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EFFECT OF BUTACHLOR (2-CHLORO-2,6 DIETHYL-N-(BUTOXY-METHYL)-ACETANILIDE) ON THE ACTIVITY OF THE INDOLEACETIC ACID OXIDASE OF MAIZE SEEDLINGS

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Abstract

The indoleacetic acid oxidase activity of roots of maize seedlings grown in a culture solution was greatly increased in the presence of 10 and 20 mg/l butachlor, a chloroacetanilide herbicide. The increased indoleacetic acid oxidase activity of roots combined with the simultaneous inhibition of the basipetal transport of auxin from the shoot may result in a high endogenous auxin level at the first internode of the shoot. This may explain the vigorous root induction observed in the presence of butachlor in the region of the adventitious roots.

Introduction

Our previous observations revealed that high concentrations of butachlor caused morphological changes in the roots of maize seedlings grown in a soil culture. The number of second-order branchings decreased and an increased formation of adventitious roots was observed at the first internode of the shoot (TARI et al., 1978). KEELY et al. (1972) reported a similar phenomenon during the development of the lateral roots of cotton treated with butachlor. Since the formation of lateral roots and root branchings are controlled by auxin, investigations concerning the auxin metabolism of maize treated with butachlor were carried out. In the present paper we deal with the decomposition of indoleacetic acid (IAA) in the roots of maize seedlings treated with butachlor.

Materials and Methods

Maize seedlings (*Zea mays* L.) var. Keszthelyi SC hybride served as test plants.

Butachlor was a commercial product of the Nitrokémia Factory. Seedlings were grown in a diluted Knop culture solution (pH 6,8) for 14 days with 16 hours of daylight, a daytime temperature of 24 °C and a nighttime temperature of 18 °C, a relative humidity of 60%, under 6500 lx. The herbicide treatment was carried out on the 14th day, applying a nutrient solution containing 10 and 20 mg/l of butachlor.

The IAA oxidase activity of the roots was measured in the 2nd, 6th, 12th, 18th and 26th hours after the treatment. The roots were excised and the fresh plant material was homogenised in 20 ml of a phosphate buffer (pH 4,5). After centrifugation at 3500 (g/20 min, 4 °C) the enzyme activity of the supernatant was measured at 28 °C. Reaction mixture contained 0,003 mmol IAA, 0,01 mmol

$MnSO_4$, 0,001 mmol dichlorophenol and 1,5 ml of enzyme extract in 10 ml of 0,15 mol KH_2PO_4 adjused to pH 4,5.

After thirty minutes incubation aliquots were mixed with Gordon-Weber reagent and absorbancies were read after 15 min. by spectrophotometer at 530 nm.

The protein content of the extract was determined by the method of LOWRY et. al. (1951).

Results and discussion

The uptake of the herbicide taken as a function of time has already been investigated by our laboratory (TARI et al., 1978) under identical experimental conditions applied in the present experiments.

The uptake of isotope-labelled butachlor by the roots proved to be insignificant within two hours after the treatment. The accumulation of herbicide increased later but in the shoot the ^{3}H-butachlor could be detected only in traces even after 24 hours.

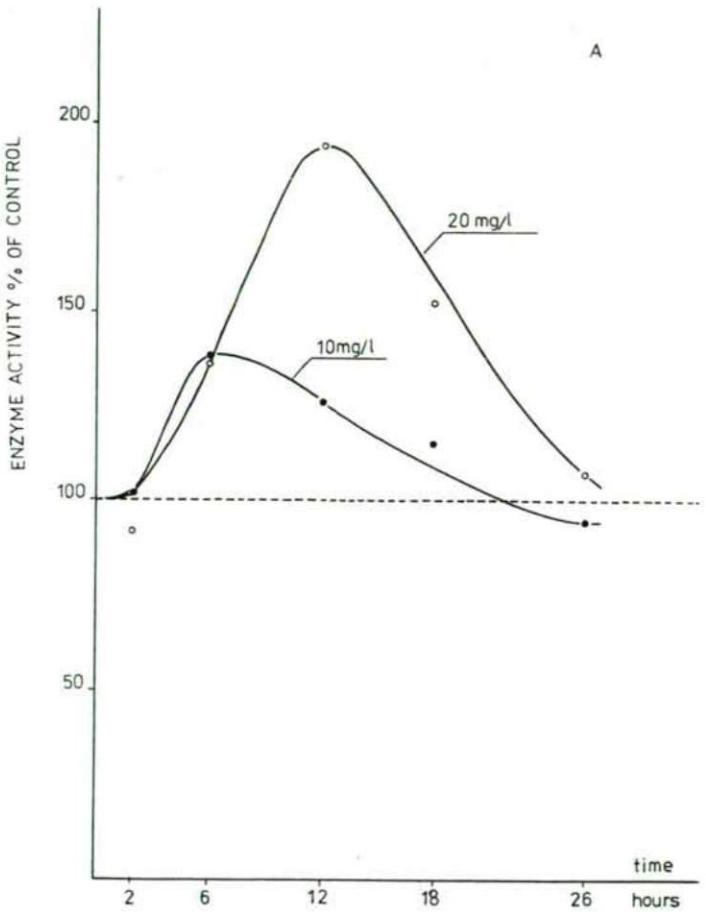

Fig. 1. a

Adding butachlor directly to the reaction mixture prepared with the untreated plant extract, enzyme activity is not affected by the herbicide over the concentration range 0,1—20 mg/l, whereas significant changes were observed in the IAA destructing activity of the enzyme preparation obtained from the plant treated with butachlor (Fig. 1).

According to Fig. 1/A. per gram fresh weight the enzyme activity changes in accordance with the changes in herbicide uptake as a function of time. In the first two hours after the treatment intensity of the decomposition of IAA does not change because the herbicide uptake via the roots can still barely be perceived in the 2-hour sample.

Later, however, significant increases can be observed in the enzyme activity in both applied butachlor concentrations. Per gram dry weight there is a decrease in

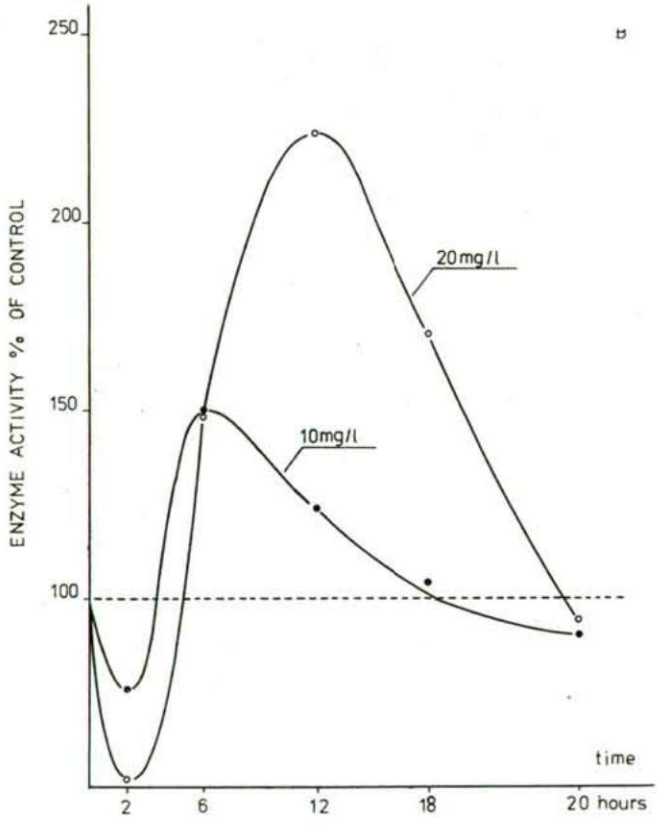

Fig. 1. b

Fig. 1. Effect of butachlor on the IAA oxidase activity of the roots of 14-day old maize seedlings on the fresh weight basis (A.) and on the dry weight basis (B.)
Enzyme activities are expressed as μg IAA oxidised per g fresh or dry weight per hour as a percent of control

IAA oxidase activity which corresponds with the significantly increased values of dry weight. It is believed that this phenomenon may be caused by the herbicide contacting with walls and membranes of root cells. So it alters their permeability which may lead to an increase in dry weight.

After 24 hours in both 10 and 20 mg/l butachlor solution a tendency of compensation can be observed. This decline in enzyme activity may be due to the lowering of the endogenous herbicide level. This is supported by the fact that the half-life of butachlor in maize proved to be 12 hours. The metabolism of chloroacetanilides in maize is very rapid and thus such an intense IAA degradation may be the consequence of an accumulation of metabolites of the herbicide. The mechanism of action of chloro-acetanilides such as butachlor is often explained by their inhibiting effect on protein synthesis (MANN et al., 1965; JAWORSKY et al., 1969; DEVLIN et al., 1970; AKOBUNDU et al., 1975; DUKE et al., 1975). However, in our experiments significant changes in protein content occurred only in the presence of 20 mg/l butachlor 12 hours after the treatment. This was preceded by an increase in the enzymatic decomposition of IAA, so the effect of the herbicide is probably not directly correlated with alterations in the protein synthesis.

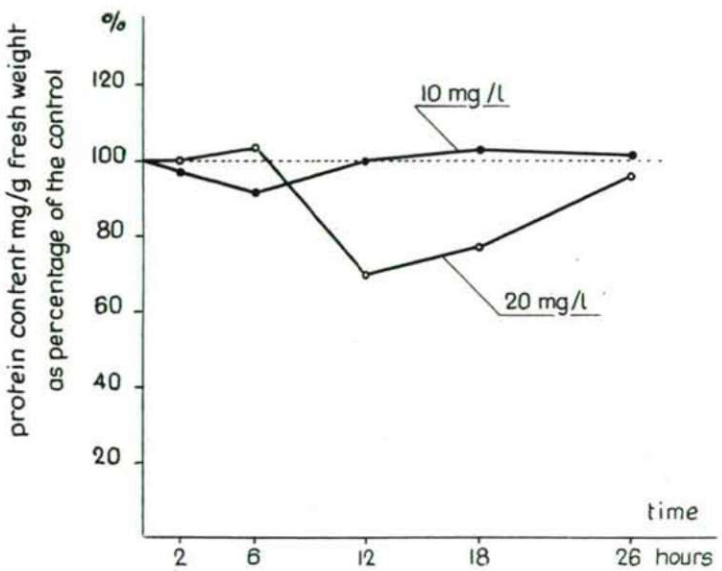

Fig. 2. Effect of butachlor on the protein content of the roots of two week-old maize seedlings
Concentrations applied: (•—•) 10 mg/l
(o—o) 20 mg/l butachlor
in a four-fold diluted Knop solution.
Incubation time: 26 hours.

According to some observations certain compounds promoting the oxidation of IAA at the same time decrease the basipetal auxin transport (STENLID, 1976). This could be proved in maize seedlings treated with butachlor where the basipetal IAA transport was inhibited by the herbicide (TARI et al., 1978) whereas the herbicide pro-

moted the IAA oxidase activity. The inhibiting effect of butachlor on the basipetal transport of IAA from the shoot when combined with the increase in IAA oxidase activity in the root can result in a low auxin level in the roots and a realtively high IAA level at the first internode of the shoot. Such an auxin distribution encourages the increased formation of the adventitious roots at the first internode-region.

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