# SECTION A, BIOLOGICAL SCIENCES

## Subsection Botany

## Effect of 2,4-D on Protein, Pectin and Sulfydryl Content of Excised Epicotyl Sections of Pea[1]

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One of the earliest reports on the action of auxin on the cytoplasm was that of Sweeny and Thimann (1938) showing that the rate of cytoplasmic streaming was affected by auxins. Northen (1942) reported that auxins caused dissociation of structural proteins resulting in viscosity changes in the protoplasm. Lundgren and Williams (1939) reported large changes in dissociation values of proteins with the onset of growth in dormant tissues.

Galston and Kaur (1959) reported 2,4-D and IAA to effect a decrease in the heat coagulability of pea stem protein. Galston, et al. (1963) indicated that this change might be due to the synthesis of large amounts of cold-water soluble pectins in the cytoplasm.

Marre and Arrigoni (1957) reported an increase in reduced glutathione (GSH) in pea internode extracts due to 2,4-D and IAA treatments. Key & Wold (1961); Spragg, Lievesley & Wilson (1962); Spragg and Yemm (1959) have indicated that the ratio of sulfhydryl to disulfide compounds in the cell is important to certain aspects of growth. Northen (1942) and others have suggested that the disulfide bonds of the protein chains may be sites of dissociation, which in turn may cause the observed changes in viscosity.

Changes in structural viscosity of cytoplasm due to dissociation of proteins, changes in the heat coagulability of the protein and changes in the oxidation-reduction state of the cell contents may not be the direct result of the auxin applied, but all of these may need to be examined in order to explain some of the observed results of auxin application. It is at least notable that all of these changes can be traced to the changes in the protein of the cell contents, either directly or indirectly. They do not, however, explain how the actual growth takes place, but merely serve to indicate that changes are being initiated in the cytoplasm, where most certainly all growth processes must be initiated and controlled.

The following experiments were conducted to ascertain if any relationship existed between growth stimulation (due to auxin) and changes in the protein content, its coagulation properties, or the nature of the sulfhydryl content of the cytoplasm of pea stem sections. Determinations of the amount of pectin in the cytoplasmic extracts was also accomplished to see if it was in any way related to the auxin growth response.

MATERIALS AND METHODS:

One cm sections of the third internode of dark-grown pea stems were removed from seven-day-old seedlings and used in these experiments. The sections were cultured in a 2% sucrose solution containing 0.02M phosphate buffer at pH 6.1. The various concentrations of 2,4-D were added to this culture solution.

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After a growth period of 18-20 hours at 30C in the dark, the fresh weight of a treatment sample consisting of 80 sections was determined.

Protein was extracted by grinding the sections in a mortar and pestle in the cold room (4C) with 0.001 M EDTA buffered to pH of 6.1 with 0.02M phosphate buffer. After filtering through several layers of cheese cloth, the solution was centrifuged in the cold room at 16,500 X G for 30 minutes. This particle-free supernatent was then used for further analysis.

Protein in the extract was determined by the methods of Lowery, et al. (1957) using the Folin-Phenol reagent. Since the Folin-Phenol reagent was used on the entire soluble extract, the data for protein reflect concentrations of free and bound protein reacting substances. Determinations were made both before and after boiling the solution for 10-minute periods to remove the heat coagulable protein.

Pectin in the particle free supernatent was determined by adapting the methods of McComb and McCready (1952) to these experiments.

Sulfhydryl content of the total extract was determined by the titration methods of Klotz and Carver (1961). This particular method determines the total amount of sulfhydryl groups in a given solution.

RESULTS AND DISCUSSION:

Table I shows the results of three separate experiments designed to determine the effect of various concentrations of 2,4-D on growth of cultured sections. Results are expressed as per cent gain over the original fresh weight of the 80 sections used for each treatment.

It can be seen that a normal auxin response (growth) is obtained, with the maximum increase in fresh weight occurring over the $10^{-6}$M ——— $5 \times 10^{-4}$M 2,4-D concentration range.

TABLE I Growth response of the sections to auxin treatment. Results are expressed as per cent gain over the original fresh weight of 80 sections.

| 2,4-D Concentration | % GROWTH OF 80 SECTIONS Exp. #1 | Exp. #2 | Exp. #3 | - - AVE. |
|---|---|---|---|---|
| NONE | 18.5% | 17.7% | 17.5% | 17.9% |
| $10^{-7}$M | 33.2 | 25.7 | 21.8 | 26.8 |
| $5 \times 10^{-7}$M | 46.5 | 51.7 | 44.9 | 47.7 |
| $10^{-6}$M | 70.8 | 67.7 | 59.4 | 65.9 |
| $5 \times 10^{-6}$M | 93.6 | 96.1 | 93.7 | 94.4 |
| $10^{-5}$M | 83.5 | 113.1 | 96.6 | 96.4 |
| $5 \times 10^{-5}$M | 44.4 | 109.6 | 99.2 | 84.2 |
| $10^{-4}$M | 30.9 | 106.2 | 89.2 | 75.6 |

Table II shows the protein-extract determinations. The results are given in optical density units as determined on a Klett colorimeter equipped with a # 54 filter.

Fig. 1 shows the average protein content and growth rates plotted against the concentration of 2,4-D. The data show that 2,4-D at the lower concentrations tend to increase the amount of total protein extracted. This is true even though the increase in fresh weight due to the low concentration of auxin was not great. In fact at the concentrations which cause the maximum increase in fresh weight there is a lesser effect on the protein content.

TABLE II Determinations of total protein in the extracts. Results are given in optical density units, as measured on the Klett colorimeter.

| 2,4-D Concentration | OPTICAL DENSITY Exp. #1 | Exp. #2 | Exp. #3 | AVE. |
|---|---|---|---|---|
| NONE | 1.04 | .830 | .840 | .904 |
| $10^{-7}$M | 1.19 | .914 | .910 | .992 |
| $5 \times 10^{-7}$M | 1.17 | .926 | .930 | 1.01 |
| $10^{-6}$M | 1.12 | .874 | .960 | .984 |
| $5 \times 10^{-6}$M | 1.16 | .890 | .934 | .994 |
| $10^{-5}$M | 1.10 | .850 | .912 | .954 |
| $5 \times 10^{-5}$M | .970 | .938 | .900 | .936 |
| $10^{-4}$M | .894 | .900 | .810 | .868 |

These data seem to support the work of Rebstock, et al. (1952), Sell, et al. (1949) and West, et al. (1960) who report that 2,4-D applied to whole seedlings by spraying acts to increase the amount of protein and nucleic acids in the tissues.

In order to ascertain if the protein differed in its heat coagulation properties, as Galston (1959) reported aliquots of the extracts were boiled for 10 minutes and then centrifuged to remove any heat coagulable protein. Table III lists the optical density values obtained on the Klett colorimeter with a # 54 filter with the supernatent solutions.

TABLE III Protein determinations on supernatent solutions after removal of heat coagulable protein by heating. Results are given in optical density units, as measured on the Klett Colorimeter.

| 2,4-D Concentration | OPTICAL DENSITY Exp. #1 | Exp. #2 | Exp. #3 | AVE. |
|---|---|---|---|---|
| NONE | .748 | .598 | .544 | .630 |
| $10^{-7}$M | .914 | .628 | .618 | .720 |
| $5 \times 10^{-7}$M | .880 | .650 | .610 | .714 |
| $10^{-6}$M | .930 | .646 | .632 | .736 |
| $5 \times 10^{-6}$M | .900 | .638 | .754 | .764 |
| $10^{-5}$M | .850 | .670 | .624 | .714 |
| $5 \times 10^{-5}$M | .674 | .780 | .600 | .652 |
| $10^{-4}$M | .608 | .608 | .620 | .612 |

The solid line of Fig. 2 represents the non-heat-coagulable protein. The dashed line represents growth at various concentrations of 2,4-D. The non-heat-coagulable protein is considerably less than the total protein (Fig. 1), however, the trend at various 2,4-D concentrations is very similar for the two protein fractions. There appears to be a maximum optical density value (indicating high amount of protein left in solution) associated with those sections treated with $5 \times 10^{-6}$M 2,4-D. This peak corresponds to the maximum growth obtained at a concentration of $5 \times 10^{-6}$M 2,4-D. These results indicate that there is a change in the heat coagulation due to auxin treatment, as reported by Galston (1959). It is, however, at a slightly lower concentration and is considerably less extensive that he reported.

The amount of soluble pectin (the cold water soluble pectin of Galston, 1959) in the extracts is tabulated in Table IV and is shown graphically in Fig. 3. Values are expressed in optical density units, as measured on the Klett colorimeter with the # 54 filter. Analyses were made before and after coagulation of protein in these samples, but it was found that the boiling period did not significantly change the amount of pectin in the solutions.

TABLE IV Pectin determinations on the supernatent extracts. Results are given in optical density units, as measured on the Klett colorimeter.

| 2,4-D Concentration | OPTICAL DENSITY Exp. #1 | Exp. #2 | Exp. #3 | AVE. |
|---|---|---|---|---|
| NONE | .426 | .336 | .332 | .360 |
| $10^{-7}$M | .490 | .376 | .364 | .390 |
| $5 \times 10^{-7}$M | .584 | .368 | .400 | .414 |
| $10^{-6}$M | .490 | .400 | .406 | .432 |
| $5 \times 10^{-6}$M | .502 | .400 | .416 | .434 |
| $10^{-5}$M | .448 | .384 | .416 | .408 |
| $5 \times 10^{-5}$M | .398 | .376 | .356 | .374 |
| $10^{-4}$M | .322 | .348 | .336 | .328 |

The solid line of Fig. 3 shows that 2,4-D at the lower concentrations acts to increase pectin content of the treated sections. This curve seems to parallel that of the protein curve for the same concentration range of 2,4-D in that respect. It does not increase pectin content at the same concentrations as those which are growth-stimulatory.

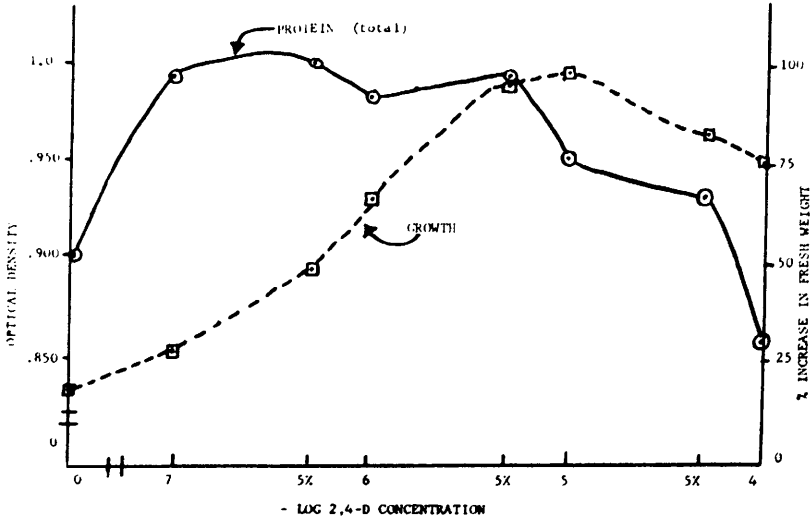


FIGURE 1. The effect of various concentrations of 2,4-D on the amount of total protein and the fresh weight of epicotyl sections of pea. (Average of 3 experiments)

Current investigations are now centered around the effects of various concentrations of 2,4-D on the sulfhydryl content of the protein extracts. A search of the literature revealed that growth stimulating concentrations of auxins, such as 2,4-D and IAA acted to increase the amount of the reduced form of glutathione (Marre and Arrigoni 1957). A $10^{-6}$M concentration of 2,4-D was chosen for the experimental groups, on the basis of their work, and compared with sections grown in the absence of it. To date, little or no variation has been measured in the sulfhydryl content of the treated sections, even though the increase in fresh weight is great.

## SUMMARY AND CONCLUSIONS:

The changes in the pectin and protein content of excised pea stem sections due to 2,4-D treatments have been determined and found to vary with the concentration applied. Pectin and protein contents increased when the lower concentrations of 2,4-D were applied, and decreased with the higher concentrations of 2,4-D. These maximum increases did not occur at the same concentrations which caused the maximum increases in fresh weights.

Sulfhydryl determinations have been made to determine the effect of 2,4-D on the oxidation-reduction state of the protein in these extracts. No significant variation with 2,4-D treatment could be detected in the amount of sulfhydryl in the extracts.

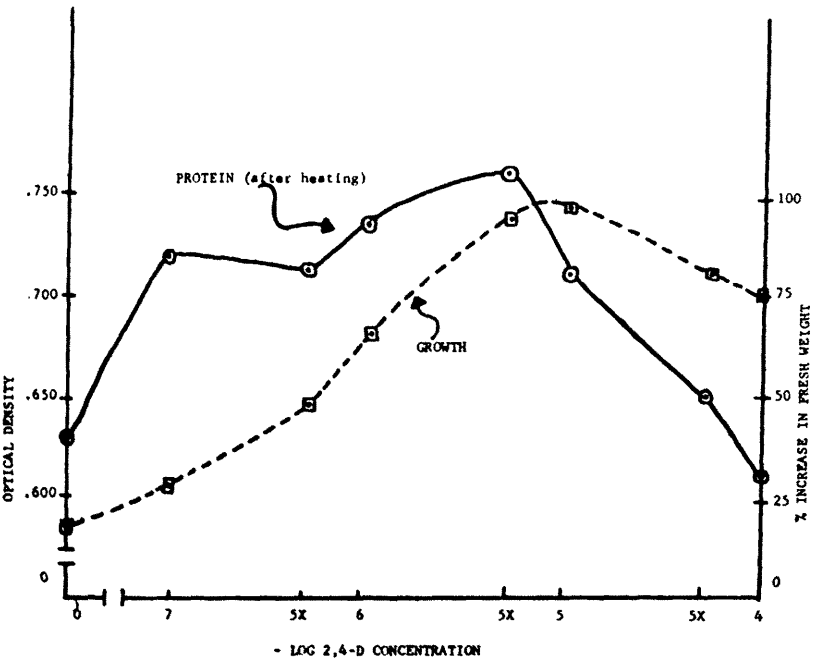


FIGURE 2. The effect of various concentrations of 2,4-D on the amount of non-heat coagulable protein and fresh weight of epicotyl sections of pea. (Average of 3 experiments)

Apparently the auxin growth effect and the increases in pectin and protein content occur at different concentrations. The increases in pectin and protein could be due to either increased degradation of particulate cellular components, or to activation of synthetic processes in the cell.

The experiments reported in this paper might serve to explain the changes in viscosity of the protoplasm as observed by Northen (1942) and others. The action of 2,4-D then might be to stimulate the synthesis of a

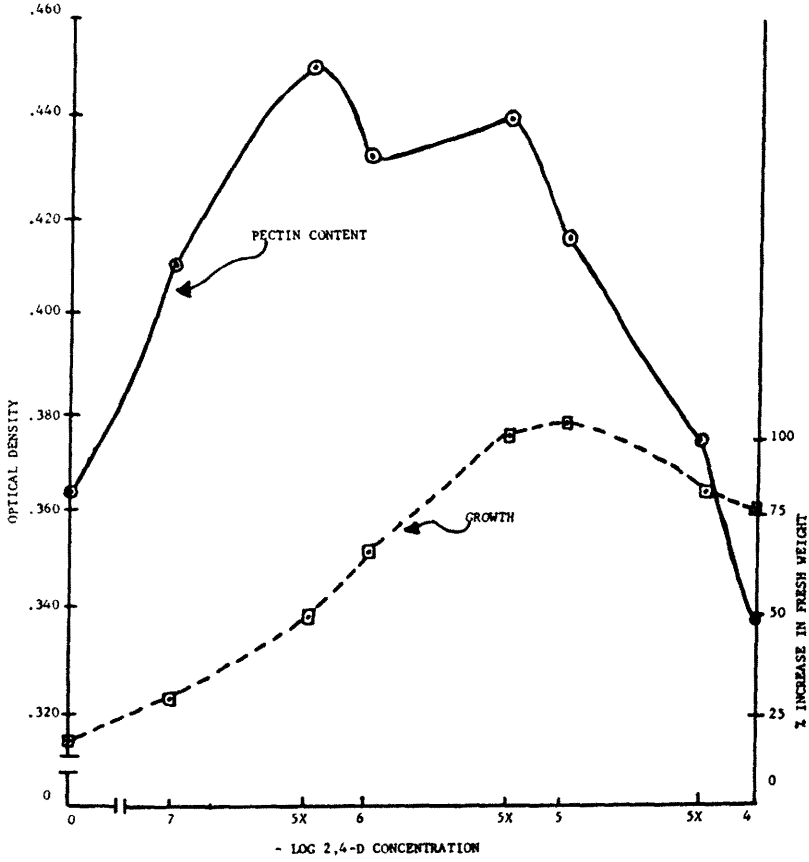


FIGURE 3. The effect of various concentrations of 2,4-D on the amount of soluble pectins and the fresh weight of epicotyl sections of pea. (Average of 3 experiments)

labile protein, as first described by Northen (1942), which is then susceptible to dissociation. Dissociation then allows greater cytoplasmic movement because of the flexibility obtained.

Since no increase in sulfhydryl groups has been consistently measured, no hypothesis can be made as to their interactions with the described processes.

As to the changes in the heat coagulability of protein, as affected by 2,4-D treatment, no conclusions can be made. It can be said that there is some indication that there is less heat coagulability shown by those proteins which have been treated with concentrations which stimulate increase in fresh weight. Whether or not this difference is due to synthesis of new heat-resistant protein or merely to changes in the old protein was not determined.

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