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EFFECT OF HERBICIDES OF 2.4-D BASE ON WATER-PLANTS

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Abstract

The destructive effect of 2.4-D induces in the cells of water-plants essentially different changes in the enzyme level from the mechanical impact or the pathogenic infection. The indole-inductivity of 2.4-D has a much more considerable influence on promoting the formation of new indole-derivatives than the indole-content of the experimental medium. In some water-plant species the 2.4-D-induced formation of the indole-derivatives may come about through the reversibility of the reaction way shikimic acid — IAA.

Introduction

The investigation into the effect of plant-protectingagents on some components and members of the living world is a real demand of the environment al conservation and as such, it extends primarily over the biotope most endangered by pesticides, the aquatic ecosystems. From among pesticides, first of all herbicides are to be taken into consideration in this respect because the development of these chemical agents is the most dynamic in our days.

A very important group of the hormonic herbicides are formed by phenoxi-alkannincarbonic acids and, within these, by phenoxi-acetic acids and their derivatives. Their most characteristic property is the so-called auxin-effect, similar to growth regulators, its essence being that auxin, which in a weak concentration is able to promote vegetable growth, applied in a strong concentration has a retarding effect. It is, however, made possible by the nearer knowledge of various effects of the auxinic compounds, to apply these compounds not only as herbicides but also as growth regulators. Agents of this character are by and large synthetized in the plants themselves, as well, resp. their formation can be influenced by other substances.

2.4—D, as a lipoid-soluble substance, changes even the permeability of the cell-membrane, and under these conditions an increasing efflux can take place, the cytoplasm may outflow through the membrane (Dexter, 1969). As at herbicide treatment the effective agents always get into touch with cell membranes, the phytotoxic effect can be measured well by the change in permeability, as well (ZSOLDOS, 1974).

Materials and Methods

Our experiments were performed on water-plants. *Myriophyllum* sp., applied from June to November, has originated from the main channel of Kórógyér belonging the irrigation system of the Kurca, while in other months of the year we worked with *Elodea canadensis* grown under artificial

conditions. The experiments were set on through different periods (between 1—35 days), with stock solution of 0.6, 3.0, 6.0, 166.0, 333.0 ppm Dikonirt (Na-salt of 2.4-D). The control was running tap water.

The determination of the peroxidase enzyme was performed by applying guaiacol reagent with spectrophotometric method, and that of indole-hydroxylation with thin-layer chromatography, according to HORVÁTH *et. al.* (1975).

Results and their evaluation

It is described by several researchers thus, among others, by FARKAS (1968) that in the tissues resp. cells, damaged mechanically or by a pathogenic infection — like a protective mechanism induced — the quantity of peroxidases and polyphenolic oxidases increases. As the considerable destruction mentioned above is to be attributed to the effect of 2.4—D, exerted on the permeability of the cell-membrane, it seemed to us justified to extend the concept of „cellular injury" over the impact of chemical agents, as well. In this case, we tried to draw a conclusion from investigating the peroxidase activity concerning the possible connection between the change in the quantity of enzyme and the 2.4—D concentration applied.

In the first part of our investigations into the peroxydase activity, we put Myriophyllum sp., developed under natural conditions, into a solution containing 2.4—D. The three treatment concentrations were chosen so that even the lowest dose should be at least one order of magnitude higher than the maximum of the level of the agent remainder, formed in the water as a result of the possible herbicide pollution, lest the herbicide concentration accumulated from the water in the course of years covers up the effect of the treatment dose. The other two concentrations were raised to an extremely high level in order to reveal if a high dose like this can even be taken up by a water-plant at all.

The first destruction took place on the 38th day in the solution of 6 ppm. As our experimental series was intended to be of comparative character as to its effect, as a result of this the further investigations were cancelled. In the water-plants treated with the other two concentrations, like in controls, for a long time there was no physical change. There manifested itself, therefore, an essential difference between the doses for the time being — at least in this respect.

It is to be seen in the figures well that the courses of all the three curves are different. The value of the peroxidase activity, measured as a result of the concentration of 6 ppm, is approximately the same on the first and last days of investigation. It never exceeds control, its changes, in the course of the experimental series, are uniform (Fig. 1).

In the intervals of the activity values of 166 ppm there is a considerable difference between the starting and ending points, the tendency of the curve is rising, it exceeds the value of control only at the endpoint of investigation, and this is, at the same time, its maximum point, as well (Fig. 2).

The activity level of the highest concentration is of declining tendency during the experimental series, exceeding the control similarly only once (what is the maximum point, here too). This takes place immediately after beginning the investigations (Fig. 3).

From among the minimum values of the three curves, those of two, and with a very little difference that of the third, as well, fell to the 24th day. The following

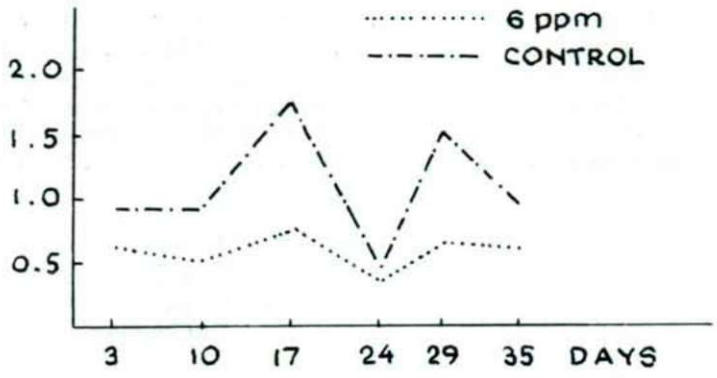

Fig. 1. Effect of a 2.4-D treatment of 6 ppm on the peroxidaseactivity in *Myriophyllum* sp.

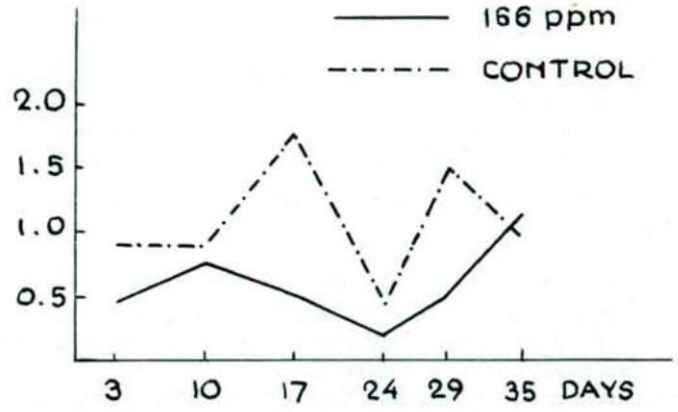

Fig. 2. Effect of a 2.4-D treatment of 166 pm on the perodase activity in *Myriophyllum sp.*

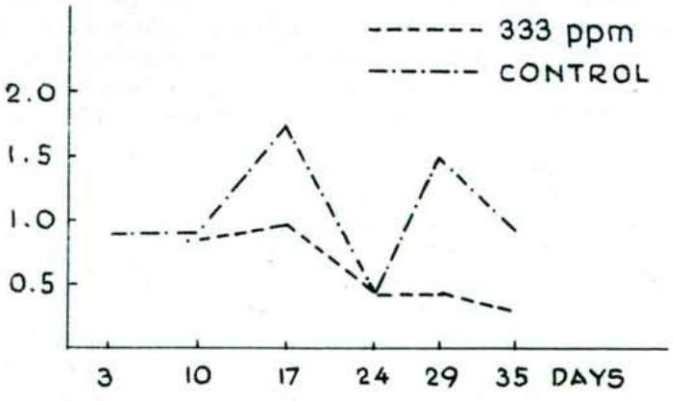

Fig. 3. Effect of a 2.4-D treatment of 333 ppm on the peroxidase activity in *Myriophyllum* sp.

measurings were concentrated, therefore, first into five, later into six days. We could no more return to the original interval because it would have fallen to the 42nd day but on the 38th day — as described before — the concentration of 6 ppm proved to be of destructive effect. We drew the conclusion partly from this, partly from the course of curves that the lowest dose proved to be the most effective. Our hypothesis became confirmed when we plotted the curves in a figure as compared to one another (Fig. 4).

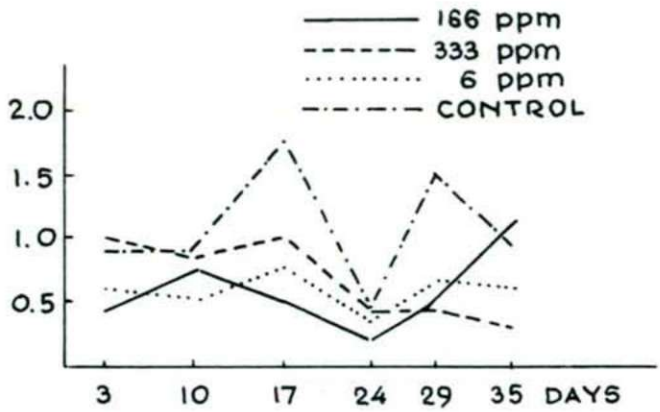

Fig. 4. Effect of 2.4-D treatment of different concentrations on the perodicase activity in *Myriophyllum* sp.

It is to be seen that the curve of enzyme activity, obtained as a result of the concentration of 6 ppm, is quasi the resultant of the other two, supporting this also by that, in case of higher doses, the herbicide effect develops, as compared with the lowest one, either very early or too late.

Wanting to investigate into the spectrum of the efficient herbicide concentration, in the further part of our experimental series concerning the enzyme activity we have not applied the 2.4-D concentration of 166 and 333 ppm but chose the doses in a way that the lowest 2.4-D concentration, of 6 ppm, which gave the previous most interesting conclusion, should be, this time, the highest one. And two more doses were chosen from a similar, or smaller but one, order of magnitude. For these, however, owing to the water polution problems outlined above, we have no more applied the test-plant mentioned before but *Elodea canadensis* which was grown under artifical conditions.

We wanted originally to extend the time of the experimental series, in this case too, to the interval used in the preceding but the destruction, similarly in case of the 6 ppm concentration, here already followed on the 19th day. Measurings were extended to the earlydates (days 3, 6, 8, etc.), as reckoned from the treatment and compared to the previous 7 days time lag, because we wanted to study the initial stage of the accumulation of the remainder of agents, as well.

It can be read from the figures that in all the three curves the initial and endpoints approximately agree but while the levels of the enzyme activity of the 0.6 ppm concentration are showing a strongly upward tendency, almost sine curve-like, exceeding the control values repeatedly and many times (Fig. 5), the values belonging

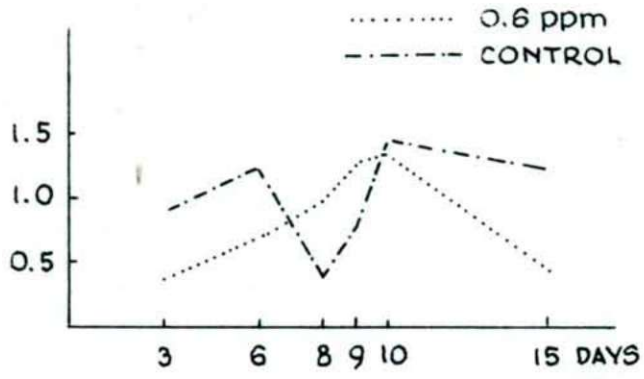

Fig. 5. Effect of 2.4-D treatment of 0.6 ppm on the peroxidase activity in *Elodea canadensis*.

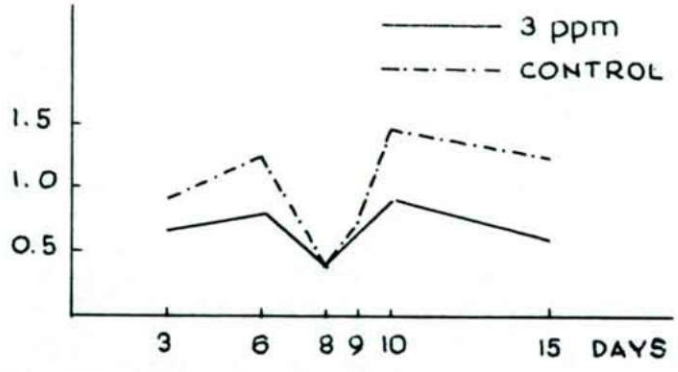

Fig. 6. Effect of 2.4-D treatment of 3 ppm on the peroxidase activity in *Elodea canadensis*.

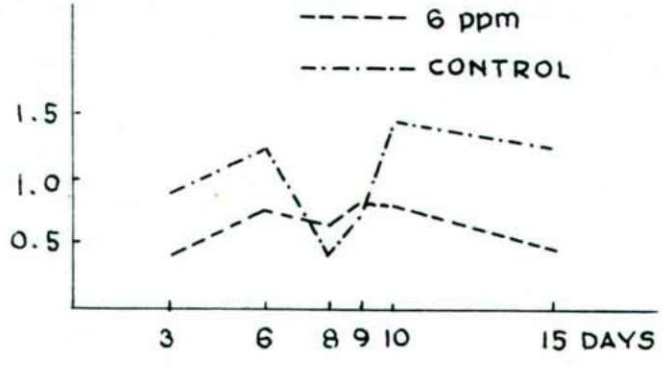

Fig. 7. Effect of 2.4-D treatment of 6 ppm on the peroxidase activity in *Elodea canadensis*.

to 3 ppm have a downward tendency (with a nearly „reversed" sinus curve character), never exceeding the control level (Fig. 6). The activity values are uniform, in these cases too, only in case of the 6 ppm dose (Fig. 7).

The correctness of our earlier supposition is also verified by plotting the values in a figure (Fig. 8).

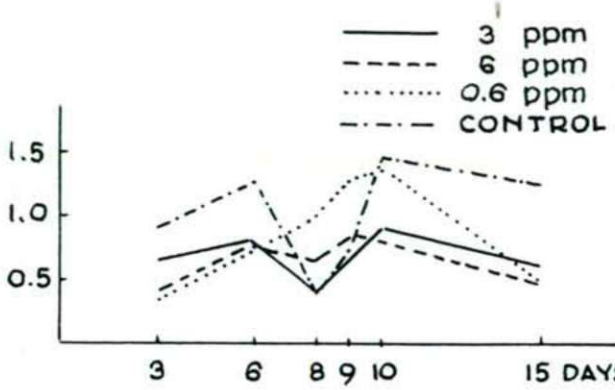

Fig. 8. Effect of 2.4-D treatment of different concentrations on the peroxidase activity in *Elodea canadiensis*.

It is apparent that the resultant of curves is given also here by the values of the 6 ppm concentration. In this way, there proved to be most efficient, here too, this dose, increased in the present case by a species-specific factor because the same concentration induced an irreversible physical change in case of *Elodea canadensis*, as compared to the Myriophyllum sp., exactly in half as much time.

From the investigations into the peroxidase enzyme activities we could, on the one hand, establish the most efficient herbicide concentration applied, on the other hand, draw the conclusion that the changes of the chemical agent-induced cell "injury" in enzyme level considerably differ from those induced by a mechanical impact or a pathogenic infection.

But by the auxin-like effect of 2.4-D not only the performance of our experimental series was considerably influenced; one of our measuring series, resp. the method used there was made justified decisively by this. In a broader sense, namely, even the compounds may be considered as auxin, which are the precursors of auxin (IAA); thus, among others, the indole itself, by which the heterocyclic basic frame is given. It is known from SÜDI's investigations (1964) that the formation of an inductive IAA precursor, *e. g.* that of the indole-acetyle-aspartat, can only take place after a pretreatment with substances of auxinic effect, thus with 2.4-D, resp. as a result of IAA exceeding the physiological concentration. We have wanted to study the effect of the 2.4-D treatment upon the formation of indole and its metabolites, on the basis of these connections.

At first we wanted to determine the indole-derivatives, having existed in waterplants already originally, without any indole induction and then with two kinds of indole administration. Settings were carried out with a middle-long treatment period

and strong 2.4-D concentration. The latter one was performed to establish the extreme limits of doses. As a test-plant, *Elodea canadensis* was applied. This meant an advantage mainly in the indole-infiltration experiments bacause of its broader leaves.

Table 1. Change in indole derivatives in *Elodea canadensis* as a result of an 8-day herbicide treatment.

Indole-derivatives	2.4-D cc. (ppm)	In phosphate buffer		In indolic buffer		In inflitrated buffer	
		R_f	γ	R_f	γ	R_f	γ
6—OH—indole	Ø	0,20	2,8	0,19	1,7	0,19	2,5
	6	0,19	2,5	0,19	2,5	0,18	3,5
	166	0,14	2,0	0,14	2,5	0,11	2,8
	333	0,11	2,8	0,16	2,5	0,16	4,0
5—OH—indole	Ø	—	—	0,32	0,5	0,31	1,8
	6	—	—	0,31	2,0	0,28	2,3
	166	—	—	0,22	1,8	0,20	2,8
	133	—	—	0,28	2,3	0,27	2.5

It may be established from the Table that in water-plants originally there can only be found the 6—OH—Indole while the 5—OH—Indole is produced by induction. The difference seen between the two kinds of indole induction is first of all that the quantity in weight of both derivatives is somewhat increased by being infiltrated; at the same time, the R_f values are a little decreased. The phenomenon may supposedly be attributed to that, in case of identical compounds, the spot of a higher „material content" runs up with more difficulty, resp. slower, in the chromatogram.

As we did not observe, according to the above data, any considerable difference between the 2.4-D concentrations applied, we have continued carrying out the experiments with small doses, beginning the investigation of derivatives from the moment of their formation, resp. from the first day of treatment. For induction, in this case, we only used the indolic buffer, leaving infiltration out of consideration.

It may be established from the Table that the quantitative change in both indole-derivatives is uneven enough, here and there even sudden. From the first day after setting, the 6—OH—Indole could not be measured, either, resp. it did not give any spot. This is referring to that, even if it was originally contained — as verified by our previous investigation — in the water-plant, it must have been of minimum quantity, giving a measurable value only after being treated for some time with 2.4-D of a certain concentration. On the second day, 6—OH—Indole and 5—OH—Indole could already been demonstrated but only if treated with 3 and 6 ppm doses. After beeng treated in a 0.6 ppm concentration or in a control, however, there could not been demonstrated any. The fluctuating character of change could most be observed on the fourth day, when the quantity of both derivatives achieved in all the three concentrations a maximum value, although the 0.6 ppm-value couldn't even be measured by the same interval before that. After the fourth day, in any case, a strong decrease, but at the end of the treatment a little increase, were to be observed.

We wanted to check the species-specificity observed at measuring the peroxidase-enzyme activity in case of this method, as well. Therefore, the experimental series was performed, under similar conditions, in *Myriophyllum* sp., too, with the difference that, because of the maximum on the fourth day, the subsequent intervals were concentrated, in order to ensure a registration as exact as possible.

We see the appearance of species-specificity, on the one hand, in that a new derivate, not-observed as yet, manifests itself: 7—OH—Indole; on the other hand, that the quantity of both induced indole-derivates (5—OH—Indole, 7—OH—Indole) can only be measured in certain phases of experiments. In case of 5—OH—Indole,

Table 2. Change in indole derivatives in *Elodea canadensis*, as a result of a herbicide treatment in indolic buffer.

Indole derivative	Period of treatment (day)	2,4-D cc. (ppm)							
		Ø	0,6	3,0	6,0	Ø	0,6	3,0	6,0
		R_f-value				γ-quantity			
6—OH—indole	1	—	—	—	—	—	—	—	—
	2	—	—	0,22	0,23	—	—	10,0	4,5
	4	0,23	0,20	0,23	0,20	5,0	18,0	18,0	30,0
	8	0,17	0,17	0,17	0,17	1,3	1,7	1,7	2,3
	15	0,27	0,26	0,25	0,25	2,3	3,3	2,1	2,4
5—OH—indole	1	—	—	—	—	—	—	—	—
	2	—	—	0,29	0,32	—	—	4,5	3,8
	4	0,32	0,28	0,30	0,28	4,8	18,0	30,0	5,0
	8	0,25	0,25	0,25	0,25	1,4	1,8	2,4	1,7
	15	0,38	0,36	0,37	0,38	2,3	1,9	2,7	2,0

Table 3. Change in indole derivatives in *Myriophyllum* sp., as a result of a herbicide treatment in indolic buffer.

Indole derivative	Period of treatment (day)	2.4-D cc. (ppm)							
		Ø	0.6	3.0	6.0	Ø	0.6	3.0	6.0
		R_f-value				γ-quantity			
6—OH—indole	4	0,22	0,19	0,25	0,24	32,0	16,0	20,0	26,0
	5	0,21	0,22	0,21	0,19	4,0	14,0	5,0	5,0
	6	0,20	0,29	0,16	0,19	14,0	10,0	3,5	5,0
	8	0,17	0,18	0,16	0,17	4,5	5,0	18,0	5,0
	12	0,20	0,21	0,21	0,21	2,0	3,0	2,2	2,7
	15	0,20	0,19	0,16	0,20	4,2	4,5	3,7	4,0
5—OH—indole	4	0,28	0,2′	0,32	0,32	20,0	10,0	16,0	16,0
	5	0,31	0,31	0,31	0,27	5,0	2,8	5,0	4,2
	6	0,27	0,39	0,29	0,30	3,7	3,8	3,3	3,8
	8	0,31	0,32	0,29	0,32	2,0	2,7	3,5	3,3
7—OH—indole	4	—	—	0,53	0,54	—	—	4,8	4,3
	5	—	—	0,49	0,51	—	—	16,0	4,8

on the 8th day we still got a spot in the chromatogram, at the next experimental point of time, on the 12th day, however, we got it no more. In case of 7—OH—Indole, the same took place on the 5th, resp. 6th day.

It can be established from our indole-hydroxylation measuring-series that the indole inductivity for the formation of new derivatives is not primarily given by the experimental medium, *e. g.* indolic buffer or infiltrated indole, but in a considerable part the 2.4-D of auxin-effect, and even species-specifical factors, may have a role in it.

By the 2.4-D treatment, according to ARTEMENKO *et al.* (1971), the IAA-content is decreased, the IAA-oxidase level, and owing to this, the IAA-decarboxylation is increased. This is somewhat contrary to SÜDI's statements (1964), quoted above, and is throwing also a broader light upon the different ways of the synthesis of the aromatic compounds. In one of these reaction ways, from shikimi acid — through indole and some other intermediaries — tryptophan, and from this — in three possible ways — IAA are induced. The question arises if this synthesis is true the other way round as well, *i. e.*, whether IAA-degrading (mainly owing to the IAA oxidase) takes place in the same way. If it does, in the above sense, the decrease in IAA-content induced by the 2.4-D treatment must also influence the increase in the quantity of indole and its derivatives. Our experiment seems to verify this latter supposition: it is visible from Tables I and II that in case of every indole-derivative the value of control is lower than that of the treated ones but only up to the 8th day. On the 15th day, they already take an intermediate place. On the basis of all these, we may draw the conclusion that in water-plants — species-specifically — there exists the „reversed" reaction way, as well (KERESZTES—HORVÁTH, 1977).

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