

EFFECT OF 2,4-DICHLOROPHENOXYACETIC ACID ON THE IN-
VERTASE, PHOSPHORYLASE AND PECTIN METHOXYLASE
ACTIVITY IN THE STEMS AND LEAVES OF THE RED
KIDNEY BEAN PLANTS¹

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Introduction

The formation of proliferated stem tissue in the red kidney bean plant induced by 2,4-dichlorophenoxyacetic acid has aroused considerable interest. SELL, LUECKE, HAMNER and TAYLOR (5, 8) have reported large differences in protein, carbohydrates, lipides, amino acids, vitamins and ash content in the normal and the tumorous stem tissue of the red kidney bean plant produced by 2,4-D.² Further studies by WELLER, LUECKE, HAMNER and SELL (9) have shown that these abnormal differences in chemical composition occurred primarily in the proliferated stem tissues. Recent observations by FELBER (1), and NEELY, BALL, HAMNER and SELL (7) have disclosed enhanced activity for peroxidase and alpha and beta amylase, respectively, in the tumorous stem tissues. The purpose of the present communication is to report the activity of other enzymes in analogous material.

Method and results

Seeds of a certified strain of the red kidney bean plants were selected for uniformity of size and planted in 4-inch pots in the greenhouse in July 1949. Each pot contained two plants that were treated when the first trifoliolate leaves were expanding, approximately 10 days after planting. Four replications of 100 plants each were used from which to obtain material of treated and non-treated plants (controls). Application of 2,4-D was made by applying one drop (.05 ml.) of a 1000 p.p.m. solution to the base of the blade of one of the primary leaves. The plants were harvested six days after treatment at which time the stem tissue had proliferated considerably but yet showed no signs of necrosis. The material was air dried at 30° C in the dark and then segregated into leaf, root and stem tissue. The hypocotyl, first internode and leaf petioles were grouped together as stem tissue. After drying, the material was ground to pass through a 60 mesh sieve, and then stored in a glass jar at room temperature and used as needed.

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² A 0.1% solution of the sodium salt of 2,4-dichlorophenoxyacetic acid acidified with orthophosphoric acid to pH 3 and abbreviated in this paper as "2,4-D."

Invertase was determined by using a modification of WILLSTATTER'S (10) method. A variety of solvents such as salt solutions and water at various temperatures and extraction times were employed for extraction. However, none of the leaf or proliferated stem tissue showed any invertase activity. This would indicate that the hydrolysis of sucrose to hexose sugars by invertase is absent in the tissues of the young plants.

The procedure of KERTESZ (4) was employed for the determination of pectin methoxylase. The results in table I show that the activity of pectin methoxylase is approximately twice as great in the normal stem tissue as in the tumorous tissue of the red kidney bean plant. The same trend was also noted in the leaves of the treated and non-treated plants. The activity of the other pectic enzymes was not determined but if the ultimate breakdown of protopectin to methanol and galacturonic acid is a series of equilibrium reactions then the increase in pectin methoxylase

TABLE I

EFFECT OF 2,4-DICHLOROPHENOXYACETIC ON THE PECTIN METHOXYLASE* ACTIVITY IN THE STEMS AND LEAVES OF THE RED KIDNEY BEAN PLANTS

| ENZYME | SAMPLE | STEMS | | LEAVES | |
|-------------|--------|---------|-------------|---------|-------------|
| | | TREATED | NON-TREATED | TREATED | NON-TREATED |
| Pectin | 1 | 6.20 | 15.81 | 3.10 | 7.90 |
| Methoxylase | 2 | 7.44 | 16.10 | 2.79 | 8.05 |
| | 3 | 8.96 | 16.27 | 3.10 | 8.20 |
| | 4 | 7.90 | 16.11 | 2.94 | 8.05 |

* Pectin methoxylase unit is defined as the number of milligrams of methoxyl split off by one gram of material in 30 minutes at 30° C.

activity would likely be associated with an increase in the breakdown of the protopectin in the cell wall. This change has been observed by MURRAY and WHITING (6) in the histological studies of the tissue of young plants treated with 2,4-D.

Phosphorylase activity was measured by a method given by GREEN and STUMPF (3). The data in table II show that the phosphorylase activity in the tumorous stem and leaf tissue of the treated plants is much less than in the normal tissue of the control plants. Although a phosphatase acting on D-glucose-1-phosphate has not been reported to occur in the tissue of the bean plants, the possibility existed that the liberation of the phosphate might be due to such a phosphatase. Therefore, a determination was made for reducing sugar by the FOLIN and WU method (2) before and after enzymatic hydrolysis to ascertain whether there was an increase in reducing sugars. The amount of reducing sugar remained constant and showed that the liberation of phosphate was due to a phosphorylase enzyme converting the D-glucose-1-phosphate to a corresponding glucose polymer. The decrease in phosphorylase activity confirms the work of previous workers (8) that treatment of plants with 2,4-D dis-

torts the carbohydrate metabolism in the cells. Since both the degradation and synthesis of starch have been observed to be reactions catalyzed by a phosphorylase and since the products of glycolysis of starch may enter the carboxylic acid cycle and would, therefore, be closely related to the synthesis of such important amino acids as aspartic and glutamic acids, it is believed that the effect of 2,4-D on phosphorylase activity may be an important point in the explanation of the basis of the distorted metabolism observed.

Summary

1. Invertase activity was absent in both the proliferated stem and leaf tissue of the red kidney bean plants treated with 2,4-dichlorophenoxyacetic acid and the controls.

TABLE II

EFFECT OF 2,4-DICHLOROPHENOXYACETIC ON THE PHOSPHORYLASE* ACTIVITY OF THE STEMS AND LEAVES OF THE RED KIDNEY BEAN PLANTS

| ENZYME | SAMPLE | STEMS | | LEAVES | |
|---------------|--------|---------|-------------|---------|-------------|
| | | TREATED | NON-TREATED | TREATED | NON-TREATED |
| Phosphorylase | 1 | 5.35 | 9.32 | 0.43 | 5.00 |
| | 2 | 5.91 | 8.76 | .73 | 3.98 |
| | 3 | 5.68 | 8.98 | .77 | 2.78 |
| | 4 | | | .51 | 3.16 |

* Units are expressed as phosphorylase units where one unit has been defined as the amount of enzyme which catalyzes the liberation of 0.1 mg. of inorganic phosphorus from D-glucose-1-phosphate in three minutes at 38° at pH 6.

2. Pectin methoxylase activity increased in both the proliferated stem and leaf tissue of the treated plants.

3. Phosphorylase activity decreased in both the proliferated stem and leaf tissue of the 2,4-D treated plants.

4. There was no indication of the presence of a phosphatase acting on D-glucose-1-phosphate.

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