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Changes in protein content and activity of proteases and acid phosphatases in needles of Norway spruce (*Picea abies* L. Karst) seedlings treated with 2,4,5-T.*

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Abstract

Effect of 2, 4, 5-T in different concentrations and time of herbicide applications on protein biosynthesis and enzymatic activity of proteases and acid phosphatases in needles of spruce seedlings was investigated. 2, 4, 5-T in all concentrations tested and applied on the seedlings during phase of intensive growth caused significant increase in soluble protein content.
After spraying the seedlings with 100 and 300 $mg \cdot l^{-1}$ of 2, 4, 5-T before bud development a small decrease in soluble protein level was noted. The herbicide caused marked disturbances in the activity of enzymatic systems of proteases and acid phosphatases. No effect of 2, 4, 5-T on dry matter accumulation was observed.

INTRODUCTION

The influence of exogenous chemicals on protein and nucleic acid biosynthesis and enzymatic activity is a point of continuing interest for many scientifists. Exploring ways of the phytotoxic action of herbicides is of great importance. Papers by Humphreys and Dugger (1957), Switzer (1957), Tonecki (1972) refer to the effect of herbicides from the group of growth regulators on respiration intensity. The authors generally note a stimulation of respiration intensity during the initial stages after treatment but this stimulating effect disapears after a few days depending on herbicide concentration and a subsequent respiration decrease can be observed. Many authors suggest that toxic effect of herbicides is the result of inhibition of oxidative phosphorylation

* Butyl ester of (2, 4, 5-trichlorophenoxy) acetic acid.

(Switzer 1957, Wedding and Black 1962, Lotlikar et al. 1968). In their papers Freed et al. (1961), Hemker (1964), Ashton et al. (1968) indicate a significant influence of herbicides from the group of growth regulators on the activity of certain enzymatic systems. Stimulatory or inhibitory effects of herbicides on protein and nucleic acid biosynthesis were investigated by Key (1964), Datta and Sen (1965), Fong and Yu (1965), Nooden and Thiman (1965) and others.

The purpose of the present work is to investigate the effect of 2, 4, 5-T on the soluble protein content and enzymatic activity of proteases and acid phosphatases in the needles of spruce seddlings depending on herbicide concentration, plant growth phase and time of exposure.

MATERIAL AND METHODS

Material

Plant material consisted of two-year old spruce seedlings grown in the nursery. Half of the plants (aprox. 5000) were treated with 100, 300 and 700 $mg \cdot l^{-1}$ of 2, 4, 5-T in spring 1970 before bud development. The same number of plants were sprayed with 2, 4, 5-T in conc. of 100, 500 and 1000 $mg \cdot l^{-1}$ in July 1970 during bud formation. Protein content and protease and acid phosphatase activity were measured 3½, 6, 8 and 13 weeks after treatment.

For the plants treated with herbicide before bud development the measurements were also made in the newly formed needles at the end of the vegetative season.

Three 20 g samples of needles, each taken from 50 plants, were taken for extractions.

The needles of seedlings sprayed with water were used as a control.

Methods

Preparing enzymatic extract

Extractions were performed at +4°C. 20 g of fresh needles from current-year shoots was homogenized with 80 ml 0.25 M Tris-HCl buffer pH 8.5 in homogenizer Unipan type 302 at 16 900 g. The homogenate was then centrifuged for 10 min at 16 900 g in Janetzki K-24 centrifuge. The supernatant was again centrifuged for 30 min at 16 900 g. In so prepared extract the measurements of soluble proteins and enzymatic activity of proteases and acid phosphatases were performed.

Protein measurements

The protein was measured by the method of Lowry et al. (1951). The protein was precipitated in 0.5 ml sample using 10% TCA for 1 hr. After precipitation the sample was centrifuged for 10 min in Unipan 302 centrifuge at 16 900 g. The pellet was then treated with 1 mln 0.1 N NaOH; to the resultant solution of proteins in NaOH 5 ml of copper reagent was added and after 10 min — 0.5 ml of Folin-Ciocialteau reagent was added. After 40 min absorption was measured in a spectrophotometer (Spectronom 202, made in Hungary) at the wave length of 750 nm against the blank sample. Protein content was determined from the standard curve using albumin (Charles Druce LTD, London) as a standard.

Protease activity determination

Protease activity was measured in 0.2 M acetate buffer pH 4.8. To 7 ml of extract 7 ml of buffer was added and the mixture was then centrifuged. After centrifuging 2 ml of supernatant were pipetted to 6 test tubes. 3 test tubes were placed in cold storage after adding 2 ml of TCA and the remaining 3 tubes were incubated at 37° C. After 1 hr. incubation TCA was added and the tubes were also transferred to cold storage. After one hour all samples were centrifuged and protein was measured as described above.

Acid phosphatase activity measurements

Activity of acid phosphatases was determined by the method of Fiske-Subbarowa (Mejbaum-Katzenellenbogen and Mochnacka 1969). For measurements 0.2 M acetic buffer pH 4.8 was used. 1 ml samples were incubated at 37° C with sodium β-glycerophosphate as a substrate. Enzymatic reaction was stopped by adding TCA. The samples where TCA was added before adding sodium β-glycerophosphate and placed into cold storage were treated as a control.

After an hour the extracts were centrifuged in Unipan 302 centrifuge at 16 900 g. Supernatant was then decanted into test tubes for inorganic phosphorus measurements. To 1 ml of supernatant 7.3 ml of distilled water, 0.5 ml of 10 N H_2SO_4, 0.8 ml of molybdate reagent and 0.4 ml of eikonogen were then added. After incubating for 10 min at 37° C absorption was measured in photocolorimeter KF-5 at the wave length of 670 nm against blank sample. Inorganic phosphorus was determined from standard curve using potassium phosphate as a standard.

RESULTS AND DISCUSSION

Proteins

Changes of soluble protein content in needles of spruce seedlings due to 2, 4, 5-T action are shown in Fig. 1. Protein content measured 3½, 6, 8 and 13 weeks after treatment rapidly increases in proportion to the concentration applied.

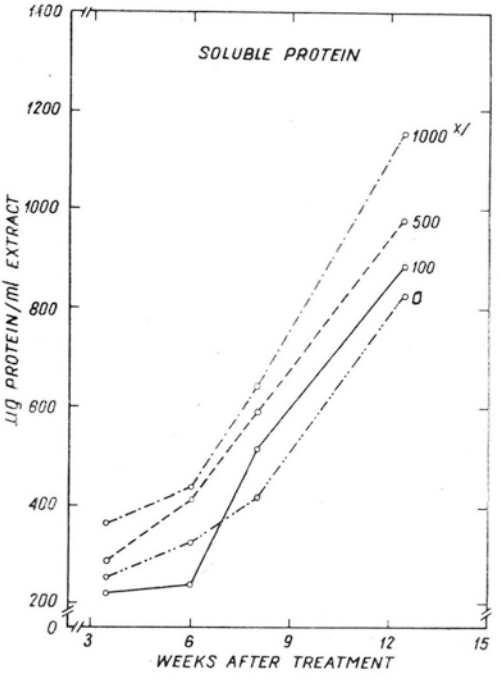

Fig. 1. Effect of different 2,4,5-T concentrations on soluble protein content in needles of two-year-old spruce seedlings treated with 2,4,5-T during bud formation. Measurements were made 3½, 6, 8 and 13 weeks after application

An increase in protein level was also observed in the needles of untreated seedlings; this is probably connected with plant preparation for winter dormancy and with the induction of frost resistance as suggested by P o m e r o y et al. (1970). Protein increase is reflected in dry matter accumulation in the needles at the end of vegetative season (Table 1).

2, 4, 5-T in conc. of 100 and 300 mg $\cdot l^{-1}$ applied before bud development causes small decrease in soluble proteins in the newly formed needles; 700 mg of herbicide slightly increases protein content — Fig. 4. It can be assumed that 2, 4, 5-T affects protein biosynthesis *in vivo* or reduces activity of the enzymes responsible for their hydrolysis or affects both processes simultaneously.

Table 1

Percentage of dry matter in needles of seedlings treated with 2,4,5-T and tested for protein content and enzymatic activity

Concentration mg·l^{-1}	% dry matter		
	time of harvesting		
	18.VIII(I)*	5.IX(II)	19.IX(III)
0	31.5	32.0	35.7
100	32.6	34.6	37.9
500	32.6	34.6	35.2
1000	31.4	33.6	36.1

Proteases

The results concerning the changes of proteolytic activity in needles treated with 2, 4, 5-T are shown in Fig. 2 and 4.

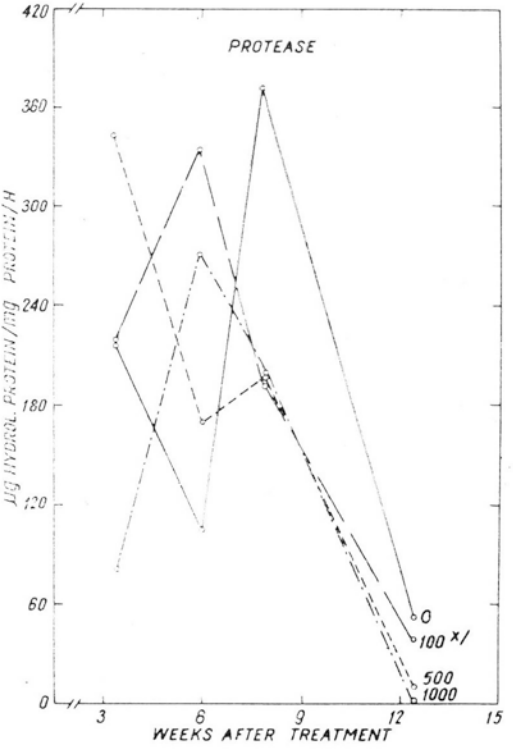

Fig. 2. Effect of different 2, 4, 5-T concentrations on activity of protease in current-year needles of two-year old spruce seedlings treated with herbicide during bud formation. Measurements were made 3½, 6, 8 and 13 weeks after application

Depending on concentration and time of exposure the herbicide stimulates or inhibits proteolytic activity. Six weeks after treatment 2, 4, 5-T in all concentrations tested stimulates protease activity but after 8

and 13 weeks inhibition in activity of this group of enzymes as compared to the control can be observed. At the end of vegetative season activity of proteolytic enzymes in the needles of untreated seedlings is also very small (Fig. 2). This can be associated with plant preparation for winter dormancy, as it was earlier suggested. Changes in soluble protein content measured on the same dates and in the same extracts were not related to changes in activity of proteolytic enzymes hence it can be assumed that the protein increase is due not only to inhibition of proteolytic system as it was suggested by K r e t ó w i c z (1964) and M a s z t a k o w et al. (1971) but maybe also to stimulation of protein biosynthesis *in vivo*.

Acid phosphatases

Changes in activity of acid phosphatase in the needles of seedlings treated with 2, 4, 5-T are presented in Fig. 3 and 4. As it shows in Fig. 3 2, 4, 5-T applied during vegetative season reduces activity of acid phosphatases responsible for hydrolysis of phosphate compounds.

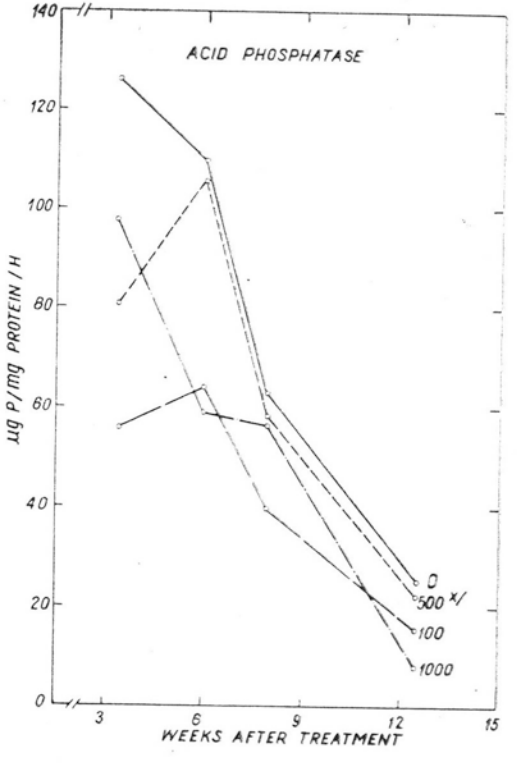

Fig. 3. Effect of different 2, 4, 5-T concentrations on activity of acid phosphatase in current-year needles of spruce seedlings treated with herbicide during bud formation. Measurements were made 3½, 6, 8 and 13 weeks after application

In case of plants treated with herbicide before bud development the stimulating effect of 2, 4, 5-T on the activity of the enzymes tested can be observed (Fig. 4).

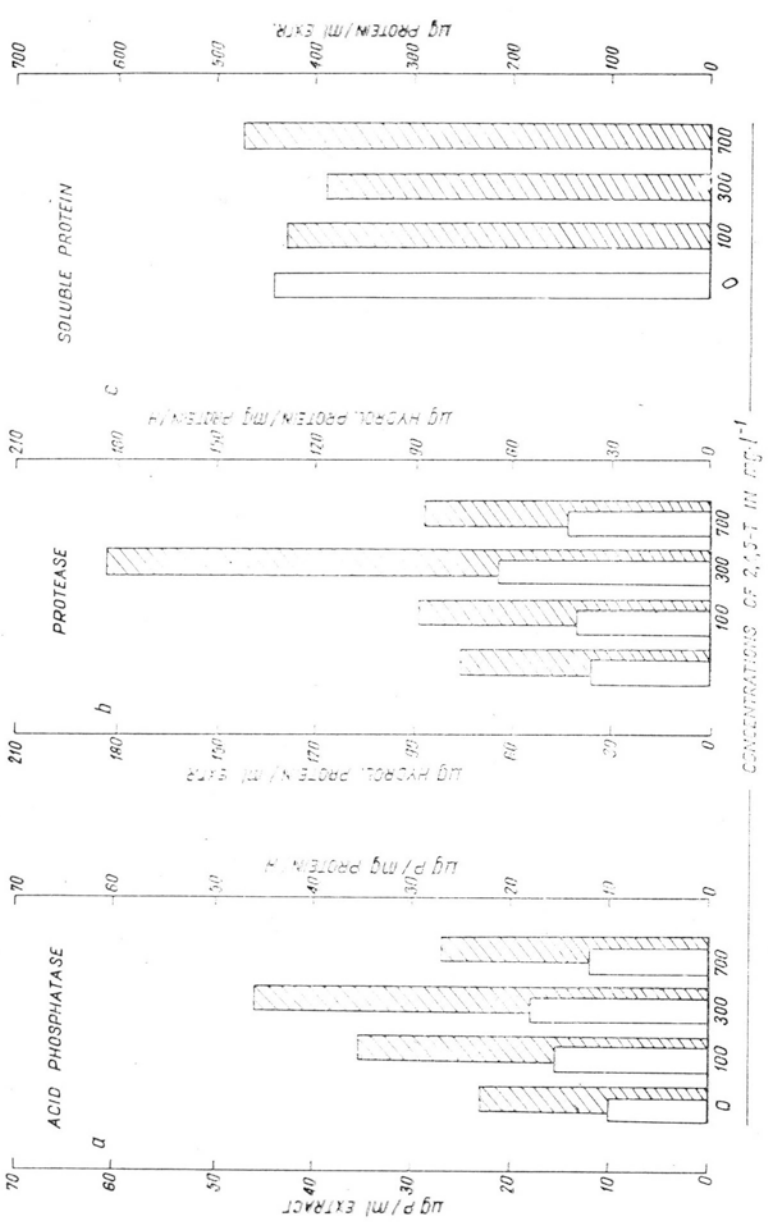

Fig. 4. Effect of different 2,4,5-T concentrations on soluble protein content and activity of protease and acid phosphatase in needles of spruce seedlings grown in nursery, treated with herbicide before bud development (24. 04. 1971). Measurements were made at the end of vegetative season (27. 08. 1971)

White graphs represent enzymatic activity per ml; bar graphs represent specific activity.

The results of the present work show that the effect of 2, 4, 5-T varies according to the growth stage and the concentration applied. Metabolism disturbances in newly formed needles as influenced by 2, 4, 5-T may indicate a possibility of herbicide translocation from old to newly formed plant organs. This confirms the results of earlier A u d u s' investigations (1964).

Conclusions

1. Butyl ester of (2, 4, 5-trichlorophenoxy) acetic acid considerable increases soluble protein content in needles of spruce seedlings.

2. Significant disturbances in proteolytic activity is observed. Depending on herbicide concentration, growth stage and time of exposure 2, 4, 5-T can stimulate or inhibit proteolytic systems.

3. 2, 4, 5-T in all concentrations tested and applied during intensive seedling growth retards activity of acid phosphatases. Applied before bud development the herbicide can stimulate activity of this group of enzymes.

4. No significant effect of herbicide on dry matter accumulation can be observed.

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REFERENCES

A s h t o n F. M., P e n n e r P. and H o f f m a n S., 1968. Effect of several herbicides on proteolytic enzymes activity of squash seedlings. Weed Sci. 16: 169—171.

A u d u s L. J., 1964. The physiology and biochemistry of herbicides. Academic Press, London and New York.

D a t t a A. and S e n S. P., 1965. The mechanism of action of plant growth substances; growth substances stimulation of amino acid incorporation into nuclear protein. Biochim. Biophys. Acta 107: 351—357.

F a n g G. and Y u T. C., 1965. Influence of auxin on *in vivo* incorporation of glycine ^{14}C in pea shoot proteins. Plant Physiol. 40 (2): 299—303.

F r e e d V. H., R e i t h e l F. J. and R e m m e r t L. F., 1961. Plant growth regulation. Iowa State Univ. Press. Ames.

H e m k e r H. C., 1964. Inhibition of adenosine triphosphate and respiration of rat liver mitochondria by dinitro-phenols. Biochim. Biophys. Acta 81: 1—8.

H u m p h r e y s T. E. and D u g g e r W. M., 1957. The effect of 2,4-dichlorophenoxyacetic acid on pathways of glucose catabolism in higher plants. Plant Physiol. 32 (2): 136—140.

K e y J. L., 1964. Ribonucleic acid and protein synthesis as essential processes for cell elongation. Plant Physiol. 39: 365—370.

K r e t o v i c h V. L., 1964. Osnovy biokhimii rastenii. Izd. Wyz. Shkola, Moskow.

Lotlikar P. D., Remmert L. F. and Freed V. H., 1968. Effect of 2,4-D and other herbicides on oxidative phosphorylation in mitochondria from cabbage. Weed Sci. 16: 161—165.

Lowry O. H., Rosenbroug N. J., Farra A. L., and Randall R. J., 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193: 265—275.

Masztakow S., Diejewa W., 1971. Działanie herbicydów na rośliny uprawne. PWRiL.

Mejbaum-Katzenellenbogen W., Mochnacka I., 1967. Kurs praktyczny z biochemii, PWN, Warszawa.

Nooden L. D. and Thiman K. V., 1965. Inhibition of protein synthesis and of auxin-induced growth by chloramphenicol. Plant Physiol. 40 (1): 193—201.

Pomeroy M. K., Siminovitch D. and Wightman F., 1970. Seasonal biochemical changes in the living bark and needles of red pine (*Pinus resinosa*) in relation to adaptation of freezing. Canad. Journal of Botany 48 (5): 953—957.

Switzer C. M., 1957. Effects of herbicides and related chemicals on oxidation and phosphorylation in isolated soybean mitochondria. Plant Physiol. 32: 42—44.

Tonecki J., The influence of butyl ester of 2, 4, 5-trichlorophenoxy acetic acid and sodium salt of 2,4-dichloropropionic acid on seedlings of Norway spruce (*Picea abies* L. Karst). Ph. D. Diss., Warszawa.

Wedding R. T. and Black M. K., 1962. Response of oxidation, coupled phosphorylation in plant mitochondria to 2,4-dichlorophenoxy acetic acid. Plant Physiol. 32 (2): 364—370.

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Zmiany poziomu białek rozpuszczalnych oraz aktywności enzymatycznej proteaz i kwaśnych fosfataz w igłach sadzonek świerkach (Picea abies L. Karst) pod wpływem 2, 4, 5-T

Streszczenie

Badano fitotoksyczny wpływ estru butylowego kwasu 2, 4, 5-trójchlorofenoksyoctowego na poziom białek rozpuszczalnych, aktywność enzymatyczną proteaz i kwaśnych fosfataz w igłach sadzonek świerka. W wyniku przeprowadzonych doświadczeń stwierdzono znaczny wzrost poziomu białek rozpuszczalnych, proporcjonalny do zastosowanych stężeń oraz istotny wpływ na aktywność enzymatyczną proteaz i kwaśnych fosfataz. Należy zaznaczyć, że wzrost poziomu białek nie jest spowodowany wyłącznie hamowaniem systemu proteolitycznego, lecz prawdopodobnie również stymulacją biosyntezy białek *in vivo*.