Inhibition of Growth and Interference with ^{14}C-Leucine Uptake and Incorporation into Protein in Non-chlorophyllaceous Sugarcane Cells by Ametryn[1]

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ABSTRACT

Non-chlorophyllaceous sugarcane cells derived from *Saccharum* spp. var. NA56-79 were used to study the effect of ametryn, a triazine herbicide, on cell growth and the uptake and incorporation of ^{14}C-leucine into protein. Cell suspensions exposed to 40 mg l^{-1} ametryn for 20 days showed 75% growth inhibition as compared to the control. Total inhibition of growth was observed in the presence of 80 and 160 mg l^{-1} ametryn. Cells pretreated for 1 hour with ametryn and incubated with ^{14}C-leucine for 2 hours in the presence of 2 to 160 mg l^{-1} of the herbicide showed a reduction in ^{14}C-leucine uptake and incorporation into protein. Pulse-chase experiments showed interference with the incorporation of ^{14}C-leucine in the presence of 80 and 160 mgl^{-1} ametryn, revealing alterations in the metabolism of proteins.

COMPENDIO

Células no clorofiladas derivadas de *Saccharum* spp. var. NA56-79 fueron utilizadas para estudiar el efecto del ametrin, un herbicida triazínico, sobre el crecimiento celular y sobre la absorción e incorporación de leucina-C^{14} en proteínas. Las suspensiones celulares expuestas a 40 mg l^{-1} de ametrin durante 20 días mostraron 75% de inhibición de crecimiento comparadas con el control. En presencia de 80 y 160 mg l^{-1} de ametrin se observó inhibición total del crecimiento. Células pretratadas con ametrin durante 1 hora e incubadas con leucina-C^{14} por 2 horas, en presencia de 2 a 160 mg l^{-1} del herbicida exhibieron una reducción en la absorción e incorporación en proteínas. Experimentos de pulso mostraron interferencia en la incorporación de leucina-C^{14} en presencia de 80 y 160 mg l^{-1} de ametrin, revelando alteraciones en el metabolismo de proteínas.

INTRODUCTION

The herbicide ametryn [2-(ethylamino)-4-(isopropylamino)-6-(methylthio)-s-triazine] inhibits the Hill reaction during photosynthesis by interacting with the electron transport carrier B of photosystem II. This interaction has been proposed as the primary mechanism of action of the triazine herbicides (9, 14). However, triazines also induce alterations in metabolic processes other than photosynthesis; for example, atrazine and propazine (10^{-5} to 10^{-3} M) have been shown to inhibit the growth of excised roots of *Lens culinaris* (18). Stimulation of alfalfa and tobacco seed germination in the presence of subtoxic concentrations of ametryn and atrazine has been observed (4). Triazines (10^{-4} M), including ametryn, were capable of inhibiting respiration to the same extent in mitochondria isolated from rat liver and *Phaseolus vulgaris* tissues (20).

Non-chlorophyllaceous plant cells cultured *in vitro* offer an advantage to the investigation of the effects of triazines on metabolic processes, as interference with photosynthesis is eliminated (7, 10, 12, 16). We previously reported different levels of inhibition of growth and a decline in the total protein content of non-chlorophyllaceous callus cultures of three sugarcane varieties grown in the presence of ametryn (6). These data suggest that ametryn disturbs protein metabolism. It was therefore of interest to investigate in further detalil the effects of ametryn on cell growth and on protein metabolism using ^{14}C-leucine as a tracer in non-chlorophyllaceous sugarcane cell suspensions.

MATERIALS AND METHODS

Cell suspensions

Non-chlorophyllaceous sugarcane cell suspensions were established from callus induced from stems of plantlets of *Saccharum* spp. var. NA56-79, as previously reported (5). The suspensions were maintained in the basal medium formulated by Murashige and Skoog (15) supplemented with the following substances: 2,4 dichlorophenoxyacetic acid (3 mg l^{-1}), arginine (60 mg l^{-1}), thiamine (1 mg l^{-1}), sucrose (20 000 mg l^{-1}), and coconut milk (100 ml l^{-1}) The pH was adjusted to 5.8.

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Growth assays

The growth assays and subsequent tests were performed using 8 to 10 day-old cell cultures under sterile conditions. The reaction mixture contained sugarcane cells with an equivalent dry weight of approximately 5 mg (d.w., calculated after drying at 115°C for 2h) and ametryn in methanol 50% (v/v) to give final concentration of 0.5, 1, 2, 5, 10, 20, 40, 80 and 160 mg l^{-1}. The concentration of methanol in all treatments and in the control was 2% (v/v), and the total volume of the reaction mixture was 5 ml (20 ml glass vials). The herbicide solutions were previously sterilized by filtration using 0.45 μm Millipore membranes. Cultures were incubated on a rotary shaker (180 rpm), at 24 ± 2°C in darkness for 20 days (time necessary to reach maximum growth under normal conditions). The cells were harvested in Miracloth discs, washed with 50 ml distilled water and dried in an oven (2h; 115°C) for dry weight determinations.

Uptake and incorporation of ^{14}C-leucine

^{14}C-leucine uptake and incorporation into protein were measured following the method described by Francki *et al*., (8). The reaction mixture contained a quantity of sugarcane cells equivalent to approximately 5 mg d.w. in 4.6 ml fresh medium plus 0.2 ml of herbicide solution or 0.2 ml of 50% methanol (control) in 20 ml glass vials. The suspensions were incubated for 1h at 24 ± 2°C in darkness on a rotary shaker at 180 rpm. Subsequently 0.2 ml of ^{14}C-leucine solution containing 0.3 μCi (^{14}C-U-leucine; 348 mCi. mmole^{-1}; purchased from Amersham Corporation, USA) was added to the reaction mixture. Incubation was continued for 2h more under the same conditions. For measurement of total ^{14}C-leucine absorbed 1 ml samples of cell suspensions were taken and the cells collected on Reeve Angel filters (grade 934 AH). The cells were washed 3 times with 3 ml volumes (3 x 3 ml) of chilled sorbitol (0.6 M) + leucine (10^{-3}M) solution.

Incorporation of ^{14}C-leucine into proteins was determined for 1 ml samples washed with 0.6 M sorbitol + 10^{-3}M leucine (3 x 3 ml), 10% trichloroacetic acid (TCA; 3 x 5 ml), and 80% ethanol (3 x 5 ml). The samples were dried at 85°C for 15 min and the radioactivity measured by liquid scintillation using a Beckman LS-230 system. The scintillation fluid employed contained 100 mg 1,4-bis 2(5-phenyloxazolyl) benzene (POPOP) + 3 g 2,5-diphenyloxazole (PPO)/ 300 ml absolute ethanol + 700 ml toluene.

Pulse-chase assays

Sugarcane cells (ca 37.5 mg d.w.) were incubated with 0.9 μCi ^{14}C-leucine in a total volume of 7.5 ml of reaction mixture in 50 ml Erlenmeyer flasks on a rotary shaker (180 rpm), at 24 ± 2°C in darkness for 2h. The suspension was centrifuged at 1500 rpm for 2 min and the supernatant (non-absorbed ^{14}C-leucine) was discarded. The cells were washed by centrifugation with fresh medium (3 x 10 ml) and resuspended. Aliquots of 2.4 ml containing 2.5 mg d.w. were dispensed into 20 ml glass vials and 0.1 ml of ametryn solution was added. The suspensions were incubated at 24 ± 2°C in darkness for 24h with agitation. The reaction was stopped by adding 2.5 ml of chilled 20% TCA. Samples of 2 ml were collected, washed, and analysed in order to measure the incorporation of ^{14}C-leucine into proteins

RESULTS AND DISCUSSION

Effect on growth

Sugarcane cells incubated in 0.5 to 5 mg l^{-1} (2.2 x 10^{-6} to 2.2 x 10^{-5}M) ametryn showed no growth inhibition (Fig. 1). At a concentration of 10-20 mg l^{-1} (4.4-8.8 x 10^{-5}M) ametryn, growth inhibition was about 7% that of the control. However, the negative effect was greater in the presence of 40 mg l^{-1} (1.76 x 10^{-4}M) of the herbicide (ca 75%

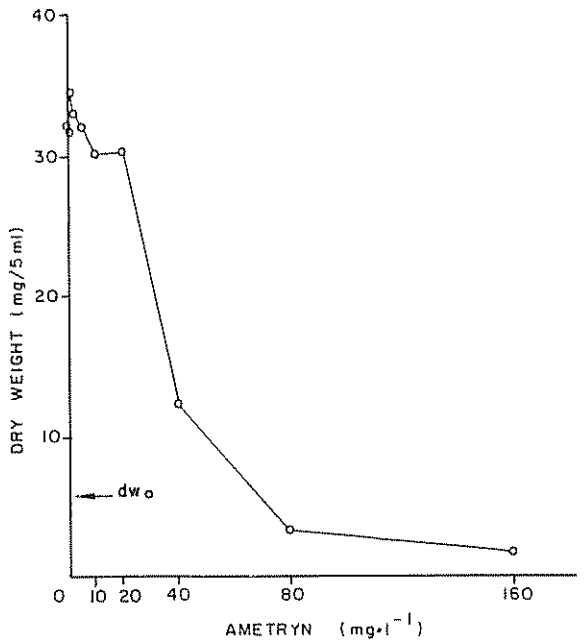

Fig. 1 Effect of ametryn concentration on the growth of sugarcane cell suspensions. dw_o indicates the cell mass of the initial inoculum. Each value is the mean of 2 replicates.

inhibition) and growth was totally suppressed by concentrations of 80 and 160 mg l^{-1} (3.5 and 7 x 10^{-4}M) ametryn. Growth inhibition of non-photosynthetic callus cultures of *Glycine max* and *Nicotiana tabacum* treated with 10^{-6} and 10^{-7}M atrazine, respectively, has been reported (7, 10). Nevertheless, subtoxic concentrations of atrazine (10^{-9} to 10^{-15}M) have been found to be effective in promoting soybean callus growth (7). Growth stimulation has also been observed in sorghum callus grown on media containing either 0.5 mg l^{-1} ametryn, atrazine, prometryn, propazine or simazine (16). These results indicate that triazines affect metabolic processes essential to all growth other than photosynthesis.

Influence on uptake and incorporation of ^{14}C-leucine

The uptake of ^{14}C-leucine decreased when ametryn was supplied at concentrations of 2 mg l^{-1} (8.8 x 10^{-6}m) and higher (Fig. 2). A similar trend was observed in relation to ^{14}C-leucine incorporation into proteins. Prometryn has also been shown to inhibit absorption and incorporation of ^{14}C-leucine into protein in cotyledonary tissues of *Cucumis sativus* (21). Several authors have reported alterations in the incorporation of ^{14}C-leucine by plant cells and tissues of different sources induced by triazines. Enzymatically isolated photosynthetic bean leaf cells showed a decrease of 13 and 35% in the incorporation of radioactivity into protein when they were treated with 10^{-5}M and 10^{-4}M atrazine respectively (1). On the other hand, subtoxic concentrations of simazine (10^{-8}M) have been shown to increase the incorporation of ^{14}C-leucine into protein in barley seedlings (19). Similar observations have been reported by Bush and Ries (2) for embryo axes of *Phaseolus vulgaris* treated with 2.2 x 10^{-8}M atrazine. These data have led to the conclusion that such herbicides affect protein metabolism. However, in all these studies, the possibility of alterations in ^{14}C-lucine incorporation through interference with amino acid uptake was not eliminated.

Pulse-chase experiments

Pulse-chase experiments were carried out in order to study the influence of ametryn on the incorporation of ^{14}C-leucine into proteins in the absence of possible interference with amino acid uptake. Sugarcane cells supplied with ametryn at concentrations of 0.5 to 40 mg l^{-1} (2.2 x 10^{-6} to 1.76 x 10^{-4}M) showed no decrease in ^{14}C-leucine incorporation (Fig. 3). However, in the presence of 80 and 160 mg l^{-1} (3.5 and 7 x 10^{-4}M) ametryn there was a reduction of approximately 30 and 100% respectively in comparison with the radioactivity incorporated into proteins in the control cells during the 24h chase, indicating alterations in protein synthesis.

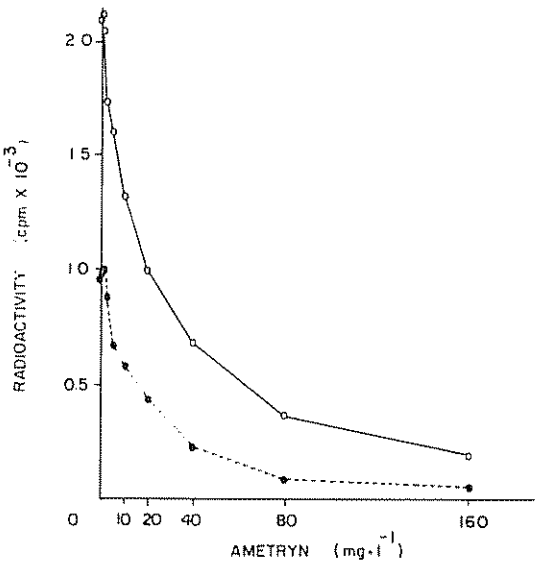

Fig. 2. Influence of ametryn concentration on the uptake and incorporation of ^{14}C-leucine into protein in sugarcane cells. (o ——— o), total radioactivity absorbed; (• -- •), radioactivity incorporated into protein. Each value is the mean of 2 replicates.

The negative effect of ametryn at concentrations between 2 to 40 mg l^{-1} on uptake and incorporation of ^{14}C-leucine into protein (Fig. 2) did not show a direct correlation with the inhibition of incorporation in pulse-chase experiments. It is possible, however, that in this concentration range ametryn may act indirectly on the incorporation of ^{14}C-leucine into protein by reducing the uptake of the amino acid into the cells. Evidence in favour of this hypothesis is reported in the work of Mann *et al.* (11). These workers used ^{14}C-α-aminobutyric acid, an amino acid that is not incorporated into proteins, and observed a decline in its uptake by barley and *Sesbania* tissues in the presence of 5 mg l^{-1} atrazine. The results in the present study suggest that ametryn might interfere with the ^{14}C-leucine uptake at low concentrations while altering protein metabolism at higher concentrations in non-chlorophyllaceous sugarcane cells.

The data obtained in the present work is not sufficient to define the mechanism of action of ametryn on amino acid uptake and protein metabolism. Nevertheless, the results do reveal an effect of ametryn on protein turnover. Ametryn might exert its influence directly on protein synthesis through interaction with some component involved in the translation process. Future experiments with an *in vitro* protein synthesis system could demonstrate this hypothesis. Alternatively, ametryn might act indirectly on protein synthesis by affecting metabolic pathways common to peptide chain formation and amino acid uptake. Since both processes are energy-dependent the most obvious candidate would seem to be ATP metabolism. Indeed, triazines have previously been reported to interfere with respiration and hence oxidative phos-

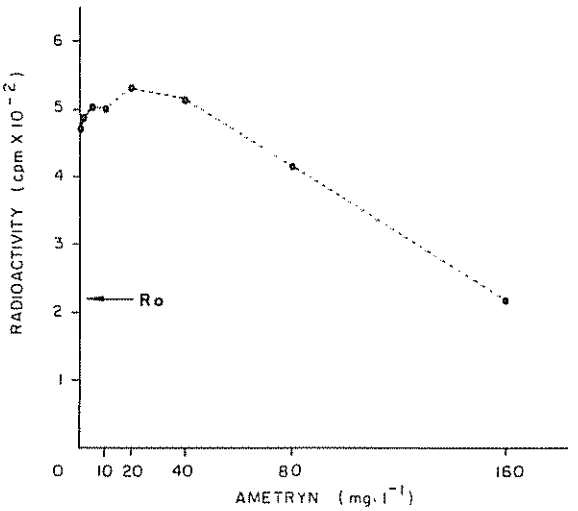

Fig. 3. Effect of ametryn concentration on the ^{14}C-leucine incorporation into protein in sugarcane cells after a 2h pulse followed by a 24 h chase. R_o indicates radioactivity incorporated following the pulse. Each value is the mean of 2 replicates.

phorylation (12, 20). Herbicides known to reduce cellular ATP levels have also been shown to be powerful inhibitors of protein and RNA synthesis (3, 13). Recently, ametryn (40 mg l^{-1}) has been demonstrated to inhibit RNA synthesis in non-chlorophyllaceous sugarcane cell suspensions (17). However, it does not appear to affect *in vitro* transcription of chromatin isolated from such cells, suggesting an indirect mechanism of action of this herbicide. The possibility that ametryn interferes with amino acid uptake, and with protein and RNA synthesis by altering ATP metabolism will be investigated in further studies.

In conclusion, these observations indicate that besides its known effect on photosynthesis, ametryn also influences several biochemical processes necessary for cell growth.

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Reseña de Libros

LEON, J. 1987. Botánica de los cultivos tropicales. San José, Costa Rica, IICA. 445 p. (Libros y Materiales Educativos no. 84).

Después de casi 20 años, el Servicio Editorial del IICA publica una nueva edición del libro originalmente titulado Fundamentos Botánicos de los Cultivos Tropicales, escrito por el costarricense Dr. Jorge León.

Esta nueva edición se titula Botánica de los Cultivos Tropicales y viene a llenar el gran vacío que quedó al agotarse el libro original, que tuvo gran acogida entre técnicos y estudiantes de las ciencias agrícolas.

La revisión del libro ha incluido una gran actualización en los aspectos de sistemática y nomenclatura. La organización del libro es la misma y sólo se hizo un reacomodo de ciertas secciones de la parte general o introductoria del libro, que se relaciona con los capítulos de diversidad genética, domesticación de plantas, origen de las plantas y la historia del origen de la agricultura.

La parte puramente sistemática viene dividida como antes en los dos grandes grupos de monocotiledoneas y dicotiledoneas y se incluyen todas aquellas familias y especies de interés en los trópicos.

El libro está dirigido a los estudiantes de agronomía de América Latina y con eso en mente se usa terminología propia de estos países, aunque para el lenguaje botánico se ha recurrido al diccionario de P. Font Quer. Por estas razones es particularmente útil al estudiante, pero debe enfatizarse que es una obra de consulta que no le debe faltar a cualquier persona cuyo quehacer esté ligado con la agricultura tropical.

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CARLOS ENRIQUE FERNANDEZ
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