## STABY - OSU

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> BIOLOGIE Physiologie

## OF THE AUXINS AND ON THE ACTIVITY OF THE OXIDASE OF IAA IN LEAVES OF TOBACCO PLANTS

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The effect of the synthetic growth regulator, maleic hydrazide (MH), on the plant organism is related primarily to the growth processes. This provides grounds to assume that the mechanism of its effect involves mainly its influence on the internal growth-regulating system, which includes the endogenous growth regulators (auxins, inhibitors, etc.), and also the oxidase of indole acetic acid (OIAA). Certain authors [4] explain the inhibiting effect of maleic hydrazide on the growth and development of plants not by the direct action of the inhibitor but by the action of suboptimal doses of auxins established in the analysed tissues of plants treated with the preparation. Unlike them, other authors examine the inhibiting effect of MH as a result of reduced content of auxins in the plants [2]. Assumption has been made, in connection with the observed decrease in the amount of auxins under the influence of MH, that the inhibitor suppresses their biosynthesis. It has been demonstrated by invitro experiments that MH does not directly effect the biosynthesis of the IAA while it raises the activity of the enzyme systems oxidating the IAA and, as a result of that, it reduces its content in the tissues [3]. Opposite results have been obtained upon studying the auxin metabolism in whole plants [7].

The aim of the work undertaken was to investigate the influence of maleic hydrazide on the level of the auxins and on the activity of OIAA in leaves of tobacco plants upon inhibiting the development of their blossoms with

the preparation.

The experiments were performed with tobacco leaves (Nicotiana tabacum), still growing or about to complete their growth, belonging to the variety of Djebelska basma 174, cultivated under greenhouse conditions. The tobacco plants were treated with maleic hydrazide at the beginning of the formation of floral buds by spraying the inflorescences with water solution of MH (diethanolamine salt — 30% solution). Each plant was sprayed with 15 ml solution containing 60 mg of active substance. The controls used were plants whose blossoms were sprayed with water, as well as plants whose blossoms had been pruned by hand.

The amount of the free auxins was determined by the method used by Nitsch a. Nitsch [6]. The activity of the individual zones from the chro-

matograms was gauged by the growth of the wheat coleoptyls [2].

The oxidase of the IAA was extracted in 0.02M solution of KH<sub>2</sub>PO<sub>4</sub> with cystein, pl1 4.5. The activity of the enzyme was determined colorimetrically after Tang a. Bonner [8]. The results adduced are mean values from 3 experiments, with 3 to 6 replications in each variant.

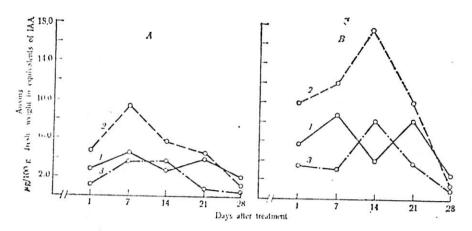


Fig. 1. Influence of maleic hydrazide on the summary auxin activity in tobacco leaves. A = middle storey; B = upper storey; I = controls; 2 = plants pruned by hand; 3 = plants treated with MB, 60 mg per plant

The influence of MH on the overall auxin activity in the leaves from the two storeys, during the reproductive development of the tobacco, is shown on Fig. 1. We see that maleic hydrazide reduces the summary auxin activity, and that throughout the entire experiment it is considerably lower than that of the control variant and of the plants pruned by hand. One exception is the maximum which appears on the 14th day after spraying the inflorescences with the preparation. The amount of the auxins in IAA equivalent in the growing leaves of the plants treated with MH in the particular case is 0.8 µg, while in the leaves of the control variant it is 4.0 µg per 100 g of fresh material. Inhibition of some of the processes taking place in the plant organism, after treatment of the plants with physiologically active substances (the inhibition then alternating with stimulation), has been observed in the investigations carried out by Key a. Hanson [5]. It is obvious that in the initial period of the operation of inhibiting doses of MH the changes in the amount of auxins differ from the changes established at later periods. The complete inhibition of the reproductive organs (the 21st day after spraying) is related to a lower content of auxins in the leaves of the experimental plants.

The established higher content of auxins in the leaves from the two storeys of the plants pruned by hand, up to the 21st day of the beginning of the experiment, can be explained by the intensified growth provoked by pruning. With the appearance of the first symptoms of ripening, the content of the auxin-type-regulators begins to decrease and, at the end of the experiment, it becomes equal to that of the MH-treated plants. Under the influence of the removal of the inflorescences, done chemically or by hand, the overall amount of the auxin varies along the pattern of a single-apex curve, whereas the control plants show two maximums. The different manners of removing the inflorescences result in changes in the general character of the dynamics of the

auxin activity during the blossoming of the tobacco.

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In connection with the changes observed in the auxin level of the leaves of plants pruned by chemicals and by hand, we studied the effect of maleic hydrazide on the activity of the enzyme controlling that level. Upon entering the plant, MH influences the enzymes in the first place — much like any

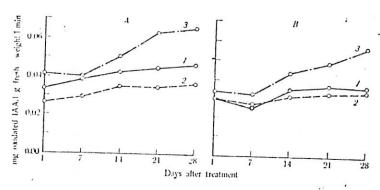


Fig. 2. Activity of OIAA in the leaves of tobacco plants treated with the preparation (in vivo).

A — middle storey; B — upper storey; I — controls; 2 — plants pruned by hand;
3 — plants pruned by chemicals, 60 mg per plant

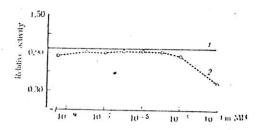


Fig. 3. Effect of increasing concentrations of MH on the IAA-oxidase activity in extract from tobacco leaves (in vitro).

1 — without effect; 2 — with effect

biologically active substance - and through them the metabolism of the various groups of substances, thereby influencing the enzyme activity as early as the 24th hour after spraying of the inflorescences (Fig. 2). A comparison made between pruning by chemicals and by hand shows that, in the variant involving MH, the activity of the OIAA in the growing leaves and in those about to complete their growth is higher than the activity of the enzyme in the leaves of plants pruned by hand. The former activity is higher compared with that of the leaves of the control variant, but the differences in the manner of removing the inflorescences do not influence the general character of auxin destruction; they affect only the intensity of that process. During in vitro experiments, maleic hydrazide, applied in a rather broad range of concentrations, does not inhibit the effect of OIAA (Fig. 3). To all probability this agent does not exercise any direct influence on the enzyme activity, but achieves such an influence by reducing the level of its inhibitors or by inducing an adaptive synthesis of the enzyme on account of limited utilization of the auxins in the growth processes [4].

The inhibiting effect of MII in relation to the development of the blossoms observed at the end of the experiment (between the 21st and 28th days after spraying) is connected with a decrease in sthe summary auxin activity (Fig. 1) and with higher auxin oxidase activity (Fig. 2). This warrants the assumption that the action of the preparation in inhibiting the reproductive development of the plants, much like its effect on the growth, is related to changes in the auxin metabolism mainly through the enzyme system of IAA oxidase.

The results adduced are in aggreement with the assumptions expressed by other authors [3] about a possible meschanism of action by MH not on the level of IAA biosynthesis but on the level of the activity of the enzyme system controlling its content in the plant tissues.

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