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# INHIBITION OF ENZYMATIC INDOLEACETIC ACID OXIDATION BY SOIL FULVIC ACIDS

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**Summary**—Fulvic acids from three different soils (cultivated, forest, meadow) inhibited IAA-oxidase activity. This effect diminished in the order: cultivated, forest, meadow fulvic acids. A direct relationship between phenolic groups and carbon contents with enzyme inhibition was found. This relationship was negative for oxygen percentages. The ash and carboxyl contents of fulvic acids were not related to their effects on the enzyme. These results suggest that phenolic groups are involved in the inhibitory action of fulvic acids on IAA–oxidase activity and there is a predominance of catechol or hydroquinone-like configuration in the fulvic molecule.

## INTRODUCTION

BASED on solubility characteristics, fulvic, humic and hymatomelanic acids can be expected to have a biological effect on plant metabolism and development. The former, which are more water-soluble and less complex in structure, can play a key role in physiological effects of humus since their absorption by higher plants has been proved (Führ and Sauerbeck, 1966).

Sladký and Tichý (1959) and Sladký (1959) reported that the lengths and fresh weights of stems and roots of *Begonia semperflorens* were markedly increased when fulvic acid solutions were sprayed on their leaves. Poapst and Schnitzer (1971) found that fulvic acids promote adventitious root formation in hypocotyl segments of *Phaseolus vulgaris*.

The effect of humic acids on plant growth can be explained, in part, by their action on the indoleacetic acid–oxidase system (IAA–oxidase) (Mato and Méndez, 1970; Mato *et al.*, 1971 and 1972). Consequently, this kind of study was extended to fulvic acids to determine any correlation between their functional group content and their effects on enzyme activity.

## MATERIALS AND METHODS

### *Soils and fulvic acids*

Fulvic acids from forest, meadow and cultivated soils from Belgium were studied. The forest soil was an $A_1$ horizon from a brown soil 'lessivé' on loess, under deciduous trees (oak, maple, hazelnut), South Louvain. The meadow and cultivated samples were the 0–10 cm layers of two sandy soils, NE Antwerp.

The soils were extracted overnight with 0·1 N NaOH under nitrogen. The humic acids were precipitated with HCl at pH 1·5 and centrifuged at 24,000 rev/min (R. de Borger, private communication).

The fulvic extracts were passed through a column of Amberlite IR 120 exchange resin in $H^+$ form. The fulvic acid solutions were evaporated to dryness under vacuum.

The elemental analysis and ash contents of fulvic acids are shown in Table 1 (R. de Borger, private communication). The functional groups of the fulvic acids (Table 2) were determined by methods described by Martin (1965).

TABLE 1. ELEMENTAL ANALYSIS AND ASH CONTENT OF SOILS FULVIC ACIDS

| Fulvic acids | Ash (%) | C (%) | H (%) | N (%) | O (%) | C/H |
|---|---|---|---|---|---|---|
| Cultivated | 9·03 | 46·27 | 4·14 | 1·25 | 48·34 | 11·2 |
| Forest | 7·72 | 44·56 | 3·74 | 1·74 | 49·96 | 11·9 |
| Meadow | 18·90 | 40·78 | 4·19 | 2·14 | 52·89 | 9·7 |

*Preparation of the crude extract and enzyme assay*

Preparation of the crude enzyme from lentil (*Lens culinaris* Medic.) seedlings roots (Mato *et al.*, 1971) and conditions for assaying IAA–oxidase activity were described by Mato (1969). Fulvic acids were dissolved in distilled water and the final concentrations were kept in the range 5–40 mg/l. Distilled water was substituted for fulvic acid solutions as the control. The initial and residual IAA were estimated spectrophotometrically at 535 nm by the modified Salkowski reaction (Pilet, 1957).

## RESULTS AND DISCUSSION

The activity of the IAA–oxidase system from lentil roots was substantially inhibited by soil fulvic acids. At the two higher concentrations (20 and 40 mg/l) the effect diminished in the order: cultivated, forest, meadow fulvic acids (Fig. 1). At the lower two concentrations (5 and 10 mg/l), however, fulvic acids of forest and cultivated soils behaved alike whereas those of meadow soils weakly promoted the enzymatic destruction of IAA.

Gorter (1969) explained the synergistic effects between phenols and auxin on root initiation by proposing that reactions inhibiting IAA–oxidase took place. The possibility that synergism between fulvic acids and IAA can be explained by such a hypothesis has already

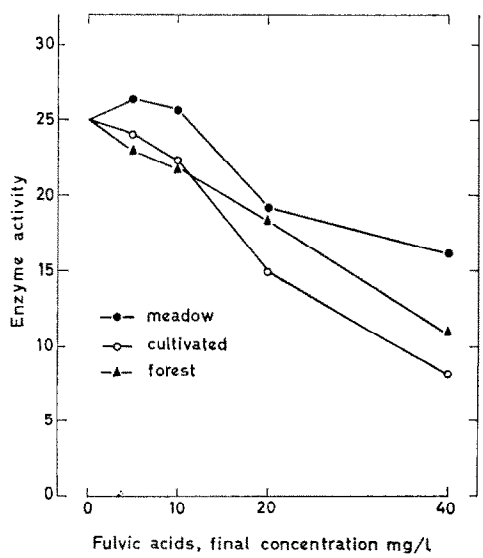


FIG. 1. Effect of soil fulvic acids on IAA oxidation by lentil roots extracts. Activities expressed in terms of $\mu$g IAA destroyed/100 mg fresh wt/60 min incubation. Each point is the mean of five observations.

been advanced (Poapst and Schnitzer, 1971). The present results which show an inhibition of IAA–oxidase by fulvic acids support that possibility, even though different effects of fulvic acids on the other enzyme systems cannot be ruled out.

As far as elemental composition is concerned (Table 1), the IAA–oxidase inhibition is correlated directly with carbon content and inversely with oxygen percentage (Fig. 2).

TABLE 2. FUNCTIONAL GROUPS CONTENT (m-equiv/g ON ASH-FREE BASIS) OF FULVIC ACIDS

| Fulvic acid from | Total acidity | Carboxyl | Phenolic |
|---|---|---|---|
| Cultivated soil | 17·3 | 7·4 | 9·9 |
| Forest soil | 13·1 | 5·0 | 8·1 |
| Meadow soil | 19·4 | 12·3 | 7·1 |

Table 2 shows the functional group composition of the preparations. No relationship between carboxyl content and enzyme inhibition was observed as Butler and Ladd (1971) found for four proteolytic enzymes. The fulvic acids with the lowest phenolic group content (meadow soil) are less effective in preventing enzymatic IAA oxidation. Thus a positive correlation was found between phenolic hydroxyls and IAA–oxidase inhibition (Fig. 2).

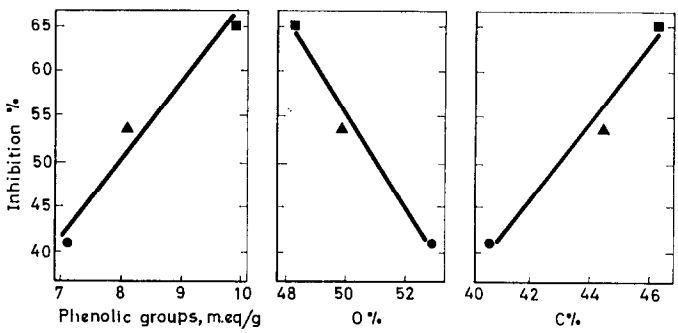


FIG. 2. Correlations between phenolic groups, oxygen and carbon contents of different soil fulvic acids at 40 mg/l and their IAA oxidation inhibition. (●) meadow; (▲) forest; (■) cultivated soils.

Our results argue in favour of a hypothesis that the action of fulvic acids on IAA–oxidase depend upon their phenolic hydroxyl content. This finding is backed by the fact that phenolic substances also modify the auxin-sparing mechanism (Tomaszewski and Thimann, 1966; Pilet and Gaspar, 1968).

In general terms, monophenols enhance the enzymatic destruction of IAA whereas polyphenols inhibit it. However, Gaspar (1966) and Pilet and Mato (1967) state that *ortho-* and *para*-substituted diphenols all inhibit IAA–oxidase, but the activity of this enzyme is increased by *meta*-diphenols.

A 'monophenolic behaviour' has been ascribed to the fulvic molecule (Poapst *et al.*, 1970) on the basis that stem elongation is inhibited by both fulvic acids and monophenols, suggesting that the former may activate IAA–oxidase activity. Since this enzyme system is inhibited by fulvic acids, our results are accounted for by the predominance of catechol- or hydroquinone-like configuration in the fulvic molecule. This apparent disagreement

could be because the concentrations used by us (5–40 mg/l) are much more lower than those of the Canadian authors, who pay special attention to concentrations near toxic levels (1000–4000 mg/l).

In the light of our recent findings with both soil humic (Mato *et al.*, 1972) and fulvic acids, *ortho-* or *para*-diphenolic groups seem to be involved in the inhibitory action of the humic substances on IAA–oxidase activity. Whether the catechol groups in the commercial humic acids (Mato and Méndez, 1970) were complexed in full by boric acid–sodium acetate, a specific chelatogenic reagent for such groups, the *para*-diphenol configuration is more probable than the *ortho*-substitution in the humic molecule.

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