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The effect of a combined treatment with retardant and auxin on mineral composition of fruits, seeds and leaves of apple trees

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Abstract
A number of factors affect the uptake of mineral elements, their movement within a plant and their accumulation in fruits. The intensity of shoot growth and the biosynthesis and distribution of auxins in the plant are of a great importance in these processes. Experiments were carried out whose aim was to assess the uptake of minerals in apple trees after shoot growth had been weakened by the use of a retardant while the force of attraction of minerals by apples had been increased by a treatment with a synthetic auxin. The results of these experiments confirmed the ambiguous and complex effect of bioregulators on the mineral composition of apple trees. The results suggest that retardants can improve the supply of calcium to apples, depending on how the preparations are applied, especially if the apple trees being treated have a low calcium content. It was found that when apples were rich in calcium, the retardant did not have any effect on the transportation of this element to them despite a clear inhibition in the growth of shoots. Paclobutrazol had a more obvious effect on the transportation of calcium to apples than daminozyd. The uptake of calcium by apples was also found to be greater after an auxin had been applied to them. Application of the retardant to shoots only did not change the effect of the auxin on the uptake of calcium by leaves, but it reduced the calcium uptake by apples. When the retardant was also applied to the apples, the effect of the auxin was greater and involved the leaves, too. The effect of the retardant and the auxin on the levels of other elements (K, Mg, P, N) also depended on these elements content in apple trees.

***Key words**:* Bioregulators, mineral composition, apple trees.

Introduction

Quality of apple fruit is closely related with their mineral composition, among them calcium is of special meaning. Ca content and relationship with other elements decide about apple resistance to physiological disorders in storage [5, 13]. The uptake of mineral nutrients, calcium in particular, was the subject of many investigations. The process is complicated and dependent on physiological state of trees [7]. Bioregulators and other preparations influence uptake and distribution of minerals, among them Ca [3, 4, 7, 10, 15].

Apple trees take the highest amount of Ca in spring at the time of intensive shoot growth. In condition of weak shoot growth fruit supply with calcium improves [12] but at strong shoot growth it worsens [14]. There are many evidences that retardants which are natural growth inhibitors, favour accumulation of Ca in fruit [6, 11, 12].

Calcium moves to fruits and shoots mainly acropetaly, however, the transport is related with basipetal movement of auxins [8]. Auxins are mainly delivered by seeds, young leaves and other intensively growing parts of plants. It is presumed that quantitative changes in fruits are due to bioregulators controlling auxins level and their transport in plant [1, 2, 9].

The present study aimed to trace the mineral nutrients uptake in apple tree in the condition of shoot growth weakened by retardant. It was assumed that their uptake by shoots would decline, too. So, shoot competition for these elements will be lessened favouring fruits. There were also trials to attract nutrient accumulation in fruits through application of synthetic auxin. To weaken shoot growth, two retardants (daminozide and paclobutrazol) were used. Both of them have different mode of action. They influence auxin metabolism in tips of shoots. To assess direct and indirect action of retardant in accumulating mineral nutrients in fruits, one of tested preparations – daminozide – was applied to whole plants or only to the shoots [4].

Material and Methods

The research was realized in three experiments, each one in different year, on 17-20 yrs old Double Red McIntosh apple trees grafted on Antonovka seedlings. To weaken shoot growth two retardants, daminozide (Alar, Uniroyal, USA) and paclobutrazol (Cultar, ICI, England), were used . Both preparations were applied two weeks after bloom at 0.2% concentration in the form of one spray. Besides, daminozide was given to shoots only in other trees. To protect fruit they were covered with aluminium bags, which were removed after preparation had dried. Chemically pure IAA (indolyl acetic acid, Koch-Light, England) was used as auxin. The auxin was given to fruit about two weeks after bloom, three days after spraying with retardants (first term) or two weeks later, i.e. about four weeks after bloom (second term). This compound was given three times in three consecutive days: first and second as submersion of fruit in the solution containing 50 mg/L with addition of Tween 20 at 0.1% concentration and the third one as injection of 0.1% IAA solution containing 50 mg/L of active ingredient without wetting agent.

Untreated trees and these treated with retardant or auxin only served as control. Also, trees with one year shoots together with undeveloped leaves tipped off at the time of retardant application in order to restrain their growth were taken as control. Another group of control trees consisting of these with one year shoots sprayed at the same time with solution of gibberellins (Gibrescol, Polfa, Poland) containing 500 mg/L of active ingredient with addition of glycerol as wetting agent, amounting 5 ml/L to achieve maximum stimulation of shoot growth. For each treatment 5 frame branches were chosen – one per tree (replicate) on parts of the tree situated in the same cardinal point. Control branches and these treated solely with retardants as well as with auxin have been on the same trees. Just after bloom fruitlets were thinned with 1 per spur left.

Description of treatments:

No.	Treatments	Week after bloom:
1	Control, untreated	
2	GA3	2
3	IAA	2
4	IAA	4
5	PB (shoots and fruits)	2
6	PB (as above) + IAA	2
7	PB (as above) + IAA	4
8	SADH (shoots and fuits)	2
9	SADH (as above) + IAA	2
10	SADH (as above) + IAA	4
11	SADH (shoots only)	2
12	SADH (as above) + IAA	2
13	SADH (as above) + IAA	4
14	Shoot thinning	2

Analytical material consisted of apples collected at harvest. Fruits from one branch (about 50 apples) served as control. Chemical analysis was made separately on flesh and seeds. Because of limited amount of seeds from replicates chemical analyses were executed on mean sample from all replicates. Leaves from the middle of terminal shoots longer than 10 cm were sampled in the middle of August. Each sample consisted of about 100 leaves taken from the same branch (5 samples per treatment).

The content of minerals Ca, K, Mg and N (in % of dry weight) was assessed in fruits, seeds and leaves. The collected samples (5 per treatment) were air-dried at 60°C and ground. Nitrogen content was assessed with Kjeldahl method. Other elements, before analyzing were mineralized in the mixture of HNO_3, H_2SO_4 and $HClO_4$. Calcium, potassium and magnesium were assessed with atomic absorption method with spectrometer Pye Unicam SP 09.

Also, the following measurements were taken: mean fruit weight, number of seeds/fruit, mean lenght of one-year terminal shoots and mineral composition of the soil. Soil was sampled according to generally accepted rules (the data are not shown in this paper).

The results concerning fruit and leaf content were analyzed statistically with analyses of variance. Significance of mean differences was assessed with Duncan's t-test at 95% probability level. The results of mineral content of seeds were not analyzed statistically because chemical analyses was made on mixed sample of 5 replicates.

Results

Changes of calcium content: Soil in the orchard showed pH 4.8 – 5.3 which indicates relatively low calcium content. Ca supply of leaves and fruits was different in experimental years (Fig. 1). Also, the influence of bioregulators on Ca content was inconsistent in separate experiments.

Ca in fruit. In Experiment 2 , apples from control trees untreated with growth bioregulators, contained almost double amount of Ca as compared to those in Experiments 1 and 3. Retardants did not affect the Ca amount in Experiment 2, when fruits were rich in this element, but in the remaining experiments retardants slightly increased its content. However, significance of differences was proven only in case of fruits and shoots after treatment with daminozide in Experiment 1 and paclobutrazol in Experiment 3. Auxin did not affect calcium content in fruits as compared to plants treated with retardant only.

Ca in seeds. The seeds contained above 2-3 times more calcium than fruit flesh. Retardants caused slight increase of this element in seeds. Auxin influenced further increase of Ca in seeds from plants treated with daminozide to shoots only in Experiment 1 and from plants treated with paclobutrazol applied to shoots and fruits in Experiment 3. In Experiment 2 seeds from trees with shoots and fruits treated with daminozide, after application of auxin contained less calcium than these treated with the retardant only.

Ca in leaves. Both retardants, alone or with auxin did not change significantly Ca content during entire period of experimenting. However, in four cases (paclobutrazol applied to shoots and fruits in Exp.1 and Exp.2) there was a tendency to decreased amount of calcium in leaves after application of auxin and treatment with retardant.

Changes of potassium content:

K in fruit. Control fruit contained the least amount of this element in Experiment 3 and the most one in Experiment 2 (Fig. 2). The greatest changes in the content were caused by bioregulators in Experiment 1. Paclobutrazol evoked significant decrease of potassium in fruits, but the effect of daminozide on fruit K content varied. Auxin influenced the increase of the element both in the control (unsprayed) and in fruits treated with daminozide (especially when given to leaves only). The reverse effect, i.e. decreased potassium content, was caused by auxin in Experiments 1 and 3 after treating previously sprayed trees with paclobutrazol and in Experiment 2, after covering leaves only with daminozide. In condition of low potassium content in fruits (Experiment 3) both daminozide and auxin did not change the amount of this element in fruit. In all experiments the greatest accumulation of this element in fruit was recorded after treatment with GA_3 and the lowest in those with shoots tipped off earlier.

K in seeds. The presented results do not describe unanimoulsy the influence of retardants on potassium content in seeds. However, auxin in many cases caused an increase of this element in seeds from trees treated with retardant, especially when used to shoots and leaves.

K in leaves. According to the presented results the influence of retardant and auxin on potassium content in leaves was inconsistent.

Changes of magnesium content:

Mg in fruits. In spite of differences in content of this element, no

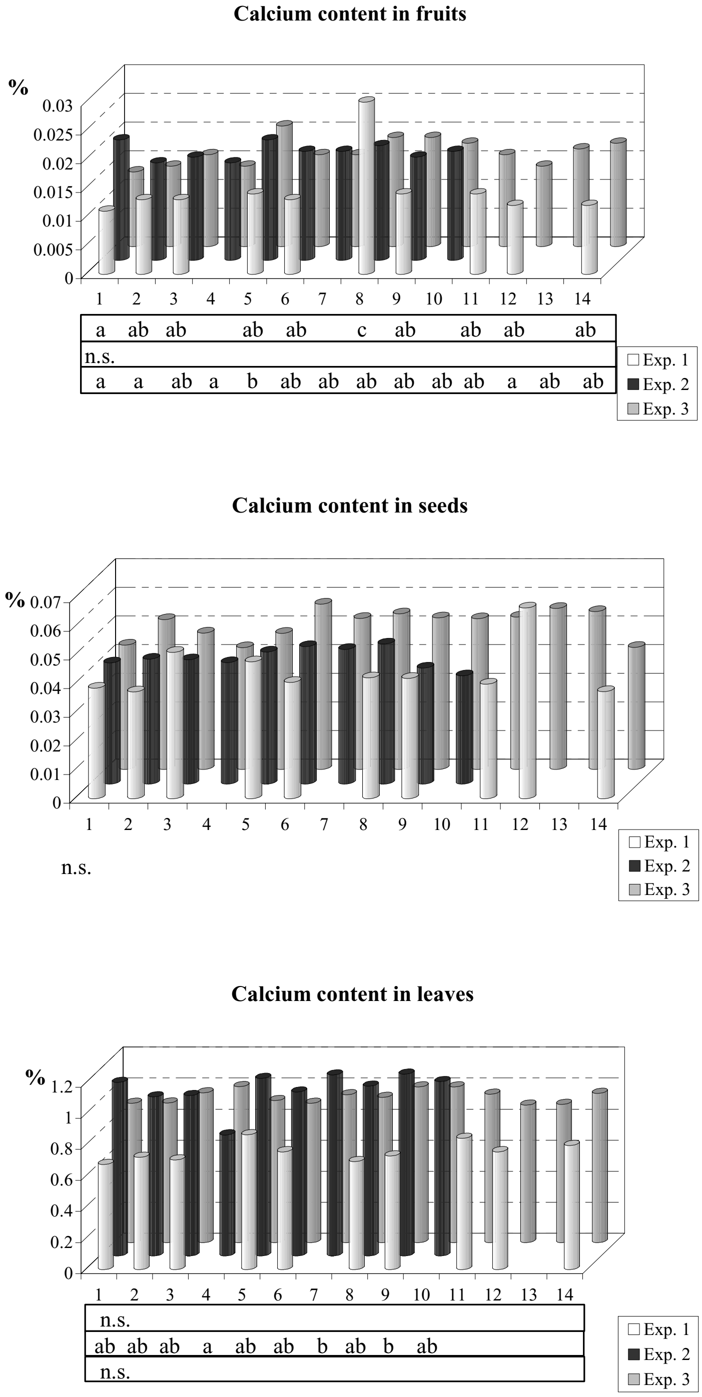

Figure 1. Effect of treatment with retardants (SADH, PB) and auxins (IAA) on calcium content in fruits, seeds and leaves of apple trees cv. Double Red McIntosh.

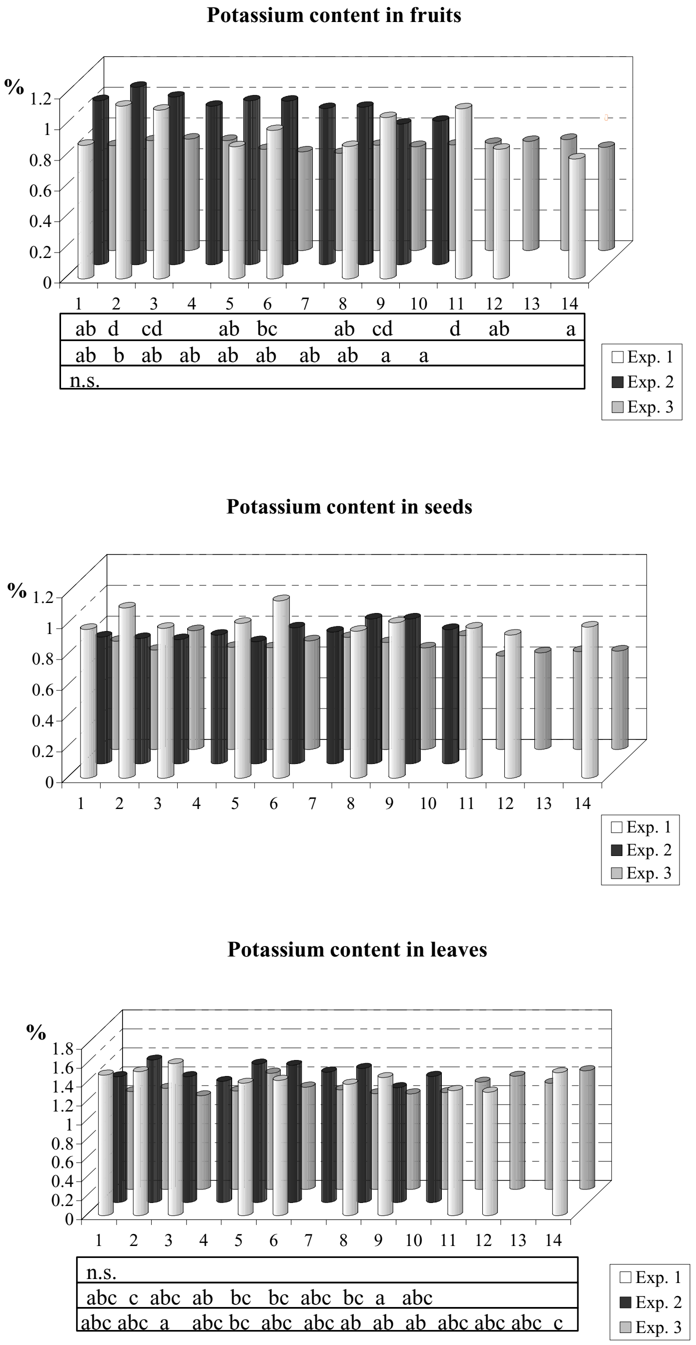

Figure 2. Effect of treatment with retardants (SADH, PB) and auxin (IAA) on potassium content in fruits, seeds leaves of Double Red McIntosh apples.

matter the kind and the way of treatment, the retardants did not affect its accumulation in fruits (Fig. 3). But auxin caused Mg decrease in apples after treatment with daminozide both in shoots and fruits in Experiment 3.

Mg in leaves. In condition of low content of magnesium both retardants and auxin, but only in case of plants untreated with retardants, generally induced small (insignificant) increase of Mg in leaves. The reverse changes were observed after application of auxin following treatment with retardant.

Changes in the content of nitrogen: Simultaneous treatment with retardant and auxin decreased the amount of nitrogen in apple leaves. It did not influence its amount in fruits and seeds (the data are not shown in this paper). The auxin alone, as well as gibberellin and shoot tipping did not change N content.

Discussion

The presented results suggest that retardants can improve fruit supply in calcium. However, it is the matter of several factors (compare Faust and Miller [7]). Daminozide did not affect Ca uptake in case of fruits rich in this element. The compound did not change calcium uptake power by fruits though through weakened shoot growth it diminished their competition for Ca. At that time fruits were small and supposedly seeds produced sufficient amount of

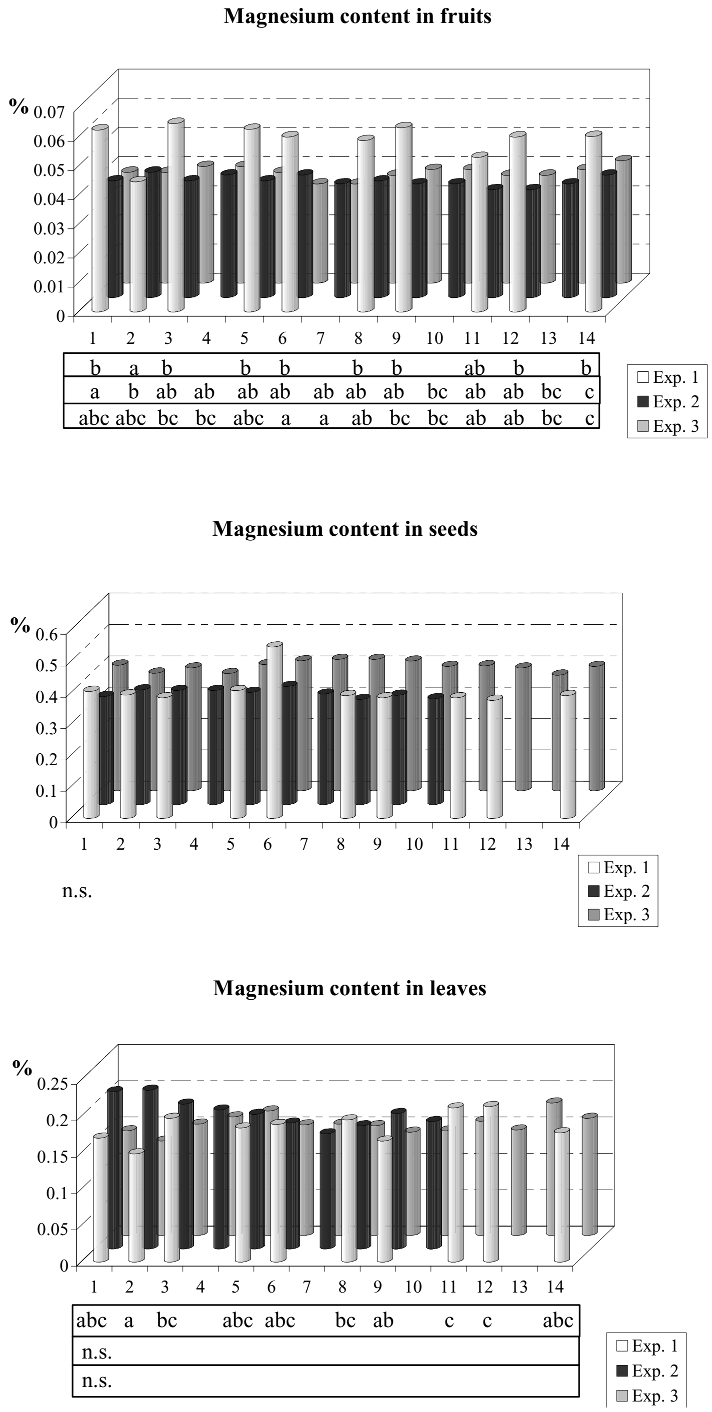

Figure 3. Effect of treatment with retardants (SADH, PB) and auxin (IAA) on magnesium content in fruits, seeds and leaves of Double Red McIntosh apples.

auxin to enable transportation of adequate volume of calcium to fruits.

There are many evidences that daminozide reduces growth of shoots and fruits. So, it could influence flow of calcium to these parts of plant [7, 12]. The presented results show that direct contact of daminozide with fruits has often changed calcium uptake by fruit and leaves. Usually, leaf and seeds uptake has been changed insignificantly. In case of fruits it was slightly increased, and decreased or did not change the amount of calcium, depending on their physiological stage during treatment with retardant. Presumably, in one experiment at the time of treatment, fruitlets were larger, though more resistant to retardant action than in another one. In case of small fruits rich in calcium the retardant did not affect the content of this element in fruit, irrespective to the way of application.

Retardants (paclobutrazol and daminozide) that have been tested influenced differently auxin transportation to tips of shoots. In the presented work daminozide evoked larger changes in the amount of calcium than paclobutrazol. Some authors report about increased Ca uptake by fruit after applying auxins [1, 10]. Also, our results confirmed that auxin can increase Ca uptake by fruits and leaves treated with retardant in shoot. However, the amount of Ca in fruit did not change. Yet, when retardant was applied to shoots and fruits auxin acted in a reverse way, i.e. slightly decreased calcium uptake by fruits and leaves. So, it seems that application of retardant to shoots only did not change auxins influence on calcium uptake by leaves and weakened the transport to the leaves. When retardant was applied also to fruits its influence on calcium uptake caused by auxin was greater and concerned leaves, too.

The retardants did not affect unanimously potassium content in analyzed plant organs. However, paclobutrazol acted more evidently than daminozide. Though, auxin has distinct influence on potassium uptake, particularly by fruits and seeds. Control fruits untreated with auxin accumulated the highest amount of potassium, but auxin did not affect unanimously potassium uptake. Probably it was due to the effectiveness of retardant. However, in many cases retardant and auxin caused an increased K content in seeds.

Joint treatment with retardant and auxin had no great effect on magnesium content in fruit. Slight increase of this element in seeds and a decline in leaves was observed.

Storage ability assessed on relationship between potassium and calcium (also potassium plus magnesium and calcium) had worsened after treatment with auxin (Table 1). Apples treated and untreated reacted similarly to auxin application. In conclusion, in case of apples with seeds producing endogenous auxins, exogenous auxin depreciate storage ability. It might be caused by a too high auxin level in fruit. Auxin at high concentration can be toxic, limiting the development of fruit and diminishing their size. It was proven in the presented work. According to Marcelle *et al.* [10], auxin stimulates Ca transport to fruit but only at certain low limited level. Exogenous auxin can intensify calcium transport to partenocarpic fruit [1]. So these apples store better. According to the presented results, auxin affected storage ability similary to giberellin A_3.

Retardant increased storage ability of apples. Similar effect was produced in condition of extreme growth inhibition through shoot tipping. So, in case of fruits with seeds, producing endogenous auxin, their supply with Ca depends mainly on sucking power of shoots. Retardants diminish level of auxin like substances in shoots, weaken their competition with fruits. As a result they facilitate flow of greater volume of mineral nutrients. The investigations on uptake and distribution of calcium using Ca isotope confirm it [3].

The presented results are a further proof of complicated influence of growth regulators on plant mineral composition [4]. However, the collected informations cannot be used to steer apple tree nutrition in practice, but they have broaden our knowledge of growth regulators influence on the uptake and distribution of minerals. The tested retardants – daminozide and paclobutrazol

Table 1. Effect of treatment with retardants (SADH – daminozide, PB - paclobutrazol) and auxin (IAA) on relation of potassium, magnesium and calcium in Double Red McIntosh apples.

Treatment	Time of treatment (weeks after bloom)	Potassium : Calcium			(Potassium+Magnesium): Calcium		
		Exp.1	Exp.2	Exp.3	Exp.1	Exp.2	Exp.3
Control untreated	-	74.9 ab	52.1 ab	52.8 b	85.1 b	53.8 a	55.7 b
GA_3	2	86.0 b	69.4 b	54.1 b	89.6 b	71.6 b	57.0 b
IAA	2	86.7 b	62.0 ab	47.3 ab	91.7 b	64.3 ab	47.2 ab
IAA	4	-	59.5 ab	53.0 b	-	60.0 ab	55.9 b
PB (shoots and fruits)	2	79.6 ab	-	43.5 ab	83.4 b	-	45.8 ab
PB see above + IAA	2+2	68.0 ab	-	49.7 ab	73.8 ab	-	52.3 ab
PB see above + IAA	2+4	-	-	51.1 b	-	-	53.8 b
SADH (shoots and fruits)	2	63.2 a	51.0 a	32.6 a	67.8 ab	52.9 a	34.5 a
SADH see above + IAA	2+2	76.1 ab	56.6 ab	39.9 ab	80.7 ab	59.4 ab	42.0 ab
SADH see above + IAA	2+4	-	56.1 ab	40.4 ab	-	58.3 ab	42.6 ab
SADH (shoots only)	2	57.4 a	51.0 a	37.4 ab	57.2 a	53.1 a	39.4 ab
SADH see above + IAA	2+2	77.9 ab	56.2 ab	38.7 ab	76.6 ab	53.8 a	41.9 ab
SADH see above + IAA	2+4	-	50.6 a	45.1 ab	-	52.7 a	47.6 ab
Shoot pinching	2	64.9 ab	-	38.6 ab	70.0 ab	-	41.1 ab

Means in column signed with the same letter don't differ significantly at P=0.05 t Duncan' test

are solely the examples of the kind of preparations. Application of the obtained results should facilitate to learn more about other new retardants.

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