was also estimated by measuring the cell volume in 2.5 ml cell suspension after centrifugation in graduated test tubes (5 min; 6000×*g*), the resulting pellet volume was determined with a 0.1 ml accuracy. The chlorophyll content of the mixotrophic spinach cell suspension culture was determined according to Mackinney.[12] All experiments were conducted using the exponential growth phase of the cell suspension cultures.

Seeds of rice (*Oryza sativa*, var. Calasparra) and spinach (*Spinacia oleracea*, var. Atlanta) were used for whole plant experiments. Two layers of filter paper soaked with 6 ml H_2O were placed in glass dishes (diameter 6.5 cm). Fifteen rice seeds were disposed on top of the filter paper and germination proceeded in the dark at 28°C. In the case of spinach 30 ml of washed sea sand instead of filter paper was used and soaked with 9 ml H_2O. Spinach seedlings were cultivated at 24°C under a 12-hr photoperiod (100 $\mu E/m^2/s^1$).

2. *Herbicide Treatment*

A stock solution of metazachlor (99.6% pure; generous gift of BASF AG, Limburgerhof, Germany), 100 mM in ethanol, was used for all assays. In the case of cell suspension cultures the stock solution was added to the suspension, keeping the maximum final ethanol concentration below or at 0.1%. Herbicide treated cells were compared with cells containing the same ethanol concentration. For the germination assays stock solution was added to the glass dishes and the solvent was allowed to evaporate before the dishes were filled with water, filter paper or sand and seeds. Shoot height of the spinach and rice seedling was measured after 10 days and 5 days of germination, respectively.

3. *Fatty Acid Extraction and Determination*

Fatty acids were extracted and methylated as reported by Couderchet & Böger;[13] shoots were cut into small pieces before saponification. Fatty acid methyl esters were separated by capillary gas chromatography as described earlier.[13]

4. *^{14}C-Metazachlor Metabolism*

[Phenyl-U-^{14}C]metazachlor (0.43 GBq/mmol; gift of BASF-AG, final concentration 0.1 μM) and ^{12}C-metazachlor were added to 100 ml of cell suspension. Samples were analyzed for labeled metazachlor and its labeled metabolites according to Fuerst *et al.*[5] In that procedure the hydrophobic parent metazachlor and hydrophilic metabolites were partitioned between hexane and water-acetone phase in the following manner. An aliquot of 5 ml cell suspension was collected, centrifuged, the supernatant was counted for radioactivity and the cells washed with fresh medium. After centrifugation the resulting pellet was resuspended in 1 ml of 70% acetone and ground with mortar and pestle. The extract was centrifuged, the supernatant collected and the pellet sequentially washed with 1 ml 70% acetone, 1 ml 20% acetone and 2 ml H_2O. The supernatants were pooled and extracted three times with 2 ml hexane. One milliliter of the resulting hexane phase that contained parent metazachlor and 0.5 ml of the water-acetone phase containing the metabolites were placed into separate vials, mixed with 4 ml of scintillation cocktail (Ultima Gold, Packard, Frankfurt, Germany) and radioactivity was determined by liquid scintillation (LKB Rackbeta II, Pharmacia, Frankfurt, Germany). After estimation of the cell volume the concentration of metazachlor and its metabolites in the plant cell was calculated. In the case of seedlings tissue slices were put into 250 ml Erlenmeyer flasks containing 100 ml H_2O, ^{14}C-metazachlor and unlabeled herbicide. After washing with 70% acetone seedlings were ground in 1 ml 70% acetone with mortar and pestle. The rest of the procedure was the same as for the cell suspension cultures. For calculation of the concentrations inside the tissue the volume of the homogenized seedlings was measured in graduated test tubes. For example 1 μM in the plant sample means 1 nmol metazachlor or metabolite per milliliter tissue or cells (Figs. 4–7).

5. *Reliability*

All experiments and determinations were repeated at least two or three times. The deviation was found <15% of the mean. Unless standard deviation is documented data from a typical experiment are presented.

RESULTS AND DISCUSSION

The mixotrophic spinach cell suspension culture grew with a doubling time of 7 days, with a chlorophyll content ranging from 35 μg/g FW (fresh weight) in the exponential phase (day 2–6 of cultivation) to 130 μg/g FW in the late stationary state. The fresh weight of the heterotrophic rice cell suspension culture doubled within 5 days.

After 4 days of cultivation with 100 μM metazachlor cell fresh weight of spinach was only slightly reduced (<10%). Fresh weight of rice cell suspension culture was not influenced even in the presence of 200 μM metazachlor (data not shown). In contrast, the herbicide induced marked growth inhibition in rice seedlings and some in spinach seedlings

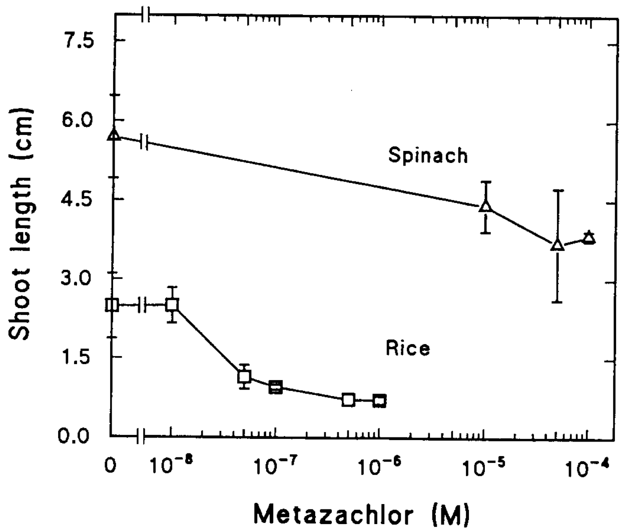

Fig. 1 Influence of metazachlor on the shoot length of spinach and rice seedlings after 5 (rice) or 10 (spinach) days of germination.

Germination rate was 65% for rice and 41% for spinach.

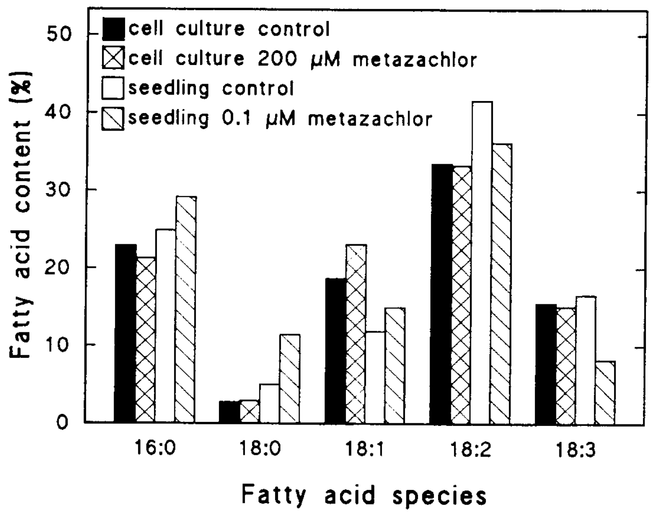

Fig. 2 Influence of metazachlor on fatty acid composition of *Oryza sativa* cell suspension culture and seedling after incubation for 4 and 5 days, respectively.

Metazachlor concentration was 200 μM in cell suspension culture and 0.1 μM in seedlings. Standard deviation was less than 5% of indicated value.

(Fig. 1). Rice shoot length was 50% reduced by 50 nM metazachlor after 5 days of germination. Herbicide concentrations higher than 0.1 μM led to plant death and soon after germination the emerging shoot failed to develop and turned brown, a typical chloroacetamide effect.[2] A more than 1000 times higher herbicide concentration was necessary to observe a similar effect on growth with spinach seedlings. Indeed, 100 μM metazachlor caused a 35% reduction of height, the seedling was still green, and no lethal herbicide concentration was reached. The seed germination rate (number of germinated seeds versus total number of seeds) for both species was not influenced at any metazachlor concentration, thus confirming earlier studies with metolachlor.[14]

Fatty acid composition of the various systems was not identical (Figs. 2 and 3). Fatty acid profiles of *Oryza sativa* were nearly the same for seedling and cell suspension culture. In rice the predominant fatty acids were 18:2 (>30%; for the fatty acid x: y, x represents the number of C atoms and y the number of double bonds), 16:0 (>20%), 18:3 (>15%) and 18:1 (>10%). The relatively low amount of 18:3 which is untypical of higher plants may be explained by the early developmental stage of the seedling and by lack of light during development.[15] When rice germinated for 5 days with 0.1 μM metazachlor present the fatty acid composition changed and a significant decrease of the polyunsaturated fatty

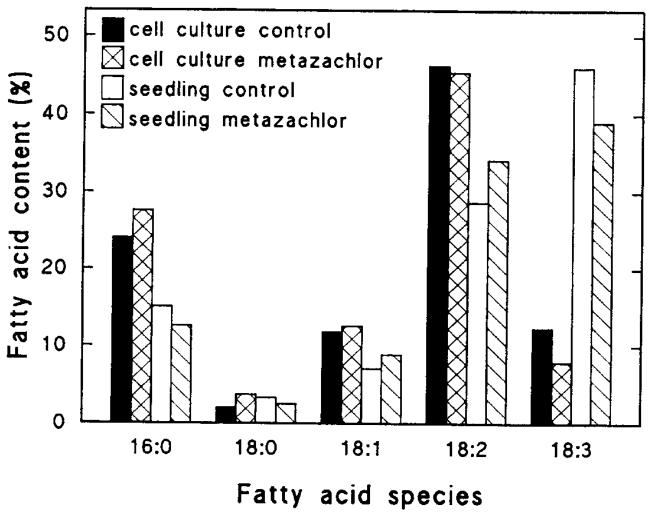

Fig. 3 Influence of metazachlor (100 μM) on fatty acid composition of *Spinacia oleracea* cell suspension culture and seedling after incubation for 4 and 10 days, respectively.

Standard deviation was less than 5% of indicated value.

acids, 18: 3 (−50%) and 18: 2 (−12%) was observed, while the relative amount of the 18: 0 species was more than doubled (Fig. 2). Qualitatively similar changes of the fatty acid profile were reported in *Scenedesmus acutus* treated with 5 μM alachlor and metazachlor.[7,8] Although the fatty acid profile of rice cell suspension culture and seedlings were comparable the effect of metazachlor on fatty acid metabolism was found different. Even in the presence of 200 μM metazachlor the rice cell suspension culture did not show any change with respect to fatty acid composition.

In the case of spinach, fatty acid composition of the cell suspension culture was not comparable with that of the seedlings (Fig. 3). In seedlings the most important fatty acid was 18: 3 (>45%) while it was the 18: 2 species (>45%) for the cell suspension culture, 18: 3 represented only 12% of the total fatty acids. Although spinach is a 16: 3-plant[16] no 16: 3 was detected, which could be due to the young age of the seedlings and to the culture conditions of the cell suspension. After treatment with 100 μM metazachlor a slight but significant decrease of 18: 3 was observed in both systems. In contrast to *Scenedesmus* and rice the relative amount of 18: 2 increased by treatment of the seedlings with metazachlor. This supports the suggestion that fatty acid desaturation may play a role in the mode of action of chloroacetamides. Further evidence is given by an experiment yielding less ^{14}C-labeled 18: 2 and 18: 3 in the presence of 100 μM metazachlor and alachlor using rice and spinach cell suspension cultures fed with ^{14}C-oleic acid (data not shown).

The totally different response to metazachlor of seedlings and cell suspension cultures within one species led us to conclude that these systems possess different tolerance mechanisms. A possible explanation for decreased response to the herbicide may be reduced uptake. Therefore, the disappearance of metazachlor from the medium in which either cells or tissue slices were incubated was investigated. It was found that uptake was high in spinach cells and seedlings and in rice cells, 22.6, 6.1, and 5.3% of the label fed had disappeared from the medium after 6 hr, respectively (Table 1). All three systems showed little or no influence of metazachlor. In contrast, treating the very sensitive rice seedlings with 200 μM metazachlor, it was not possible to detect any diminution of the radioactivity in the medium. A slow disappearance of the label from the medium (4 nM in 6 hr) was only found when the seedlings were fed with 0.1 μM metazachlor (Table 1).

The radioactivity in the medium may include the herbicide applied, the herbicide that has leaked out of the cells or even metabolites of the herbicide. No analysis of the medium was conducted and it is not possible to definitely conclude that all radioactivity recovered in the medium was herbicide that has not been taken up by the cells or tissue. Nevertheless, even if the medium were contaminated by leakage, the results show a low uptake by the sensitive system opposed to a higher uptake in the less or not sensitive systems. This contradiction rules out a role of uptake as a tolerance mechanism.

Plant tolerance to chloroacetamides is ex-

Table 1 Decrease of metazachlor concentration in the medium after uptake by cells and seedlings of *Spinacia oleracea* and *Oryza sativa*.[a)]

Incubation time (hr)	Metazachlor left in the medium (μM)				
	Spinacia oleracea		*Oryza sativa*		
	Cell culture	Seedlings	Cell culture	Seedlings	
0	200.0	200.0	200.0	200.0	0.100
1	168.6	193.8	198.6	200.0	0.097
6	154.8	187.8	189.4	200.0	0.096

a) For incubation with ^{14}C-metazachlor 1 ml suspension culture contained 0.1 ml *Spinacia oleracea* cells or 0.015 ml *Oryza sativa* cells in the logarithmic phase.

plained by metabolism of the herbicide *via* glutathione S-transferase[1] and the first metabolite of metazachlor[17] and other chloroacetamides like acetochlor and propachlor[18,19] was identified as the glutathione-conjugate. It is generally thought that there are no qualitative differences in the metabolism of pesticides between cell suspension cultures and corresponding plants.[20] Thus, quantitative differences in the metabolism of the herbicide should explain the different sensitivity between cells and seedlings. Accordingly, it was necessary to investigate the fate of labeled metazachlor in our systems.

Uptake of labeled metazachlor that was demonstrated in all systems by disappearance of the parent herbicide in the medium (Table 1) was confirmed by increase of label in the plant material (Figs. 4–7). As it was hypothesized for most undissociable herbicides,[21,22] the uptake kinetics indicate that metazachlor uptake mechanism by cell suspension culture and plant tissue is passive diffusion. In spinach cell suspension culture the concentration of label in the cells increased rapidly during the first hour and then slowly approached that of the medium, as can be seen by adding the concentrations of metabolite and parent herbicide (Fig. 4). In long-term experiments of 72 hr (*e.g.* Fig. 4) the label accumulated in the cells probably because continuous and fast metabolism maintained a concentration gradient between cell and culture medium, allowing for more metazachlor to enter the cells. Such a phenomenon was also observed in spinach seedlings (Fig. 5) and in corn treated with BAS 145-138, a safener of metazachlor.[23] Uptake of metazachlor by rice cell suspensions was comparable to that of spinach cells, incorporation of the label was rapid during the first hour and slower thereafter (Fig. 6). The more gradual incorporation observed in spinach

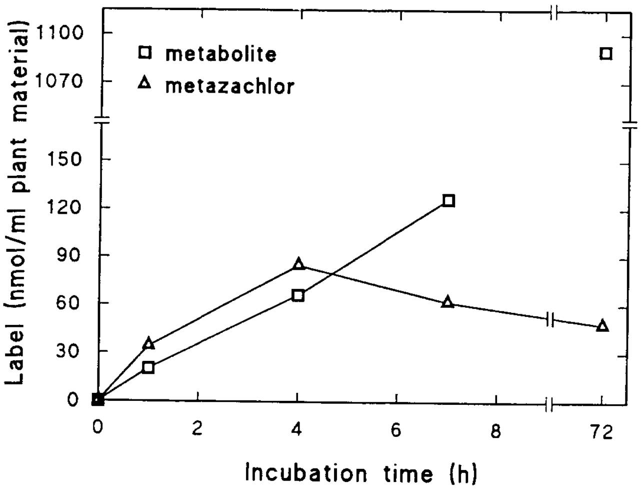

Fig. 5 Metabolism of metazachlor in *Spinacia oleracea* seedlings.

Initial metazachlor concentration in the medium was 200 μM.

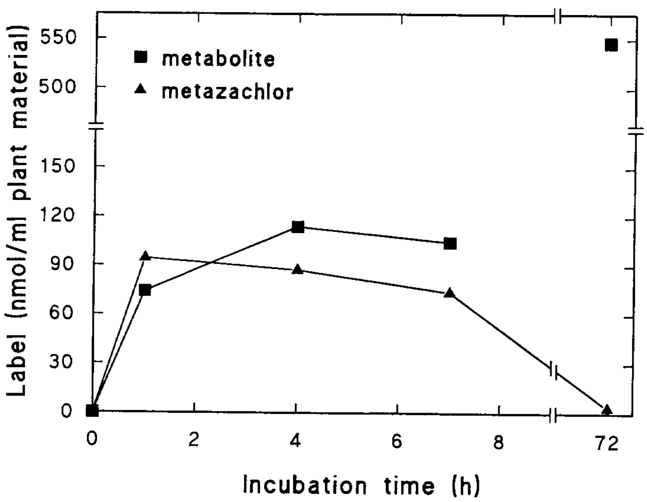

Fig. 4 Metabolism of metazachlor in *Spinacia oleracea* cell suspension culture.

Initial metazachlor concentration in the medium was 200 μM.

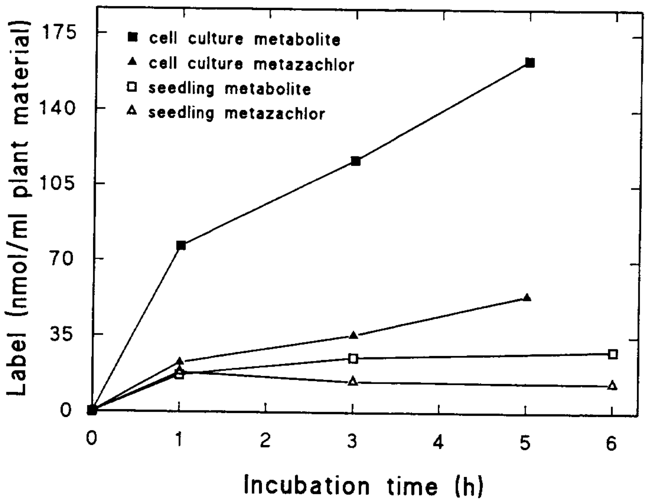

Fig. 6 Metabolism of metazachlor in *Oryza sativa* cell suspension culture and seedling.

Initial metazachlor concentration in the media was 200μM.

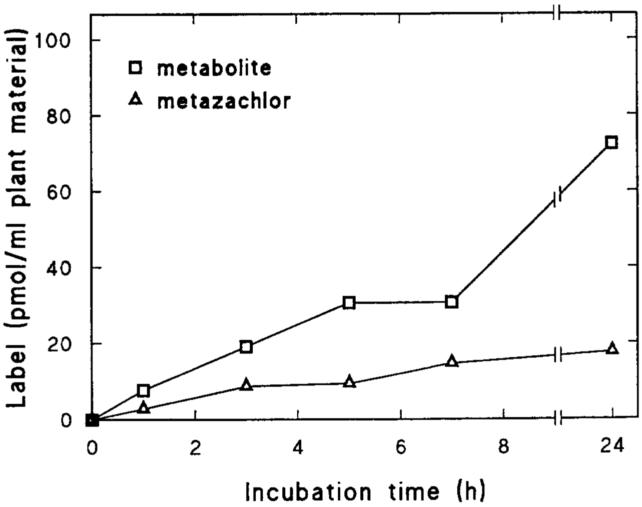

Fig. 7 Metabolism of metazachlor in *Oryza sativa* seedlings.
Initial metazachlor concentration in the medium was 0.1 μM.

seedlings may be explained by the time necessary to reach the middle of the leaf section.

Metazachlor metabolism started immediately in all samples. More than 30% of the label was determined to be metabolite after 1 hr of incubation. Formation of metabolite was proven to be enzymatic since a heated cell suspension culture (30 min at 100°C) did not metabolize the herbicide (data not shown). Metazachlor metabolism was quantitatively comparable in spinach cell suspension culture and seedlings (Figs. 4 and 5). In both systems, metazachlor was rapidly incorporated and metabolized. Parent metazachlor concentration in the plant material decreased steadily. For the cell suspension culture metabolism was nearly complete after 72 hr (3.3 nmol/ml left in the plant material), thus explaining the small herbicidal effect on cell growth. With spinach seedlings after 72 hr the concentration of parent metazachlor was higher (48 μM) which may account for the slight sensitivity of the germinating seedling to metazachlor (Fig. 1).

Quantitative differences between metabolism in cell suspension culture and seedling were found in the case of rice (Fig. 6). Feeding rice cell suspension culture with 200 μM metazachlor resulted in a rapid herbicide uptake (Table 1, Fig. 6); metabolism started promptly and continued at a steady rate throughout the incubation time (Fig. 6). In contrast, when rice seedlings were incubated 1 hr with 200 μM metazachlor little uptake could be detected inside the seedlings (confirming the result of Table 1) and approximately half of the label recovered was not hexane-soluble (Fig. 6). Metabolism of the herbicide proceeded further with a low rate during the following 2 hr and stopped. Although 200 μM metazachlor was not effective in cell suspension cultures, it was much above the lethal concentration for seedlings, that probably explains the rapid interruption of uptake and metabolism in seedlings.

To allow for comparison between seedlings and cell suspension culture the metabolism of a sublethal herbicide concentration was investigated. When 0.1 μM ^{14}C-metazachlor was fed to rice seedlings the decrease of label measured in the medium (Table 1) was confirmed by an increase of radioactivity in the tissue (Fig. 7). Uptake and metabolism of the herbicide were continuous throughout the 24-hr assay period but could not be followed further, 0.1 μM metazachlor being deleterious to the tissue in the long run. Within the assay period metabolism was not complete and after 24 hr 18 nM parent metazachlor was still present in the tissue (Fig. 7). Since an outside concentration of 0.1 μM metazachlor yielded over 50% growth inhibition (Fig. 1), 18 nM of parent herbicide inside the tissue was enough to obtain more than 50% phytotoxic efficiency. According to the distribution of metazachlor inside maize tissues[5] the concentration might even be lower at the site of action. The extremely low effective concentration supports the conclusion that there is a very specific primary target for the herbicide.

In spinach and in rice cell suspension cultures high parent metazachlor concentrations caused little or no effect compared to rice seedlings. For example, the parent herbicide concentration was over 1000 times higher in spinach (48 μM) than in rice (18 nM) seedlings, yet metazachlor was more effective in rice. One could speculate on the existence of an unknown metabolic pathway yielding hexane-soluble compounds that would contaminate the hexane fraction in spinach. At the moment we have not investigated further the metabolites. However, such a pathway seems unlikely since Fuerst's analysis[5] of the hexane extract showed only parent metazachlor. Similarly, Lamoureux & Rusness (personal communication, 1993) found only one peak that cochromatographed with parent metazachlor in an HPLC analysis of metazachlor metabolites in a methylene chloride fraction, comparable to our fraction. Furthermore, the only detoxification pathway in plants that does not involve GSH conjugation of chloroacetamide has been described for acetochlor[24] and metolachlor.[25] It seems to be under control of a cytochrome P450 mixed function oxidase.[25] This detoxification involves a splitting of the oxyalkyl functional group and should not occur with metazachlor where no oxyalkyl group is present.

Protection mechanisms for chloroacetamides in tolerant plants might exist, which are not yet known. Binding sites at the primary site of action may be highly different in rice and spinach or these sites may be unimportant or absent in non-sensitive species or cell cultures. It is not possible, however, to conclude on a general difference between mono and dicotyledons since rice cell cultures also are insensitive to the herbicide and some dicotyledonous weeds can be controlled by metazachlor.

A possibility for lack of sensitivity in the rice cell suspension culture may be the heterotrophic cultivation. Little response of heterotrophic plant cell suspensions to chloroacetamides was explained by a possible additional mode of action that involves photosynthetic processes.[6] However, our studies with mixotrophic cell suspension cultures of tobacco, soybean, *Chenopodium album* (common lambsquarter) and photoautotrophic spinach did not lead to more sensitive reactions, although at least for soybean the concentration of parent metazachlor was still over 20 μM after 24 hr (data not shown). Our experiments were conducted in the logarithmic phase and the results may differ in the stationary phase; however, since chloroacetamides have been found to affect cell division the cell cultures should be even less sensitive to metazachlor in the stationary phase.

A general tolerance of higher plant cell suspension cultures to chloroacetamides could be suggested, since no highly susceptible culture has yet been found. Cell suspension cultures consist mostly of non-organized and undifferentiated cells. This could be a reason for lack of sensitivity provided the target site of chloroacetamides exists only in specialized cells. In any case, the primary mode of action is still to be found and further comparative studies of cell suspension culture and corresponding plant may provide some clues toward elucidation of the problem.

Cell suspension cultures may be a good model for plants in testing the mode of action of herbicides, as was demonstrated with the spinach cell suspension culture. However, as shown in this study a rice cell suspension culture and rice seedling are no comparable systems, indicating that confirmation of results by whole plant systems remains necessary.

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要　約

クロロアセトアミド系除草剤 metazachlor の効果と代謝：細胞懸濁培養と幼苗の比較

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Metazachlor の生物活性およびその消長を，イネとホウレンソウの細胞懸濁培養，およびそれぞれの幼苗で比較した．イネの細胞培養での生長は，metazachlor 200 μM で阻害されず，ホウレンソウでは 100 μM でわずかに阻害された．対照的に，幼苗の生長はイネでは metazachlor 0.1 μM で 50% 阻害され，ホウレンソウでは 100 μM で 35% 阻害された．イネ幼苗においては，0.1 μM の metazachlor は脂肪酸の不飽和化を著しく減少させたが，一方 100〜200 μM でも他の 3 種の試験系ではほとんど何の影響も示さなかった．細胞懸濁培養はそれぞれの幼苗の試験系に比較して，metazachlor に対する感受性がより低かった．最も感受性の高かったイネ幼苗において，薬剤吸収が最も小さかったことから，吸収で感受性の差異を説明することはできなかった．すべての試験系で metazachlor の代謝が認められたが，ホウレンソウでは 200 μM の metazachlor 投与 72 時間後で，細胞培養では 3.3 μM，幼苗では 48 μM の親化合物がそれぞれ残存していた．また，0.1 μM で培養したイネ幼苗では，24 時間後で 18 nM の metazachlor が残存していた．Metazachlor はホウレンソウには高濃度で残存するが活性を示さず，一方イネ幼苗においてはその残存量が少ないにもかかわらず活性を示す．このことはグルタチオン抱合による薬剤代謝が耐性植物における唯一の保護機構ではないことを示唆している．