

# DIFFERENTIAL UPTAKE OF 2,4-D ACID AND ITS OCTYL ESTER BY SEEDLING CORN ROOTS AND COLEOPTILE SECTIONS<sup>1, 2</sup>

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Although much research has been directed toward the uptake of inorganic ions by roots, the entry of organic compounds has received little attention. The present study concerns a basic difference in permeability between seedling corn roots and coleoptile sections as indicated by a differential uptake of ionized 2,4-D acid and the apolar octyl ester of 2,4-D.

It is known that the addition of an alcohol through an ester linkage drastically changes the physical properties of the 2,4-D molecule (5). In any study of the effect of molecular changes on toxicity, it is important to determine whether the changes, if any, affect the inherent toxicity or merely affect the ability of the molecule to reach the site of activity. It seemed possible that the transformed dosage-response curve, as applied to spore toxicity by Rich and Horsfall (6), could provide this information.

The inhibition of primary root growth was determined for corn seedlings grown in buffered solutions which contained graded concentrations (on an acid equivalent basis) of either 2,4-D acid or its octyl ester. The experiments were conducted at pH 7, with a 24 hour incubation period. The difference in slopes of the resultant lines established by linear regression was not statistically significant, implying that the inherent toxicity of the 2,4-D molecule is not changed by esterification. However, a large difference was found for the ED<sub>50</sub> (concentration necessary for 50% inhibition of root growth) i.e., 4.5 mg/l for 2,4-D and 9.7 mg/l for the octyl ester. Theoretically the ED<sub>50</sub>'s should reflect the ability of the toxicant to reach the site of activity (6) since the mode of action is unchanged.

## MATERIALS AND METHODS

Uptake of the acid and ester was measured directly. The octyl ester was synthesized by means of the acid chloride from 2,4-D-2-C<sup>14</sup> obtained from Nuclear-Chicago Corp. Both the final 2,4-D solution and the ester emulsion were of the same concentration (500

mg/l acid equivalent) and specific activity (2,200 cpm/ml). The solutions were prepared in 0.01 M sodium phosphate buffer at pH 7 and 0.05% Tween 80. Corn seedlings germinated 72 hours were grown on filter papers in Petri plates containing 5 ml of the solution to be tested. At intervals of 1, 2, and 3 hours seedlings were removed, washed in ethanol, and prepared for counting. The roots were ground in a mortar, transferred to a planchet, dried, and the radioactivity counted directly at infinite thickness. Due to the low specific activity in the remainder of the seedling, the seeds and shoots were ground and extracted with ether. The ether extract was evaporated directly on the planchet. A Baird Atomic automatic sample changer with a detector of the Sugarman type was used with a pre-set count to provide 1% accuracy.

Corn coleoptile sections, prepared similarly to the *Avena* coleoptiles of Johnson and Bonner (4), were floated in 20 ml of the same solutions as used for the root studies. Twenty sections were removed at intervals of 1, 2, and 3 hours, washed in ethanol, dried in the planchet, and counted directly as above.

## RESULTS AND DISCUSSION

The curves for uptake of 2,4-D acid and ester for both intact roots and coleoptile sections (figs 1 and 2), follow those of Johnson and Bonner (4) quite closely. Severe growth inhibition appeared between 3 and 6 hours after treatment. Radioactivity appeared in the seeds and shoots in the same ratio as the roots. From measurements of radioactivity in the plant tissue and inhibition of root growth, it was apparent that the quantity of octyl ester entering the roots was considerably less than that of the acid. This same result was observed with intact cucumber roots and excised corn roots. On the other hand, more radioactivity from the octyl ester appeared in coleoptile sections; increased growth of coleoptile sections also indicated a greater uptake of the octyl ester. Uptake of the ester can be attributed partially to an *in vivo* conversion to the acid. Such a conversion would maintain the concentration gradient of 2,4-D ester in favor of continued uptake. Plant enzymes have been shown to catalyze the de-esterification. The results of this work will be published elsewhere.

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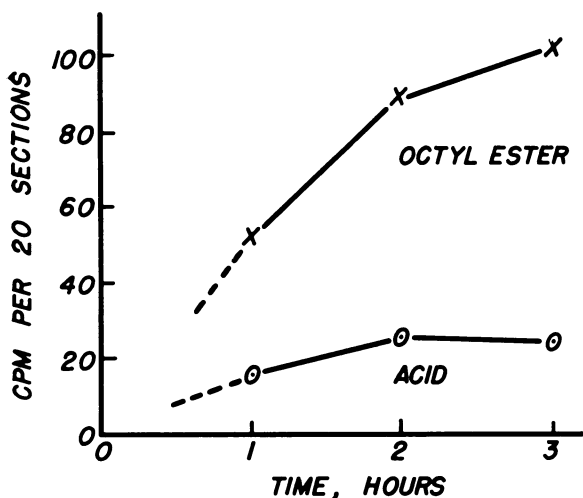


FIG. 1. Uptake of 2,4-D-2-C<sup>14</sup> and 2,4-D-2-C<sup>14</sup> octyl ester by intact corn roots. Data include radioactivity measurements for the entire seedling so that total uptake was measured. Each point represents an average of three replications of ten corn seedlings grown under uniform conditions.

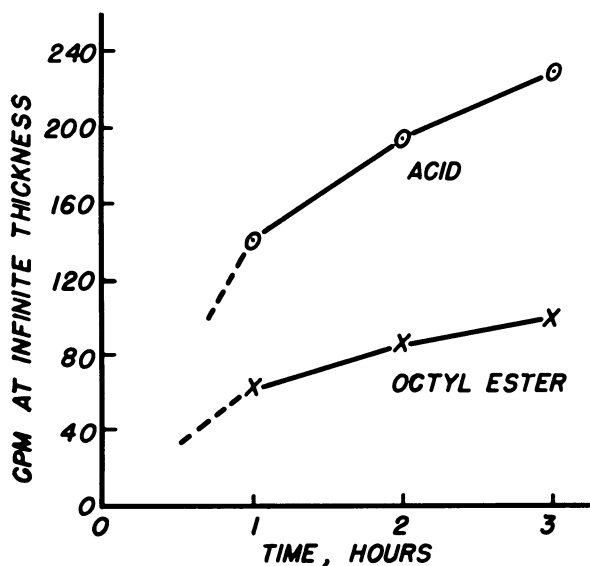


FIG. 2. Uptake of 2,4-D-2-C<sup>14</sup> and 2,4-D-2-C<sup>14</sup> octyl ester by excised corn coleoptile sections. Each point represents an average of duplicate counts from 12 replications of 20 coleoptile sections taken from corn seedlings grown under uniform conditions.

It might be argued that the observed differences in uptake are due to the fact that the ester was supplied as an emulsion. This interpretation is discounted since the slope of the line representing 2,4-D ester inhibition of corn roots is not changed through a range of concentrations which involved both true solutions and finely divided emulsions stabilized by the addition of Tween 80. Spontaneous hydrolysis undoubtedly occurs but apparently has no major effect on the results. It should be recognized that the acid is near complete ionization at pH 7 (7) but the ester is still less effective than the acid in inhibiting root growth at pH 5.6, the lowest pH tested.

When the relative solubilities of 2,4-D acid and ester in water and organic solvents are considered (5), the measurements of uptake by roots are not in agreement with the Overton-Meyer theory but confirm the suggestion of Crafts (3) that roots are more adapted to the uptake of ionized compounds. Conversely, results with coleoptile sections are consonant with the classical view that uptake of the apolar ester should be greater than for the ionized acid (2).

It is interesting to note in this regard that Åberg (1) demonstrated that the activity of the methyl ester of 2,4-D in inhibiting the growth of flax roots was higher than the acid. This suggests that the observations reported here may apply only to esters with high oil-water partition coefficients.

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