Journal of Experimental Botany, Vol. 28, No. 103, pp. 468–479, April 1977

Absorption, Efflux, and Metabolism of the Herbicide [^{14}C]Buturon as Affected by Plant Nutrition

AJAZ-UL HAQUE, I. WEISGERBER, AND W. KLEIN

Institut für ökologische Chemie der Gesellschaft für Strahlen- und Umweltforschung mbH, München, D-8042 Neuherberg, Federal Republic of Germany

Received 29 April 1976

ABSTRACT

Mineral deficiencies can reduce the absorption and translocation of the urea herbicide [^{14}C]-buturon and its metabolites in wheat. The efflux of [^{14}C]buturon and its metabolites from mineral-deficient plants was increased as compared to normal-grown plants.

The metabolism rate of herbicide in the roots of normal-grown plants increased for 24 h, then decreased again, whereas in shoots a continuous increase was observed for 7 d; at this time, the metabolism rate was 25·6 per cent of the radioactivity present in the roots and 23·7 per cent in the shoots. In the nutrient-deficient plants, the metabolism of buturon was significantly increased as compared to the plants grown in normal medium. In the N-deficient nutrient medium, the ratio of metabolites to parent compound was higher than in the complete medium.

Four groups of metabolites were observed in plants: a small group of carbamates (metabolite group I), a group of mainly unstable compounds decreasing rapidly in roots (II), a group comprising, among others, *p*-chloroacetanilide (III), and a group of conjugates (IV); the groups III and IV increased continuously during 7 d. The metabolites were isolated through column and thin layer chromatography and identified by comparison with reference compounds and combined gas chromatography/mass spectrometry analysis.

INTRODUCTION

Urea herbicides are being used in agriculture to control weeds in various crops. It is important to know their mode of action, absorption, movement, and degradation in crops in which they are used. The degree of penetration of a synthetic compound into plant roots and the extent of its subsequent translocation into other plant parts are both functions of the particular plant, soil type, and physicochemical properties of the compound. Several investigators have reported nutritional influences relative to the penetration and translocation of pesticidal compounds within plants (Hacskaylo and Ergle, 1955; Yu and Morrison, 1969; Talekar and Lichtenstein, 1971; Al-Adil, White, McChesney, and Kilgore, 1974). They observed either positive or negative correlations between the nutrient supplies and the uptake of pesticides into roots or shoots. However, due to differences in the chemical structure of various pesticides, the rate and mechanism of uptake and translocation are different for each pesticide and the plant species used for the study.

The objective of the present study was to determine the effects of nutritional conditions upon absorption, efflux, and metabolism of buturon (*N*-(*p*-chlorophenyl)-*N'*-methyl-*N'*-isobutinylurea, Fig. 8) in wheat plants.

MATERIAL AND METHODS

Culture of plants

Seeds of spring wheat (*Triticum aestivum* L., cv. Kolibri) were germinated in moist paper towels for 3 d. After the first leaf emerged from the coleoptile, about 100 seedlings were transferred into each of five black-painted 1 l jars containing $CaCl_2$ solution (1 mmol l^{-1}), and all the jars were placed into a growth chamber under a light intensity of 20 klx with 16 h photoperiod. The temperature was maintained at 24 °C during the day and 16 °C during the night. The relative humidity was about 60 per cent.

One week after the seedlings were depleted of their endogenous minerals, they were transferred into modified Hoagland–Arnon half-strength nutrient solutions (Hoagland and Arnon, 1938), one being a full nutrient solution and the other four being deficient in N, K, P, or Mg. The compositions of the nutrient media were as follows:

1. Full nutrient solution: $Ca(NO_3)_2.4H_2O$, 0·821 g; KNO_3, 0·506 g; KH_2PO_4, 0·136 g; $MgSO_4.7H_2O$, 0·120 g; in 1 l of water.
2. N-deficient solution: K_2SO_4, 0·871 g; $Ca(H_2PO_4)_2$, 0·117 g; $CaSO_4.2H_2O$, 0·344 g; $MgSO_4.7H_2O$, 0·060 g; in 1 l of water.
3. K-deficient solution: $Ca(NO_3)_2.4H_2O$, 1·231 g; $Ca(H_2PO_4)_2$, 0·117 g; $MgSO_4.7H_2O$, 0·241 g; in 1 l of water.
4. Mg-deficient solution: $Ca(NO_3)_2.4H_2O$, 0·821 g; KNO_3, 0·506 g; K_2SO_4, 0·436 g; KH_2PO_4, 0·136 g; in 1 l of water.
5. P-deficient solution: $Ca(NO_3)_2.4H_2O$, 0·821 g; KNO_3, 0·253 g; K_2SO_4, 0·435 g; $MgSO_4.7H_2O$, 0·241 g; in 1 l of water.

Microelements were given to all the plants (for each pot 0·5 ml of the following stock solution: H_3BO_3, 2·50 g; $ZnCl_2$, 0·50 g; $CuCl_2.H_2O$, 0·05 g; MoO_3, 0·05 g; $MnCl_2.4H_2O$, 0·50 g). Iron was supplied as Fe-EDTA salt (5 mg l^{-1} Fe). The nutrient solutions were aerated continuously throughout the experiment. Before treatment with the herbicide, the nutrient solutions were renewed and thereafter maintained at a constant level with distilled water.

Treatment with [^{14}C]buturon

When 15 d old and showing moderate deficiency symptoms, the plants received 84 μCi of [^{14}C]buturon per pot through the roots in the nutrient medium, corresponding to about 3·5 mg l^{-1} medium. The ring-labelled [^{14}C]buturon used (specific activity 5·0 mCi $mmol^{-1}$) was synthesized in this Laboratory (Attar, Ismail, Bieniek, Klein, and Korte, 1973) and was of 99·2 per cent radiochemical purity at the time of application. The herbicide was applied by dissolving in a small amount of acetone and adding to the nutrient solutions. Thereafter the nutrient solutions were bubbled extensively to eliminate acetone.

Absorption and translocation studies

After supplying the plants with the herbicide [^{14}C]buturon, seven plants from each pot were collected at 2, 4, 24, 48, 72, 96, and 168 h intervals. The roots were washed in a mixture of water and methanol (1:1, by vol.) and blotted dry between folds of filter paper. They were then separated into roots and shoots and weighed. There were variations in the fresh weights of individual plants, and, to reduce these, the mean weight of two plants was taken. The deficiency in plants was of moderate order and there were no substantial differences in fresh weight between sets of deficient plants when compared to those grown in complete nutrient medium.

The radioactivity taken up was determined in each part by separate combustion of two plants from each pot in an automatic oxidizer (Packard, Model 306), where the $^{14}CO_2$ liberated was trapped in a toluene-based scintillation solution with phenethylamine, and the radioactivity was counted in a liquid scintillation counter (Packard, Tricarb 3380). The average of two such determinations was taken to calculate the total concentration in each plant part (Fig. 1). The remaining five plants collected from each pot were extracted fresh in a mixture of chloroform and methanol (1:1, by vol.) by homogenizing with an Ultra Turrax. The extracts were filtered and dried over Na_2SO_4. The residue was washed several times with the same solvent mixture. The shoots were extracted similarly to the roots. All extracts were concentrated in a rotary evaporator cooled with methanol. They were stored at 0 °C till further use.

Efflux studies

After uptake of [^{14}C]buturon for 2 d, five plants from each pot were transferred into each of five vessels containing nutrient solutions identical to those in which they were grown previously. Roots were rinsed with distilled water before transfer. The plants were allowed to grow in the growth chamber under the conditions described above. Samples from the aqueous medium were taken at intervals and the radioactivity measured (liquid scintillation counters Tricarb, Packard 3375 and 3380; scintillant based on dioxane). An average of three determinations was made. The volume of the nutrient solutions was maintained with distilled water.

Separation and isolation of plant metabolites

To determine the metabolism rates in plants, the extracts obtained from the absorption and translocation studies were spotted on precoated, thin layer chromatography (TLC) silica gel plates (20 cm × 20 cm) (Merck, Darmstadt) and separated with a dichloromethane solvent system (developed twice). Standard [^{14}C]buturon was spotted on TLC plates parallel with each set of extracts in order to compare the position of the parent compound. The radioactive components on the plates were localized by means of a dot-print scanner (Berthold/Frieseke, Germany). Each radioactive region was then scraped off from the plate and radioactivity in them was counted by liquid scintillation counting (scintillant based on dioxane).

To isolate the metabolites, all the root extracts were combined and preliminary cleaning and separation by column chromatography was made. A silica gel (70–200 mesh) column and different gradient elution systems were utilized. Dichloromethane was the starting solvent system with increasing proportions of ethyl acetate, and the second system consisted of ethyl acetate with increasing proportions of methanol. The column was connected to a scintillation counter (Berthold/Frieseke, Germany) and the flow of radioactivity in the elute passing through the counter was registered on a chart. Each radioactive fraction recovered was concentrated on a rotary evaporator and purified on precoated and self-made silica gel plates. The most hydrophilic fraction was eluted from the column with methanol and hydrolysed in aqueous medium with 6 N HCl for 8 h. The hydrolysate was extracted with ether and the extract separated and purified by T.L.C. with various solvent systems. The substances were identified by gas–liquid chromatography (GLC) and combined gas chromatography/mass spectrometry (GLC/MS; for details see Haque, Weisgerber, Kotzias, Klein, and Korte, 1976).

Separation and isolation of conversion products in the nutrient media

At the end of the experiment (7 d), the complete and the N-deficient nutrient media were separately freeze-dried; the residues were taken up in methanol and separated by TLC (method as for plant extracts, dichloromethane solvent).

An aliquot of the separated fractions was used for determination of radioactivity; the remaining radioactivity of the non-polar metabolite fraction was purified by repeated TLC and identified by GLC and GLC/MS.

RESULTS AND DISCUSSION

[^{14}C]*Buturon translocation and growth of plants*

Moderate deficiency symptoms were apparent on the plants grown in various deficient nutrient solutions at the time of [^{14}C]buturon treatment in nutrient solutions. After an uptake period of 2 d, [^{14}C]buturon caused a reduction in both shoot and root fresh weight of wheat whether the plants were grown in a complete nutrient solution or in a solution deficient in N, K, P, or Mg. After 5 d growth, the fresh weight of shoots and roots at all levels of nutrition showed a tendency to decrease. This may be due to high accumulation of [^{14}C]buturon and its metabolites which continued to increase both in root and shoot tissues irrespective of growth (Fig. 1).

Although no evidence is presented, it appears that the translocation of buturon is largely a passive process which is closely associated with the movement of water

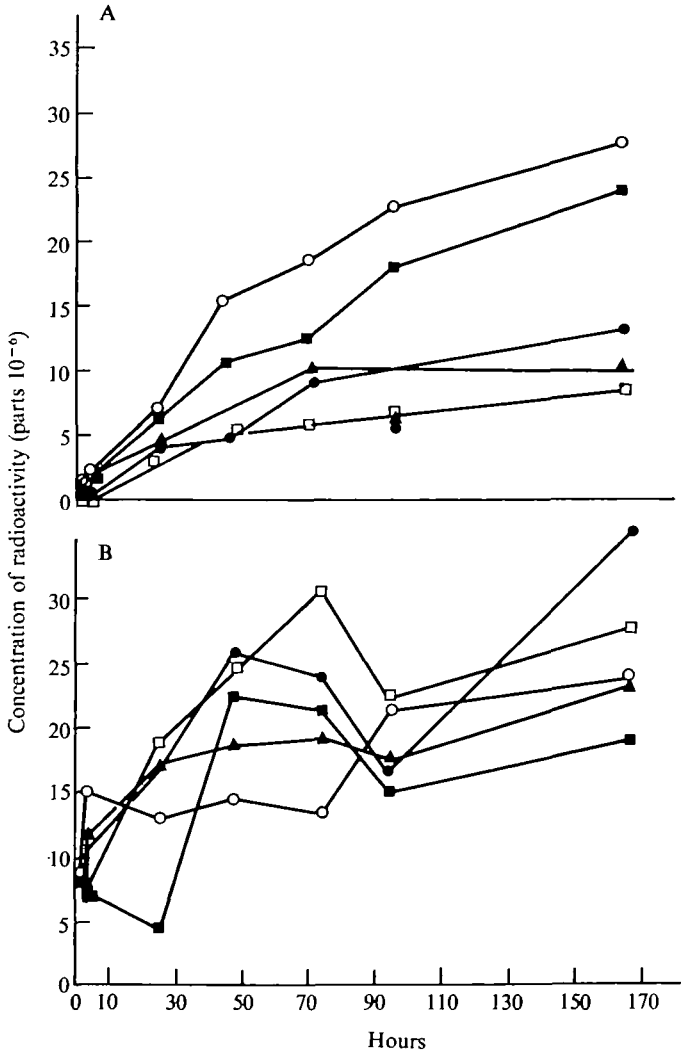

FIG. 1. Absorption of [^{14}C]buturon and conversion products by wheat plants from various mineral-deficient nutrient media. Concentration of radioactive substances are in μg g^{-1} fresh plant weight. The nutrient media contained 3·5 mg l^{-1} [^{14}C]buturon at the beginning of uptake. A: shoots; B: roots. Complete nutrient medium (○); complete medium lacking: P(■), K (●), Mg (▲), N (□).

in the transpiration stream. With the absence of one of the nutrient elements the transpiration is probably altered which in turn also affects the uptake of buturon. Shone and Wood (1972) examined the absorption and translocation of simazine by barley and found that the herbicide decreased the transpiration rate as well as reduced the uptake of phosphate and rubidium ions. In the present experiment the situation may be accentuated in that both mineral deficiency and herbicide possibly influence the transpiration as well as the metabolic processes of the plants.

Within 2 h the concentration in the root tissues rose to a value greater than that applied to the ambient medium (Fig. 1). In the absence of one of the nutrient elements, within 4 h the concentration of [^{14}C]buturon was variably different. The con-

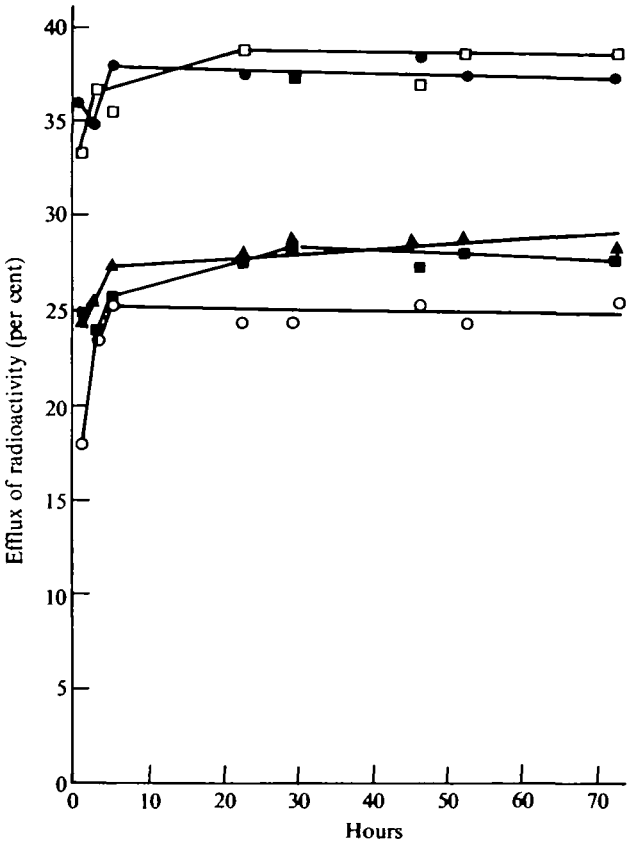

FIG. 2. Efflux of radioactive substances into various mineral-deficient nutrient media after a period of 2 d of [^{14}C]buturon uptake. After the uptake period, the plants were transferred into non-radioactive buturon-free media. For key to symbols see Fig. 1.

centration in the shoot tissues increased slowly and after 24 h reached a concentration between 3·0 parts 10^{-6} in N-deficient shoots and 7·4 parts 10^{-6} in complete-nutrient shoots (Fig. 1).

After 2 d growth the concentration of [^{14}C]buturon and its metabolites in deficient roots reached a maximum which was at least five to eight times higher than that in the ambient medium. The concentration in the roots increased further after a lag phase of 1–2 d. The roots in the complete nutrient solution did not show such a lag phase, and [^{14}C]buturon concentration increased continuously but was lower than in K- and N-deficient roots. It appears that the herbicide was retained in the root tissues and, after a period of time, released into the growth medium causing a lag phase in the root concentration. This lag phase may be true of deficient plants. However, it does not seem that the transpiration plays any role in causing the lag phase as no such lag phase or increase in shoot concentration was observed during the same period. The concentration in shoots of complete-nutrient plants rose faster than in the deficient shoots and showed the highest concentration of 27·8 parts 10^{-6} in contrast to 8·5 parts 10^{-6} in N-deficient shoots (Fig. 1).

Efflux of radioactivity

The low uptake of radioactivity in mineral-deficient plants as compared to normal-grown plants after [^{14}C]buturon application prompted us to find whether a faster elimination of radioactivity occurs in mineral-deficient plants. Therefore, the efflux of radioactivity from the roots into the nutrient medium was measured.

Figure 2 shows the efflux of [^{14}C]buturon from roots into nutrient solutions deficient in N, P, Mg, and K. The amount of loss of radioactivity was determined from five plants in each set of experiments. From the initial amount of radioactivity present in the complete-nutrient plants, an average of 24 per cent radioactivity was lost into the nutrient medium during the 72 h period. This loss was mainly during the first 5 h of the experiment. For the plants grown in mineral-deficient solutions, the elimination of radioactivity was greater and also occurred mainly during the first 24 h.

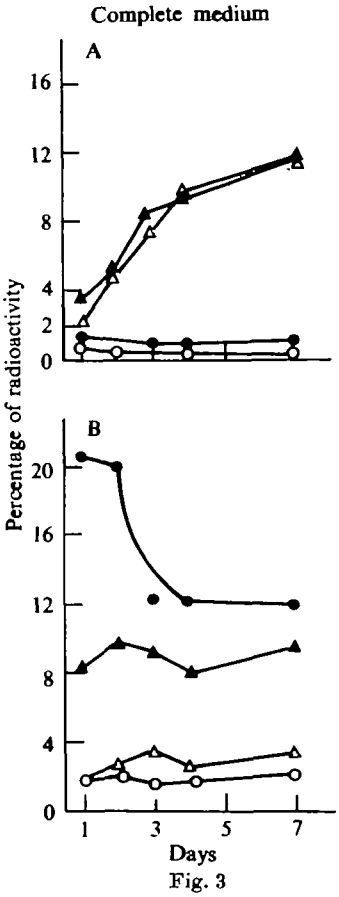

Fig. 3

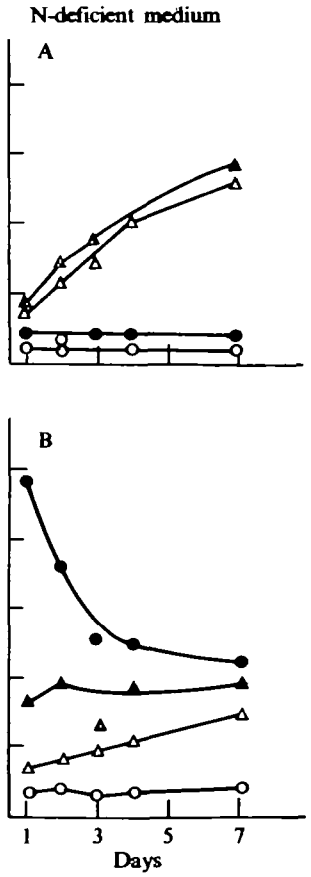

Fig. 4

FIGS 3–7. Time-course study of metabolite groups from wheat plants grown in various mineral-deficient nutrient media. Metabolite groups were quantitatively determined after separation of extracts by silica gel TLC (developed twice with dichloromethane). The percentage of metabolites are based on the total radioactivity recovered from the extracts. The metabolite groups had the following R_F values: I, 0·7–0·8; II, 0·4; III, 0·1–0·2; and IV, < 0·1. The unconverted buturon (R_F 0·48) is not shown. A: shoots. B: roots. Metabolite group I (○); II (●); III (△); IV (▲).

[^{14}C]Buturon metabolism in plants

The time course sequence of [^{14}C]buturon metabolism in wheat plants grown in various deficient nutrient solutions was followed. A continuous supply of [^{14}C]-buturon in nutrient solutions resulted in a rapid metabolism in complete-nutrient plants which after 24 h was 32·7 per cent of the total radioactivity extracted in the roots and 8·3 per cent in the shoots (Fig. 3A, B). Further growth of plants in the same medium for a period of 7 d increased the total percentage of metabolites of the shoots to 23·7 per cent, while that of roots decreased to 25·6 per cent. This suggests that the metabolism of [^{14}C]buturon may be occurring in the shoots and roots independently. The herbicide and the metabolites of the roots probably are partly transported through the transpiration stream to the shoot and partly leached out into the growing medium, which results in the decrease of the total metabolites in the roots and increase in the shoots.

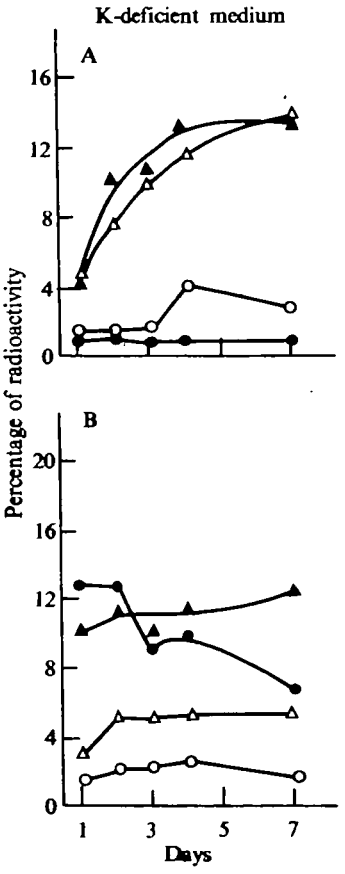

Fig. 5

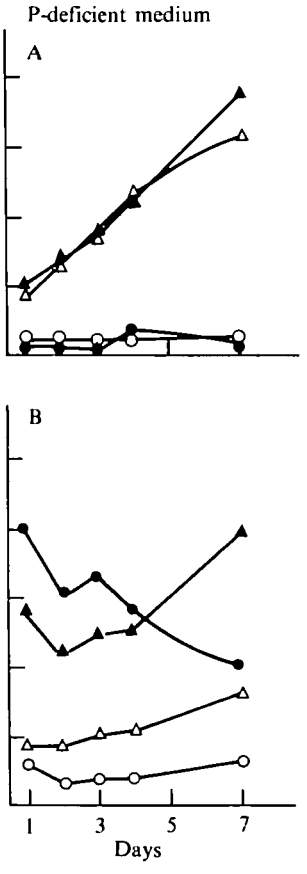

Fig. 6

The rate of metabolism in the various nutrient-deficient plants was generally higher compared to the complete-nutrient plants. The N- and K- and P-deficient plants (Figs 4–6) showed 24·0, 31·5, and 29·5 per cent, respectively, of the total radioactivity as metabolites in the shoots, while in the roots it was 24·3, 26·0, and 32·7

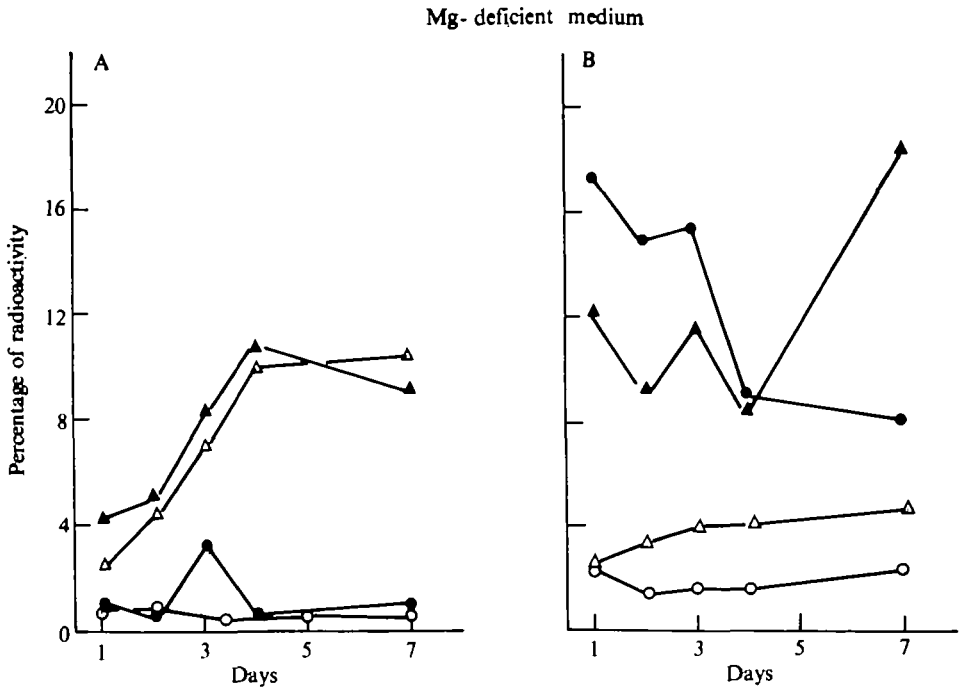

FIG. 7

per cent, respectively, towards the end of the 7 d experiment. Only for Mg-deficient plants (Fig. 7A, B), was the metabolism rate in the shoots comparable to that of the normal plants (21·4 per cent after 7 d), whereas in roots it was higher than for normal plants, which is in line with the observations in the other deficient plants.

The groups of metabolites designated as I, II, III, and IV were separated by TLC (Figs 3–7); the concentration of these was followed with time in various plants grown in nutrient-deficient media. Metabolite group I (R_F 0·7–0·8), which was in the lowest concentration, remained almost constant both in the shoots and roots. Metabolite group II (R_F 0·4), which appeared to be, in the beginning, in major concentration in the roots, fell rapidly after one day's growth of plants, whether they were grown in complete or deficient nutrient solution. In the shoot this group, which was below 2 per cent in concentration, stayed constant during the growth of the plants. It appears that group II contains metabolites formed first in the roots. Metabolite groups III (R_F 0·1–0·2) and IV (R_F <0·1) increased to a greater amount in shoots than in roots. This pattern of metabolism was the same whether the plants were grown in complete or deficient nutrient medium.

Identification of root metabolites

The metabolites from the combined root extracts were isolated and identified. From the metabolite group I (Figs 3–7), two metabolites were identified by GLC-MS. By mass spectrometric analysis, one metabolite (Fig. 8, formula A) gave a molecular ion of 185 and was identical to an authentic sample of *N*-(*p*-chlorophenyl)-*O*-methyl carbamate; the most significant mass spectral fragments were

at *m/e* 153 (M^+-CH_3OH) and 127 (*p*-chloroaniline). The other metabolite (Fig. 8, formula B) gave a molecular ion of 199 and was identical to *N*-(*p*-chlorophenyl)-*N*-methyl-*O*-methyl carbamate. The more significant mass spectral peaks were as follows: 153 (M^+-OCH_3-CH_3), 140 (M^+-CO-OCH_3), and 127 (*p*-chloroaniline, base peak). These two metabolites were also identified in a previous work with winter wheat under outdoor conditions (Haque *et al.*, 1976). They occur only in low concentration in all experiments.

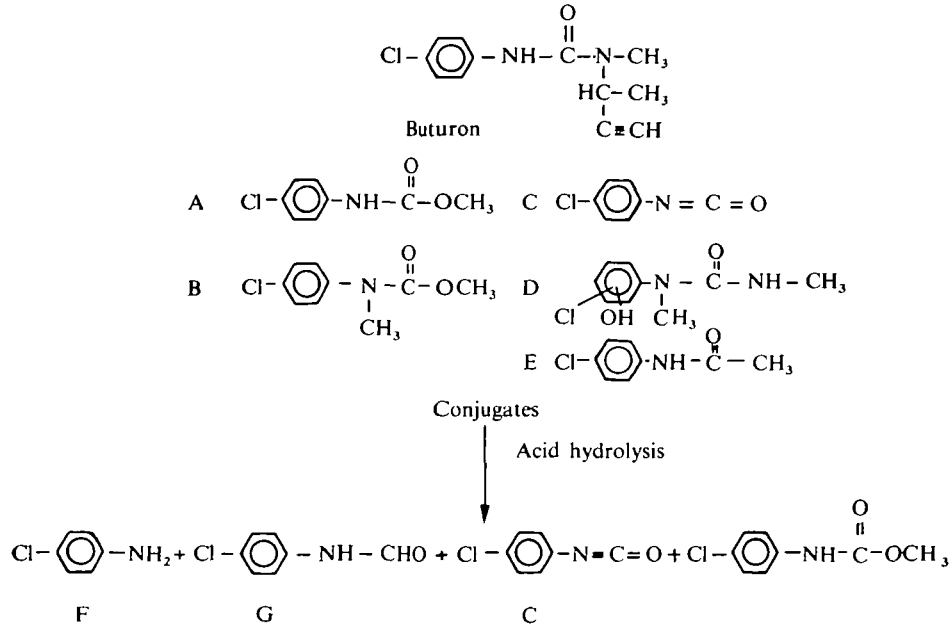

FIG. 8. Structural formulae of buturon and conversion products in wheat roots in hydroponic culture.

The metabolite group II in Figs 3–7 was separated by TLC into at least two components. The major metabolite upon GLC/MS was decomposed and formed *N*-(*p*-chlorophenyl) isocyanate (Fig. 8, formula C). These reactive properties of the main component of group II probably cause the decrease of this group in roots during the experiment. A small component with R_F 0·57 in hexane/ethylacetate/acetone (10:2:1, by vol.) solvent gave a molecular ion of 214. On the basis of its fragmentation this metabolite was tentatively identified as *N*-chlorohydroxyphenyl-*N*-methyl-*N'*-methylurea (Fig. 8, formula D). The more significant mass spectral peaks of this metabolite were as follows: 214 (M^+), 199 (M^+-CH_3), and 169 (M^+-CH_3-$NHCH_3$). The proposed structure is a methylation and hydroxylation product of the debutynylated buturon, a metabolite isolated from microorganisms (Wallnöfer, Safe, and Hutzinger, 1973), higher plants (Haque *et al.*, 1976), and in u.v. studies (Kotzias, Klein, and Korte, 1974). The enzymatic introduction of hydroxyl groups into urea herbicide molecules seems to occur preferably into the side chains (Frear and Swanson, 1972; Schuphan, 1974); however, for buturon this has not been observed (Haque *et al.*, 1976).

From buturon, a ring-hydroxylated derivative was isolated from leaching water of buturon-treated soil; however, its biological origin could not be unequivocally demonstrated (Haque *et al.*, 1976). The abiotic formation of such derivatives was demonstrated by u.v. irradiation studies (Kotzias *et al.*, 1974; Crosby and Tang, 1969). Nevertheless, the biological origin of ring-hydroxylated buturon derivatives is possible since ring hydroxylation is a well known general enzymatic process. Ernst and Böhme (1965) and Böhme and Ernst (1965) demonstrated ring hydroxylation in 4-monochlorinated urea herbicides from rat urine. In monuron and monolinuron the hydroxylation was preferably in the *ortho* and to a smaller extent in the *meta* positions. For the metabolite described here, the position of the hydroxyl group is proposed according to the mass spectrum.

The metabolite group III contained, among other substances, a product with R_F 0·06 in benzene/ethylacetate (10:1, by vol.) on TLC and a molecular ion of 169 upon mass spectrometry. Upon comparison of its mass spectrum with an authentic reference compound, this metabolite was identified as *p*-chloroacetanilide (Fig. 8, formula E).

Some conjugates (metabolite group IV) have also been found in the present study. Their percentage of total radioactivity increased during the experiment, as did the metabolite group III. The nature of conjugation was not examined. The conjugate fraction, upon acid hydrolysis, gave several products, four of which were identified by GLC/MS analysis and comparison with reference compounds. These products were: *p*-chloroaniline (Fig. 8, formula F), *p*-chlorophenyl isocyanate (Fig. 8, formula C), *N*-(p-chlorophenyl)-*O*-methyl carbamate (Fig. 8, formula A), and *p*-chloroformanilide (Fig. 8, formula G). The formation of artefacts during the hydrolysis process cannot be excluded.

[^{14}C]*Buturon conversion products in the nutrient media*

Of the different nutrient media used in this study, only the complete and the N-deficient media were analysed for conversion products.

Fig. 9 shows thin layer chromatograms of both media, along with a chromatogram of parent [^{14}C]buturon. In this figure, the start peak (on the right side) corresponds to the metabolite groups II–IV of the plants (Figs 3–7), the middle peak corresponds to unchanged buturon, and the less polar peak (at left) to the carbamate fraction (group I in Figs 3–7). Upon isolation, the carbamate fraction was found to be only one substance, *N*-(*p*-chlorophenyl)-*O*-methyl carbamate (Fig. 8, formula A). Whereas, in all plant extracts, this substance amounts to only a few per cent of the total radioactivity present, in the aqueous medium it equals the sum of all other metabolites. From this fact it may be concluded that the *N*-(*p*-chlorophenyl)-*O*-methyl carbamate is formed preferably in the nutrient medium and taken up by the plants. In the plants, this carbamate is accompanied by its *N*-methylated derivative (Fig. 8, formula B), indicating that *N*-methylation of the carbamate A occurs in plants. However, the possibility that both carbamates are formed in the roots cannot be completely excluded.

In the N-deficient solution, the percentages of all metabolite groups are significantly higher than in the complete nutrient solution. The same is true for the plant

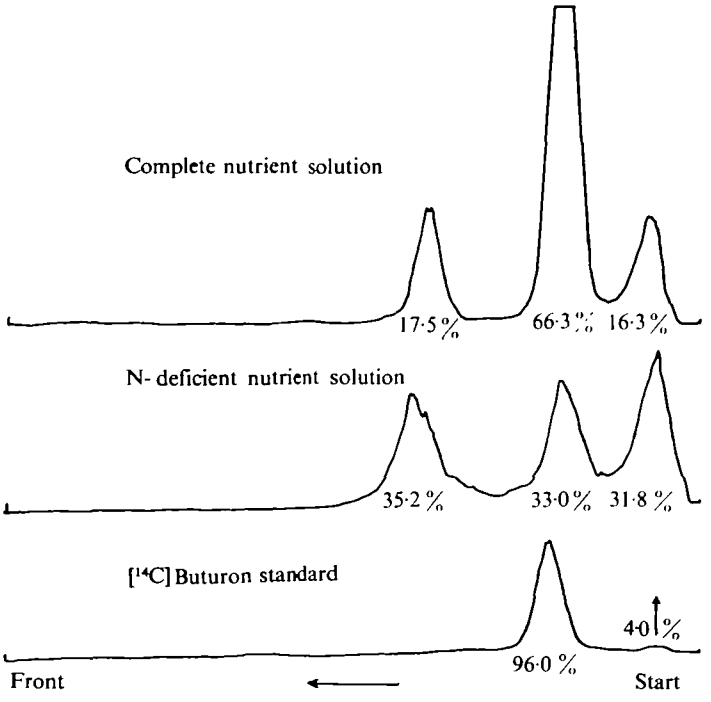

FIG. 9. Thin layer chromatograms of complete and of N-deficient nutrient media 7 d after application of [^{14}C]buturon and growth of wheat plants.
Dichloromethane solvent; chromatograms recorded with TLC-scanner from Berthold/Frieseke, Germany.

samples (Figs 3–7). As to the increase of carbamates in the N-deficient solution and plants, there remains the interesting question whether microorganisms and/or plants are able to consume the side-chain nitrogen of the buturon and leave the remaining molecule moiety as carbamate in the nutrient medium.

From these experiments it is evident that mineral deficiency reduces the uptake of buturon and increases its efflux from the roots and its metabolism.

The present results appear to be comparable with the work of other authors who found a positive correlation between the mineral supply and pesticide uptake (Yu and Morrison, 1969; Talekar and Lichtenstein, 1971; Al-Adil *et al.*, 1974).

LITERATURE CITED

AL-ADIL, K. M., WHITE, E. R., MCCHESNEY, M. M., and KILGORE, W. W., 1974. *J. agric. Fd Chem.* **22**, 242–4.
ATTAR, A., ISMAIL, R., BIENIEK, D., KLEIN, W., and KORTE, F., 1973. *Chemosphere*, **2**, 261–6.
BÖHME, C., and ERNST, W., 1965. *Food Cosmet. Toxicol.* **3**, 797.
CROSBY, D. G., and TANG, C.-S., 1969. *J. agric. Fd Chem.* **17**, 1041–4.
ERNST, W., and BÖHME, C., 1965. *Food Cosmet. Toxicol.* **3**, 789.
FREAR, D. S., and SWANSON, H. R., 1972. *Phytochemistry*, **11**, 1919–29.
HAQUE, A., WEISGERBER, I., KOTZIAS, D., KLEIN, W., and KORTE, F., 1976. *J. environ. Sci. Health*, Part B **11**(3), 211–23.
HACSKAYLO, J., and ERGLE, D. R., 1955. *Bull. Texas agric. exp. Stn.* **821.**
HOAGLAND, D. R., and ARNON, D. J., 1938. *Circ. Calif. agric. exp. Sta.* **347**, 39.

KOTZIAS, D., KLEIN, W., and KORTE, F., 1974. *Chemosphere*, **3**, 161–6.
SCHUPHAN, I., 1974. Ibid. **3**, 131–4.
SHONE, M. G. T., and WOOD, A. V., 1972. *J. exp. Bot.* **23**, 141–51.
TALEKAR, N. S., and LICHTENSTEIN, E. P., 1971. *J. agric. Fd Chem.* **19**, 846–50.
WALLNÖFER, P. R., SAFE, S., and HUTZINGER, O., 1973. *Pest. Biochem. Physiol.* **3**, 253–8.
YU, S. J., and MORRISON, F. O., 1969. *J. econ. Ent.* **62**, 1296–1304.