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RESPONSE OF DANDELION ROOT TISSUE TO TREATMENT WITH 2,4- AND 3,5-DICHLOROPHENOXYACETIC ACIDS

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Key Word Index—*Taraxacum officinale*; Compositae; dandelion; root; inulin; respiration; hydrolases; auxins; 2,4-dichlorophenoxyacetic acid; 3,5-dichlorophenoxyacetic acid.

Abstract—Treatment of tissue disks prepared from roots of the dandelion (*Taraxacum officinale*, Weber) with 10^{-5} M solutions of the auxin 2,4-dichlorophenoxyacetic acid (2,4-D) induced marked physiological and biochemical changes. There was a large uptake of water, decrease in carbohydrate reserves and an increase in the rate of respiration, the level of total soluble protein and in hydrolase activity. Similar treatment with the isomer 3,5-dichlorophenoxyacetic acid (3,5-D), which is inactive as an auxin, produced little or no response.

INTRODUCTION

PREVIOUS studies[1] have indicated a correlation between physiological response and enzyme activity when tissues from certain inulin-storing roots and tubers were treated with the highly active growth regulator 2,4-dichlorophenoxyacetic acid (2,4-D). More detailed work using tissue from Jerusalem artichoke tubers[2] and chicory roots[3] has related the effect of 2,4-D on the hydrolysis of oligosaccharides to simpler sugars to the activity of some enzymes of carbohydrate metabolism.

Apart from some early reports of the 2,4-D treatment of whole dandelion plants (*Taraxacum officinale*, Weber) resulting in the depletion of carbohydrate reserves[4] and an increase in invertase activity,[5] relatively little work has been carried out on the effect of 2,4-D on Dandelion tissue. Since the enzymes resonsible for the depolymerization of inulin to simple sugars during the cold storage of dandelion roots[6] have recently been described,[7] it seems appropriate at this stage to examine in more detail the effect of 2,4-D on this material. Such a study is of interest since 2,4-D is used in various herbicidal formulations for the control of dandelions.[8]

RESULTS

Figure 1 shows the effect of duration of treatment on the magnitude of water uptake induced in disks cut from dandelion roots when treated with water or 10^{-5} M solutions of the inactive growth regulator 3,5-dichlorophenoxyacetic acid (3,5-D) or 2,4-D. The water and 3,5-D treatments induced only a small uptake of water and this occurred mainly during the first 2 days; 10^{-5} M solutions of 2,4-D however, caused large amounts of water to be taken up after longer periods of treatment.

[1] A. E. FLOOD, P. P. RUTHERFORD and E. W. WESTON, *Nature, Lond.* **214,** 1049 (1967).
[2] P. P. RUTHERFORD, E. W. WESTON and A. E. FLOOD, *Phytochem.* **8,** 1859 (1969).
[3] A. E. FLOOD, P. P. RUTHERFORD and E. W. WESTON, *Phytochem.* **9.** 2431 (1970).
[4] L. W. RASMUSSEN, *Plant Physiol.* **22,** 377 (1947).
[5] E. HOFMANN and B. V. SCHMELING, *Naturwissenschaften* **40,** 23 (1953).
[6] P. P. RUTHERFORD and E. W. WESTON, *Phytochem.* **7,** 175 (1968).
[7] P. P. RUTHERFORD and A. C. DEACON, *Biochem. J.* **126,** 569 (1972).
[8] C. LONNBERG, *Naatalous Koetdiminta* **18,** 173 (1964).

The storage carbohydrates in the dandelion root form a continuous series of β-D-fructofuranosides[9] with a degree of polymerization (D.P.) from 1 (hexose) to about 35 (inulin). Continuous extraction for 6 hr with 80% boiling ethanol[6] gave a soluble fraction containing fructosans with D.P. up to 8; the insoluble fraction containing higher D.P. fructosans.

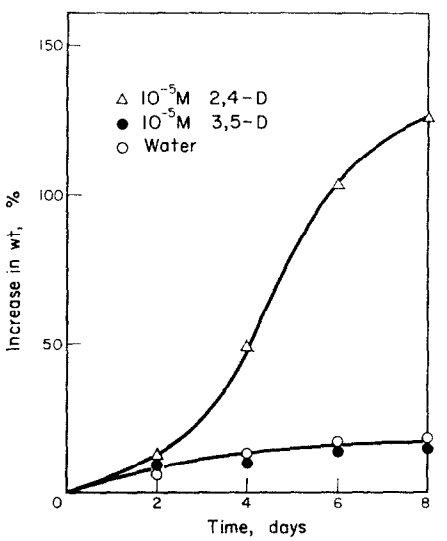

FIG. 1. THE WATER UPTAKE INDUCED IN DISKS OF DANDELION ROOT BY TREATMENT AT 25° WITH WATER OR 10^{-5} M SOLUTIONS OF EITHER 2,4-D OR 3,5-D FOR VARYING PERIODS OF TIME.

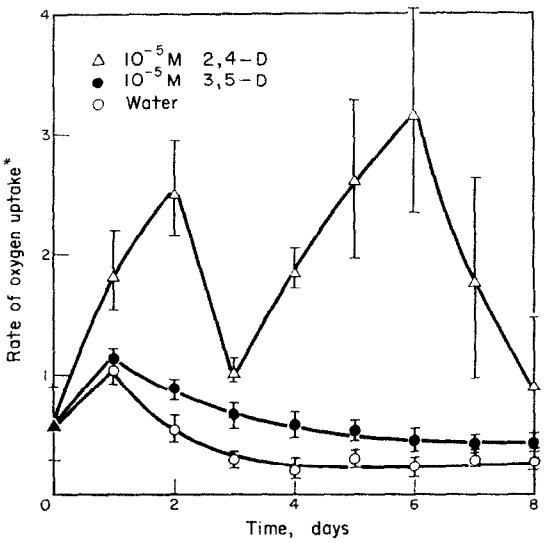

FIG. 3. CHANGES IN THE RATE OF OXYGEN UPTAKE BY DANDELION ROOT DISKS TREATED AT 25° WITH WATER OR 10^{-5} M SOLUTIONS OF EITHER 2,4-D OR 3,5-D FOR VARYING PERIODS OF TIME.
* ml of oxygen taken up/hr/g initial dry matter. Mean values shown with 95% confidence limits (5 observations).

Figure 2 shows the change in both total and insoluble carbohydrate content of disks after various times of treatment with 10^{-5} M 2,4-D, water and 10^{-5} M 3,5-D. After a lag period of 2 days, 2,4-D treatment resulted in a rapid decrease in total carbohydrate; this decrease did not occur with either water or 3,5-D treatment. The values for duplicate determinations of total carbohydrate (expressed as a % of initial dry matter) were initially 76·9 and 85·1 respectively; after 8 days of treatment with water were 66·3 and 63·5; with 10^{-5} M 3,5-D 64·6 and 61·7; with 10^{-5} M 2,4-D 14·4 and 21·4. Although there was clearly a significant difference between 2,4-D and the other treatments, the scatter was too great to demonstrate any difference between water and 3,5-D treatment. The soluble carbohydrate content of the disks (initially 10% of the dry wt) did not change significantly over the 8 day period with any of the treatments used. The free reducing sugars present (initially 0·6% of the dry wt) increased simultaneously with all three treatments to reach after 8 days approx. 4·3%. However, only 2,4-D treatment produced a rapid decrease in insoluble carbohydrate (Fig. 2) and this accounts for the observed change in total carbohydrate content.

[9] J. S. D. BACON and J. EDELMAN, *Biochem. J.* **48,** 114 (1951).

The rate of oxygen uptake by disks was measured daily over a period of 8 days to discover if increased respiration could account for the observed decrease in carbohydrate content (Fig. 3). An initial short burst in respiration was observed with water and 3,5-D treatment during the first day followed by a gradual decrease. This initial short burst in respiratory activity was attributed to wound metabolism as a direct result of preparing the disks. A similar initial increase but larger occurred upon 2,4-D treatment and fell off after 2 days; this was followed by an even greater second burst of respiratory activity.

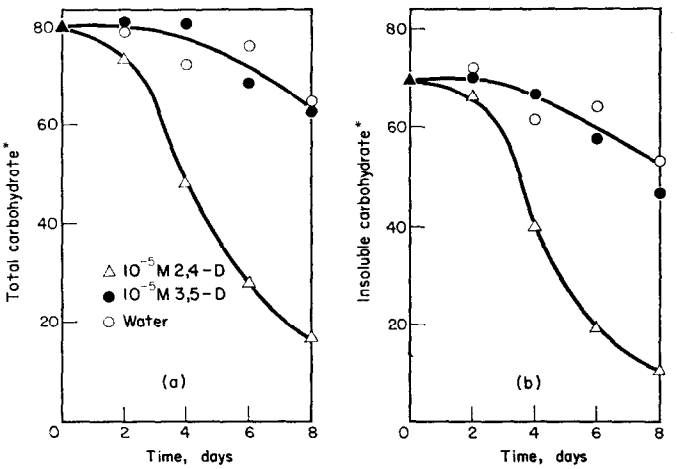

FIG. 2. CHANGES IN TOTAL AND INSOLUBLE CARBOHYDRATES AS % INITIAL DRY WT OF DANDELION ROOT DISKS AFTER TREATMENT AT 25° WITH WATER OR 10^{-5} M SOLUTIONS OF EITHER 2,4-D OR 3,5-D FOR VARYING PERIODS OF TIME.
* Results expressed as % initial dry matter. Symbols in Fig. 2b same as in 2a.

To verify that this increase in respiration was sufficient to account for the observed depletion of carbohydrate reserves, the amount of carbohydrate which would have to be respired away to produce the total oxygen uptake which occurs over an 8 day period was

TABLE 1. THE UTILIZATION OF CARBOHYDRATE RESERVES BY DANDELION ROOT DISKS DURING 8 DAYS OF TREATMENT AT 25° WITH WATER OR 2,4-D

Carbohydrate fraction	Carbohydrate expressed as % of initial dry matter	
	Water	10^{-5} M 2,4-D
Untreated	81·0	81·0
After treatment:		
(i) remaining in disks	72·8	23·8
(ii) used for respiration	10·7	50·8
(iii) leaked into external medium	0	2·9
Total accounted for	83·5	77·5

calculated. The resulting balance sheet (Table 1) shows that increased respiration satisfactorily accounts for the exhaustion of carbohydrate reserves; negligible leakage of sugars occurred into the external medium.

Since 2,4-D treatment resulted in the depletion of insoluble carbohydrates, the activities of the hydrolytic enzymes responsible for the depolymerization of fructosans[7] were examined. Figure 4 shows a very large increase in the hydrolase activity of soluble protein extracts prepared from disks treated with 2,4-D, coinciding with the decrease in insoluble carbohydrate reserves and increase in respiration; water and 3,5-D treatment, however, produced only a slight increase in hydrolase activity. The values for duplicate estimations of hydrolase activity (expressed as μmol $\times$ 10^2 of hexose liberated/min/g initial dry matter) were initially 6·6 and 9·4 respectively; after 8 days of treatment with water were 9·4 and 15·1; with 10^{-5} M 3,5-D 18·2 and 23·6; and with 10^{-5} M 2,4-D 109 and 80. Therefore 2,4-D produces a massive increase in hydrolase activity compared with any of the other treatments. The scatter of the results is insufficient to account for the small differences in activity observed between the water control and 3,5-D treatment.

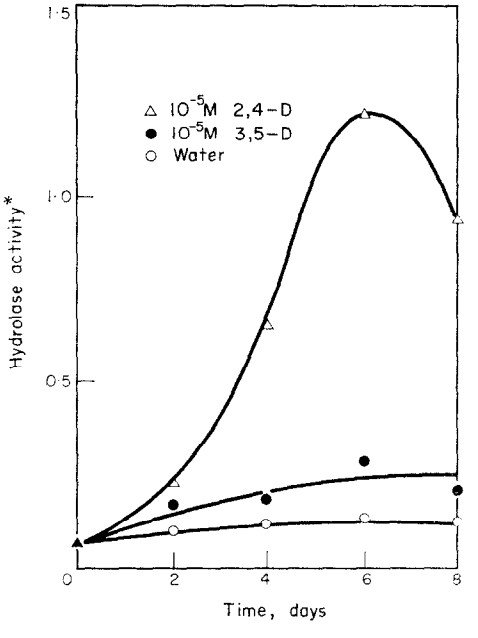

FIG. 4. CHANGES IN HYDROLASE ACTIVITY OF SOLUBLE PROTEIN EXTRACTS PREPARED FROM DANDELION ROOT DISKS TREATED AT 25° WITH WATER OR 10^{-5} M SOLUTIONS OF EITHER 2,4- D OR 3,5-D FOR VARYING PERIODS OF TIME.

* One unit of hydrolase activity represents the liberation at 25° and pH 4·5 of 1 μmol of hexose/min/g initial dry matter.

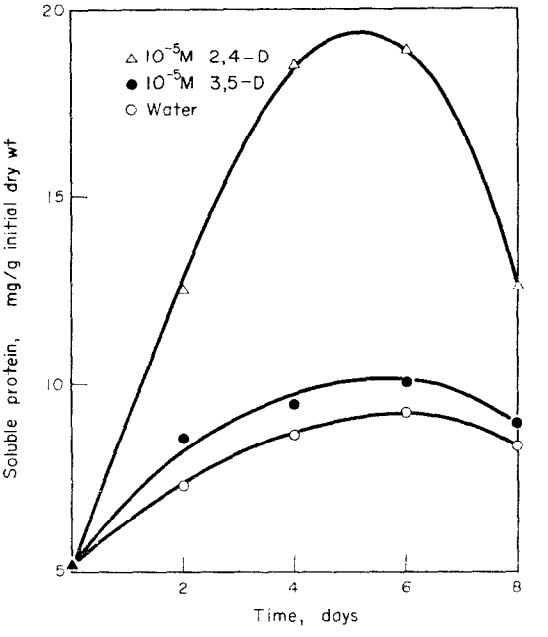

FIG. 5. CHANGES IN SOLUBLE PROTEIN OF EXTRACTS PREPARED FROM DANDELION ROOT DISKS TREATED AT 25° WITH WATER OR 10^{-5} M SOLUTIONS OF EITHER 2,4-D OR 3,5-D FOR VARYING PERIODS OF TIME.

We have previously shown[7] that dandelion roots contain two hydrolases (A and B) which are separable by chromatography on DEAE-cellulose. Table 2 shows that the activity of both hydrolases A and B increase simultaneously upon 2,4-D treatment, contrasting to the finding with Jerusalem artichoke[2] that nearly all of the increase was associated with hydrolase B.

TABLE 2. FRACTIONATION ON DEAE-CELLULOSE[7] OF SOLUBLE PROTEIN PREPARATIONS OBTAINED FROM DANDELION ROOT DISKS TREATED AT 25° WITH 2,4-D FOR VARYING PERIODS OF TIME

Duration of treatment (days)	Activity in hydrolase peaks (units* × 10^2)			
	Hydrolase A	Hydrolase B	Total A and B	Unfractionated
0	3·5	0·5	4·0	6·6
2	12·5	1·6	14·1	23·5
4	60·8	4·7	65·5	67·6
6	61·6	6·8	68·4	97·7
8	79·6	12·0	91·6	109·0

* For units, see legend of Fig. 4.

Figure 5 shows that 2,4-D treatment resulted in an increase in the soluble protein extracted from the disks; water and 3,5-D produced a smaller change. The magnitude of this increase was much less than that in hydrolase activity, i.e. there was an increase in hydrolase specific activity. Addition of 2,4-D at a final concentration of 10^{-5} M to extracts of soluble protein prepared from untreated and 2,4-D treated disks, failed to produce an increase in hydrolase activity.

DISCUSSION

Treatment of dandelion root tissue disks with either 10^{-5} M solutions of the inactive growth regulator 3,5-D or with water has comparatively little effect on either the water uptake or the carbohydrates present in the tissue. However, treatment of the disks with 10^{-5} M solutions of the highly active growth regulator 2,4-D not only induces a large uptake of water, but leads to a considerable decrease in carbohydrate reserves. This effect, together with the others described above, is therefore an auxin induced effect. 3,5-D was found to produce some of the effects of 2,4-D treatment, but much smaller in magnitude; this may be due to slight contamination of 3,5-D by some active constituent which is not detected in the standard growth tests[10] or to 3,5-D itself having some slight activity.

Since addition of 2,4-D to extracts of soluble protein did not produce the increase in hydrolase specific activity observed with the intact tissue, it seems unlikely that 2,4-D has any direct effect upon the enzyme. It would, however, be premature to conclude that the increase in hydrolase activity observed here is due to enzyme induction.[11]

The consistency of our results suggests that 2,4-D increases the hydrolase activity in the tissue, resulting in the depolymerization of the storage carbohydrate inulin; the free sugars released do not accumulate but are immediately utilized by the tissue. The net result is an increase in the rate of respiration and the depletion of carbohydrate reserves. The latter effect may well be related to the herbicidal action of 2,4-D on the dandelion.

EXPERIMENTAL

Biological material and treatment. Dandelion plants were clonally propagated (by a method to be published) in April 1971, lifted in November 1971 and used within 24 hr. The roots were washed gently and thoroughly in cold running H_2O and peeled with a flamed stainless steel knife. Cylinders of tissue, *ca.* 12 mm dia. were cut parallel to the central axis with a flamed scalpel and weighed. The disks, 3·0–6·0 mm

[10] R. L. WAIN and F. WIGHTMAN, *Ann. Appl. Biol.* **40**, 244 (1953).
[11] P. FILNER, J. L. WRAY and J. E. VARNER, *Science* **165**, 358 (1969).

in thickness (0·2 and 0·6 g), contained both steel and cortex since dandelion roots are too small to prepare disks from a single tissue as is possible with other inulin-storing plants.[12] Disks were cut from 6 roots of the same age and clone, and selected at random from different parts of each root system for each treatment. Test solutions in boiled H_2O were in 8·5 × 1·5 cm Petri dishes with a flat-bottomed 5·0 cm watch-glass in each to support a double layer of 7·0 cm filter paper at the surface of the solution. Each treatment was carried out at 25 ± 1° and consisted of 3 dishes each containing 9 disks. After the required time, the disks were removed, dried between soft filter paper and weighed. 2 disks were used for carbohydrate determinations; the remainder were bulked and used for the preparation of protein extracts. All experiments were performed in duplicate and the results given in the figures are the mean values.

Extraction and determination of 'sugars'. Extraction and hydrolysis of 'sugars' was carried out by the method described by Rutherford and Weston.[6] Reducing sugar in extracts and hydrolysates was determined by the cuprimetric method of Nelson[13] as modified by Asatoor and King.[14]

Protein extraction, fractionation, determination and assay of hydrolase activity. Soluble protein was extracted and fractionated on DEAE-cellulose columns by methods already described.[7] The hydrolase activity of unfractionated and fractionated protein was measured[7] and the amount of protein determined[15] with bovine serum albumin as standard.

Measurement of oxygen uptake. Oxygen uptake was determined manometrically at 25° by Warburg's direct method. Each flask contained 1 disk (prepared as described above) in 2 ml of medium (H_2O or 10^{-5} M solutions of either 3,5-D or 2,4-D) in air. The daily rate of O_2 uptake was determined by taking measurements over a 3-hr period. 5 replicates were set up for each treatment.

Acknowledgement—A.C.D. thanks the Science Research Council for a Research Studentship.

[12] P. P. RUTHERFORD, C. M. GRIFFITHS and R. L. WAIN, *Ann. Appl. Biol.* **58,** 467 (1966).
[13] N. NELSON, *J. Biol. Chem.* **153,** 375 (1944).
[14] A. ASATOOR and E. J. KING, *Biochem. J.* **56,** xliv (1954).
[15] O. H. LOWRY, N. J. ROSEBROUGH, A. L. FARR and R. J. RANDALL, *J. Biol. Chem.* **193,** 265 (1951).