INFLUENCE OF DEFOLIATING AGENTS ON THE LEVEL OF SOME SECONDARY METABOLITES IN COTTON-PLANT LEAVES

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The change in the composition of the secondary metabolites (SMs) of cotton-plant leaves under the influence of two modifications of defoliating agents in the period of leaf fall has been studied. It has been shown that, in contrast to the dynamics of the SMs in genetic deciduous lines, up to the period of leaf-fall the amount of free sterols in the petioles decreases, while in the leaf blades it remains unchanged. In experiments with cotyledons, the opposite effect was observed. In all cases, the amount of polyprenols decreased substantially.

In the search for natural ageing factors for cotton-plant leaves the dynamics in the change in the amounts of free and bound sterols and triterpenoids during the phases of ontogenesis in the leaf blades and petioles of two deciduous selection lines and the control variety Tashkent-1 (T-1) have previously been compared [1]. The greatest differences between the deciduous lines and the control consisted in a shift in the direction of an earlier phase for the maximum level of free sterols saturated in the side chain of C-17 in the leaf blade and the minimum level of all the sterols analyzed in the petioles of the deciduous lines. In the leaf-shedding phase of the line L-470 a sharp increase in the concentration of the SMs of this species was observed in its petioles.

The existence of the features mentioned impelled us to continue investigations in the direction of evaluating the influence of the exogenous treatment of the control plants with standard defoliating agents on the level of SMs in the same organs of variety T-1 with a simultaneous increase in the number of components to be determined quantitatively in the total extractive substances.

The main aim of the work was to compare the influence of genetic and exogenous factors on the biosynthesis of the SMs in connection with the process of leaf shedding in an attempt once more to evaluate how important a role change in their balance in the later phases of ontogenesis may play in this process.

As the defoliating agents we used two modifications of the preparation Dropp (thidazuron): from the firm of Schering (DSch), and the domestically produced defoliant (DS) containing carboxy derivatives and diphenylurea as impurities. The standard of consumption was 0.6 kg/ha and the date of treatment September 6, 1989. Fifteen days after the DSch treatment ~40% of the leaves fell, while in the case of DS it was ~80%. Such an unusual difference in the activities of the two preparations could be due to seasonal-climatic factors [2]. It was also reflected in certain indices characterizing the materials treated. The yield of total extractive substances from the petioles decreased considerably but differently: at 9% in the control plants, it fell to 7% (DS) and 5% (DSch). At the same time, this index did not change in the leaf blades (14% for the control, 14% for DS, and 15% for DSch). The ratio between the weight of the leaf blade and the petiole averaged 9.1 in the case of the treatment with DSch, and 4.9 under the action of DS (for the control plants it was 4.6). The results of observations lead to these conclusions: 1) the artificial defoliation described in a recent monograph [3], in particular, as a process of the evacuation of metabolites from the leaf, acts in this sense, rather, on the petioles; and 2) under the action of

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TABLE 1. Levels of SMs (mg/g of ADM) in the Leaf Blades and Petioles of Variety T-1 (control) in Plants Treated with DSch and DS in the Maturation Phase

Secondary metabolites	Organ	T-1 control	T-1 + DSch	T-1 + DS
Sitosterol	Leaf blades	0,93	0,88	1,32
	Petioles	2,43	1,10	1,23
Stigmasterol	Leaf blades	0,36	0,41	0,64
	Petioles	1,08	0,67	0,63
Campesterol	Leaf blades	0,08	0,08	0,16
	Petioles	0,50	0,29	0,28
24-Ethylidene-cholesterol	Leaf blades	0,36	0,37	0,39
	Petioles	0,42	0,31	0,29
α-Tocopherol	Leaf blades	1,32	1,17	1,16
	Petioles	1,69	0,86	0,65
β- and γ-Tocopherols	Leaf blades	0,23	0,18	0,35
	Petioles	0,46	0,14	0,23
Amyrin	Leaf blades	0,44	0,38	0,65
	Petioles	0,62	0,49	0,43
Undecaprenol	Leaf blades	32,6	17,4	10 9
	Petioles	8,6	5,1	5,2
Dodecaprenol	Leaf blades	18,9	10,3	6,4
	Petioles	4,5	2,7	2,9

DSch a degeneration of the petioles, including the separating layer, preceding the fall of the leaf may take place.

Let us now pass to a quantitative estimate of the influence of DSch and DS on the level of concrete SMs. The results of determinations by the procedure of [1] in milligrams of SMs per gram of air-dry mass (ADM) of the leaf blades and the petioles are given in Table 1.

The amounts of free sterols, tocopherols, and amyrin in the leaf blade remained at practically the same level under the action of DSch, while on treatment with DS they rose somewhat, with the exception of 24-ethylidenecholesterol and α-tocopherol. More appreciable changes in the levels of all the SMs mentioned took place in the petioles (decrease by a factor of 1.5-2), i.e., the effect was the opposite of that which took place in the petioles of the deciduous line L-470 on passing from the maturation phase to leaf-shedding phase [1].

Special attention must be devoted to the polyprenols – in the present case, undeca- and dodecaprenols. Lipophilic properties permit their derivatives to be incorporated in the structure of the cell membrane and to act as the lipophilic acceptor of carbohydrate residues [4, 5]. Their level in the materials under consideration was 1-2 orders of magnitude higher than that of the other SMs. At the same time, in contrast to that of the other SMs, the concentration of polyprenols in the petioles of the control plants was approximately one quarter of that in the leaf blades. Under the action of both the defoliants it decreased by an average of 1.6-fold. On the leaf blade the exogenous effect appeared to a greater degree: The amount of polyprenol decreased by factors of almost two (DSch) or three (DS).

Thus, a preliminary conclusion can be drawn on the important influence of defoliating agents under consideration on the processes of the biosynthesis or transport of the SMs. At the same time, this still does not give grounds for stating that the observed dynamics is directly responsible for ageing and leaf-shedding.

It is considered that defoliating agents accelerate the occurrence of natural processes involved in the transformation of the cells of the separating layer in the petiole, which leads to their mechanical breakdown and the subsequent fall of the leaves [6]. If it is established that in this process the level of SMs changes in a certain way, it would be completely appropriate to determine whether a similar effect of exogenous action was specific only for the maturation phase of the cotton plant. This is all the more urgent since to en-

TABLE 2. Amounts of SMs (mg/g of ADM) in the Leaves and Stems of the Cotyledons of T-1 (control) and Plants Treated with DSch and DS

Secondary metabolites	Organ	T-1 control	T-1 + DSch	T-1 + DS
Sitosterol	Leaves	2,05	1,22	1,02
	Stems	0,59	0,90	0,85
Stigmasterol	Leaves	0,62	0,75	0,57
	Stems	0,22	0,41	0,42
Campesterol	Leaves	0,56	0,36	0,26
	Stems	0,16	0,23	0,29
α-Tocopherol	Leaves	2,20	1,80	3,20
	Stems	0,20	0,28	0,27
β- and γ-Tocopherols	Leaves	0,25	0,23	0,57
	Stems	0,06	0,06	0,05
Amyrin	Leaves	0,08	0,05	0,11
	Stems	0,04	0 04	0,05
Undecaprenol	Leaves	9,9	3,4	4,7
Dodecaprenol	Leaves	4,6	1,1	1,7

sure year-round tests on defoliant activity cotton-plant cotyledons grown under laboratory conditions are usually used as the test objects. However, the basically different structure of the leaves of plant in this phase and the absence of a formed petiole, of a separating layer, and of a system of protection from external actions that are characteristic of the mature plant, together cast doubt on the possibility of similar response to treatment with a defoliant in the young and the adult plant.

In order to determine the probable indeterminancy of the action of defoliating agents on the cotton plant in the various phases of ontogenesis that have been mentioned, we determined the levels of eight compounds in the cotyledonous leaves and stems of variety T-1 (control plants and plants treated with DSch and DS in a dose of 0.6 kg/ha). The amounts of SMs in mg/g of ADM were determined in materials gathered 15 days after treatment (Table 2).

The levels of sterols and tocopherol in the cotyledonous leaves were considerably higher than in the leaves gathered in September (see Table 1). In contrast to the leaves of mature plants, in the cotyledons both defoliating agents considerably decreased the level of sterols with a saturated side chain at C-17. However, the level of stigmasterol, an increase in which is sometimes considered as sign of the ageing of the leaves [7], did not fall in either phase under the action of DSch and DS.

Taking the tocopherols as an example, the difference in the example of the action of the two modifications of defoliants was appreciable: On the use of DS, the level of these compounds in the cotyledonous leaves decreased considerably. Attention is attracted by the low level in the same material of amyrin, which, according to some statements [8], together with other compounds of this class, may serve as a protective agent from pests and stress situations.

So far as concerns polyprenols, their amount in the cotyledonous leaves while remaining highest among the SMs determined, was substantially (by a factor of 3-4) smaller than in the mature leaves. Under the action of DSch and DS, moreover, it fell 3- to 4-fold and 2- to 3-fold, respectively (see Table 2). There is no doubt that such a substantial suppression of the level of polyprenols is one of the factors disturbing the physiological processes in cotyledonous leaves, as a result of which on the 15th day they suffered a 79% (DSch) or 77% (DS) fall. We may note, additionally, that the stems of the cotyledons contained only traces of polyprenyl alcohols. At the same time, as can be seen from Table 2, the decrease in the level of sterols in the cotyledonous leaves was accompanied by an increase in the amount of compounds in the stem, i.e., an outflow of sterols from the leaves into the stems is possible.

Before comparing the action of defoliating agents on the levels of the SMs determined in two organs in young and mature plants, we may note that, with respect to their distribution over the phases, these SMs are divided into two groups. The level of the compounds of the first group is fairly high (free sterols, tocopherols) and in the early phases of on-

togenesis they are partially subjected to biosynthetic transformations the products of which have multiform physiological functions. The accumulation of the SMs of the second group (oxidized triterpenoids, polyisoprenoids) takes place in later periods of development.

As a rule, the treatment of plants with DSch and DS in both phases leads to a redistribution of the compounds of the first group between the organs. The same effect is brought about in mature plants in relation to amyrin. At the same time, the changes in the level of substances of the first group under the action of identical factors in the cotyledons and mature plants are in the main, opposite in sign. The example of sitosterol is the most indicative (see Tables 1 and 2).

A monotypical effect – in the direction of a decrease in some degree of the level of polyprenols – was observed in all samples treated with the defoliants and did not depend on ontogenetic differences. If it is borne in mind that genetic leaf fall shows a dynamics of the change in the level of free sterols in the leaf blades and petioles [1] opposite to that brought about by treatment with DSch and DS, it is possible to put forward two versions, requiring further confirmation, of the version of the ageing process that are connected with the participation of the SMs: 1) the mechanisms of genetic leaf fall and that caused by defoliants of the given class are different; and 2) the dynamics of the biosynthesis of polyprenols is of decisive significance in processes of leaf loss. It must be followed through all the vegetation periods in the leaf blades and petioles including those of deciduous lines. The search for an identification of bound polyprenols must be made.

EXPERIMENTAL

Material (1 g) from young and mature plants (control and defoliant-treated) that had been dried in the air were extracted as described in [1]. The levels of SMs were determined on a MKh 1310 spectrometer by the method of multipeak monitoring [1, 9]. The sterols and tocopherols and amyrin were investigated in the 300-450 a.m.u. interval with sitosterol and α-tocopherol as standards. Polyprenols were investigated in the 700-850 a.m.u. interval with undecaprenol as standard.

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