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Possibilities to use growth regulators in winter oilseed rape growing technology
1. The effect of retardant analogues on oilseed rape growth

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Abstract. The effect of growth regulators – derivatives of the diethylamine chloride 3-DEC and morpholinium chloride 17-DMC – on the growth and productivity of the winter oilseed rape 'Kasimir F_1' was studied.

3-DEC and 17-DMC have been found to exert positive effect on the growth and development of the oilseed rape 'Kasimir F_1' in autumn: it induced the growth of root collum, accumulation of monosaccharides in its tissues, leaf and root system formation, enhanced the endurance of this culture to wintering. Under the effect of these compounds applied in spring, stem growth was retarded and stem diameter as well as stem primary cortex ring and stele width increased, resulting in enhanced endurance to lodging. Thus, the compounds 3-DEC and 17-DMC, by modifying oilseed rape growth in autumn and influencing oilseed rape growth in spring, influenced the development of productivity elements. The extra seed yield under the effect of 3-DEC (250 g ha^{-1}) and 17-DMC (500 g ha^{-1}) in autumn was 350 and 455 kg ha^{-1}, and in spring 496 and 406 kg ha^{-1}, the control yield being 2,300 kg ha^{-1}.

Key words: *Brassica napus* – morpholinium derivative – 17-DMC, dietylamine derivative 3-DEC, growth, productivity

INTRODUCTION

Brassica napus is one of the principal crops for oil and protein production. However, data on oilseed rape growth and development as well as on the possibility to regulate plant growth and productivity elements formation are yet scarce. The formation of spring and partly winter oilseed rape productivity elements is determined by the peculiarities of the vegetative growth and generative development of plants (Bouille et al., 1989; Dienpenbrock & Grosse, 1995; Novickienė et al., 1998).

At the Laboratory of Plant Physiology of the Institute of Botany, special effects of original quaternary ammonium salts have been studied (Merkys et al., 1993). Of all compounds tested, the growth of oilseed rapes shoots was markedly inhibited by the compounds 3-DEC and 17-DMC (Miliuvienė, 2000). The physiological (retardant) activity of the compound 3-DEC is predetermined by two methylene groups located between two diethylamine radicals as well as by benzyl groups joined to nitrogen atoms. The molecule of the derivative of dimethylmorpholinium chloride (17-DMC) comprises two methylene groups which are separated with CHOH groups between two morpholinium rings, and allyl groups attached to the nitrogen atoms (Fig. 1). We supposed that these compounds, with their narrow specific effect, could modify the growth and productivity formation of oilseed rape. We had data on the positive effect of growth regulators (analogues of auxin and quaternary ammonium salts) on the growth and formation of winter wheat and the winter oilseed rape ‘Accord’ in autumn and on preparing for wintering (Gavelienė et al., 1998).

The aim of this work was to study the effect of the original compounds 3-DEC and 17-DMC, quaternary ammonium salt derivatives, on the peculiarities of oilseed rape growth in autumn and spring and on the formation of productivity elements.

MATERIALS AND METHODS

The experiments on the winter oilseed rape (*Brassica napus* L. ssp. *olifera biennial)* hybrid ‘Kasimir F_1’ were carried out in field trials at the Lithuanian Institute of Agriculture in Dotnuva - Akademija 250 mg kg^{-1} (2000–2002) on a light loamy carbonaceous gleyic soil, pH 7.0, containing: 2.2% of humus, 250 mg kg^{-1} of mobile phosphorus, 225 mg kg^{-1} of mobile potassium. Chemical fertilisers were applied at the rate of $P_{60}K_{60}$ in autumn and N_{90} in spring before plant vegetation renewal, and additionally N_{60}. The size of accounted plots was 22 m^2. The trials were performed in four replications according to the following scheme:

Scheme: Field trial of winter oilseed rape

Test variant	Application time / development stage
Control (H_2O)	Autumn rosette of 4–5 leaves
3-DEC (250 g ha^{-1})	
17-DMC (500g ha^{-1})	— “ —
Control (H_2O)	Spring 5–6 true leaves
3-DEC (250 g ha^{-1})	
17-DMC (500g ha^{-1})	— “ —

The test compounds were applied in autumn at the rosette stage of 4–5 leaves and in spring, at the stage of 5–6 true leaves, as aqueous solutions at the rate of 300 l ha^{-1} (3-DEC 250 g ha^{-1} and 17-DMC 500 g ha^{-1}), and 1 l of solution for each plot (Fig. 1). The optimum concentrations of the compounds, which were established in the previous trial on spring oilseed rape, were used for winter oilseed rape plants (Miliuvienė et al., 1999; Милювене, 2000). In autumn, 30 days after the application of the compounds under study, the following morphometric parameters of plant development were detected: leaf number, root collum diameter, the diameter of main root, the number of lateral roots, dry roots mass.

Monosaccharides were determined in the root collum after U. Jensen (Jensen, 1965). These carbohydrates were extracted from oilseed rape root collum tissues (dry substance) with ethanol. After centrifugation, monosaccharide content was detected by the orcinin method on a spectrophotometer. The content of the carbohydrates was calculated according to a standard curve formed on the basis of glucose.

The retarding effect of the compounds was assessed from stem height, diameter, and anatomic structure. Samples for determining the anatomical structure of stems were taken in the flowering phase. Anatomic structure was studied in the second storey, where growth is particularly strongly inhibited by retardants. The stem segment samples were fixed with a mixture of formalin, acetid acid and ethanol (1:1:20), embedded in paraffin and cut into slices, which were stained with the Schiff reagent (Kublickienė, 1998). The width of the primary cortex and the stele rings was measured and the number of vascular bundles was counted. The measurements were made with an eyepiece micrometer and a MBI–3 light microscope.

Stem resistance to lodging was evaluated on a five-point score (5 points – no lodging, 1 point – final lodging; and yield structure was assessed in the phase of complete ripeness.

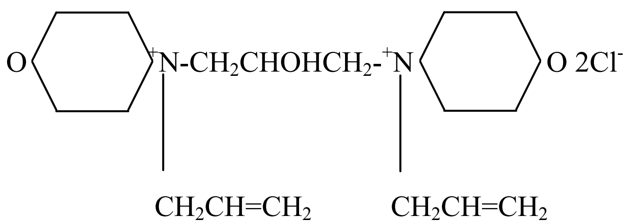
Structural formula	Code	M.m.
$(CH_3CH_2)_2 - N^+(CH_2 - C_6H_5) - CH_2 - CH_2 - N^+(CH_2 - C_6H_5) - (CH_2CH_3)\ 2\ Cl$	3-DEC	455,61
	17-DMC	383,36

Fig. 1. Structural formulae of test compounds.

The data were treated statistically. The tables list the mean values and their standard errors. The significance of difference was assessed by Student's test; for yield, the least significant difference (LSD) was determined at the 95% confidence level (Songailienė & Ženauskas, 1985).

The statistical analysis of siliquae and seeds was carried out in the phase of complete ripeness before harvesting by calculating the arithmetical mean or evaluating the differences at $P \leq 0.05$ (Songailienė & Ženauskas, 1985).

RESULTS AND DISCUSSION

Based on the literature (Bouille & Sotta, 1989) and on our own data (Gavelienė et al., 1998; Милювене, 2000; Novickienė et al., 1998), according to which oilseed rape growth and the formation of its productivity elements are modified by growth regulators of quaternary ammonium salt type, we studied the growth and development of the winter oilseed rape 'Kasimir F_1', and the modification of these processes by the compounds 3-DEC and 17-DMC, which are derivatives of quaternary ammonium salts. According to the data of primary screening and field trials on spring oilseed rape, these compounds were physiologically active as regards oilseed rape stem and root growth, morphogenesis, and productivity element formation (Miliuvienė, 1999; Милювене, 2000). We supposed that development of oilseed rape in autumn could be modified by the retardant compounds 17-DMC and 3-DEC. An analysis carried out one month following the application of these compounds demonstrated that 3-DEC and 17-DMC had an effect on the formation of oilseed rape leaves: their number increased by 11% and 10%, respectively, as compared to the control.

Table 1. Effect of the compounds 3-DEC and 17-DMC on development of the winter oilseed rape 'Kasimir F_1' in autumn (mean for one plant).

Test variant	Leaf number per plant	Diameter of main root, mm	Number of lateral roots	Dry roots mass, g	Root collum diameter, mm	Mono-saccharide content in root collum, mg g^{-1}
Application in 4–5 leaf rosette stage						
Control (H_2O)	6.7±0.27	5.1±0,50	5.3±0.41	0.90±0.08	4.3±0.8	69±1.1
3-DEC, 250 g ha^{-1}	7.5* ±0.36	5.3±0.22	5.9±0.31	1.03*±0.05	5.0*±0.5	80*±0.5
17-DMC, 500 g ha^{-1}	7.4±0.32	5.9*±0.45	6.2*±0.43	1.04*±0.99	5.8* ±0.5	84*±0.5

Note: 40 plants were used per treatment

* Data are statistically reliable at significance level $P \leq 0.05$.

Table 2. Effect of the compounds 3-DEC and 17-DMC on the winter oilseed rape 'Kasimir F_1' stem growth and the diameter of the lower layer (I, II, III) in the phase of flowering.

Test variant	Stem height		Stem diameter					
			I		II		III	
	cm	%	mm	%	mm	%	mm	%
Application in autumn - 4–5 leaf rosette stage								
Control (H_2O)	89.0±1.26	100	4.3	100	4.5	100	4.4	100
3-DEC (250 g ha^{-1})	80.0*±1.40	89	4.7	109	4.9	109	4.9*	112
17-DMC (500 g ha^{-1})	78.0*±1.52	88	4.7	109	4.8	107	4.8*	110
Application in spring - 5–6 true leaves stage								
Control (H_2O)	89.0±1.26	100	4.3	100	4.5	100	4.4	100
3-DEC (250 g ha^{-1})	77.5*±5.1	87	4.8*	112	4.9	109	5.1*	115
17-DMC (500 g ha^{-1})	76.0*±8.8	85	4.8*	112	5.0	111	5.2*	118

Note: 40 plants were used per treatment.
* Data are statistically reliable at significance level $P \leq 0.05$.

According to the literature (Šidlauskas & Rife, 2000), the number of fully expanded leaves in winter oilseed rape during the autumn vegetative growth period is closely related to the number of days after emergence and accumulated growing degree days, as well as interactions among these factors. The compounds 3-DEC and 17-DMC showed a strong effect on root system formation at the end of October: the diameter of the main root increased by 15% and 16%, respectively, the number of lateral roots by 11% and 17%, while dry root mass increased by 13% and 15% as compared to the control (Table 1). Important for plant preparation for wintering is also the root collum development. Under the effect of 3-DEC, the diameter of root collum increased by

19% and 17-DMC by 18% as compared to the control. Under the effect of the compounds not only the diameter of root collum increased, but also monosaccharide accumulation in its tissues. Studies on monosaccharide content in plants exposed to compounds 3-DEC and 17-DMC tested in autumn demonstrated that monosaccharide accumulation in oilseed rape root collum increased by 15% and 21%, respectively (Table 1).

Thus, the compounds 3-DEC and 17-DMC exert a positive effect on the development of plant structure in autumn. The effect of these compounds was observed also in spring: the number of leaves formed in autumn was preserved and that of new leaves exceeded the control. The effect of the compounds proved also to be positive on root growth and the formation of lateral roots.

The aim of spraying these compounds in autumn and spring included determination of the optimum time of their application. Applied in autumn, the compounds showed a positive effect on the development of root collum and thus increased oilseed rape winter endurance while, applied in spring, they enhanced the resistance of oilseed rape stem to lodging and even the formation of productivity elements.

We studied the physiological effect of 3-DEC and 17-DMC on the linear growth and anatomic structure development of oilseed rape stem. Under the influence of the compounds applied in autumn, the diameter of the lower stem increased by 9–12% and by 9.7–10% respectively, as compared to the control. Under the effect of 3-DEC, in spring stem height was hindered by 13% and, under the effect of 17-DMC, by 15% while stem diameter increased by 12, 9, 15% and 12, 11, 18%, respectively, as compared to control (Table 2). An analysis of the second storey stem microtomic sections showed that the inhibition of stem linear growth and the increase of stem diameter were accompanied by a larger primary cortex ring (12–8%) and central stele cylinder (19–10%) and a greater number of vascular bundles in the stele (5–3%) (Table 3).

Table 3. Effect of the compounds 3-DEC and 17-DMC on the anatomical structure of stems of the oilseed rape 'Kasimir F_1' at the end of the flowering phase.

Treatment Application in spring (5–6 true leaves stage)	**Ring width**				**Number of vascular bundles in stele**	
	primary cortex		stele			
	µm	%	µm	%	n	%
Control (H_2O)	367±4.8	100	2405±8.9	100	39±1.0	100
3-DEC (250 g ha^{-1})	410*±3.2	112	2865*±9.1	119	41±0.0	105
17-DMC (500 g ha^{-1})	397±4.1	108	2652*±7.5	110	40±1.0	103

Note: 30 plants were used per treatment

* The difference between treated and untreated plants was significant at $P \leq 0.05$.

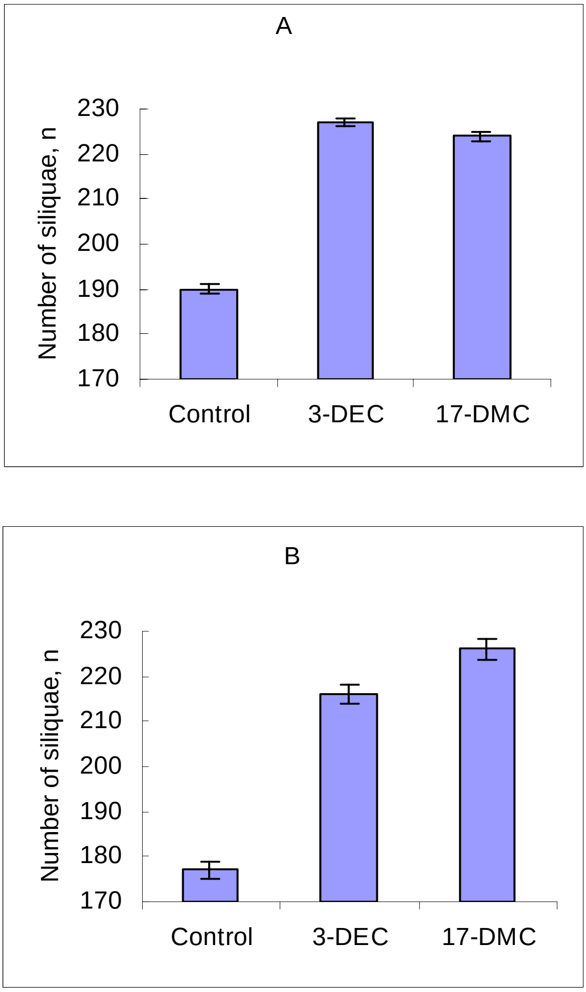


Fig. 2. Effect of the compound 3-DEC (250 g ha^{-1}), 17-DMC (500 g ha^{-1}) (A-applied in autumn at 4–5 leaf rosette stage, B-applied in spring at 5–6 true leaves stage) on winter oilseed rape siliquae number in the phase of full ripeness.

Due to the positive effect of the compounds on the anatomical structure of oilseed rape stems, oilseed rape stem resistance to lodging increased. It was visually seen that the resistance of oilseed rape crops to lodging increased by 1–2 grades. Thus, the increase in the resistance of oilseed rape stem to lodging is associated with hindering stem shooting growth, increase in stem diameter and changes in stem anatomical structure.

To evaluate the effect of the compounds on the formation of yield productivity elements, the number of siliquae, seed number and mass are important indices. The effect of 3-DEC was significantly less as compared to the effect of 17-DMC and significantly higher as compared to the control (Fig. 2). It is of interest that the seed number per siliqua in the control plants proved to be identical both in the main and lateral branch racemes. At the same time, under the effect of 3-DEC in autumn the number of siliquae in the main stem raceme was by 44%, and in the racemes of lateral branches by 63% higher as compared to the control. The retarding effect of 3-DEC and 17-DMC on stem growth and the stimulation of siliquae and seed production resulted in greater seed productivity.

Evaluation of oilseed rape seed yield per plot and calculation of yield per ha in the presence of humidity showed that exposure of plants to the test compounds both in autumn and spring resulted in a statistically reliable extra yield under the effect of 3-DEC 350 and 496 kg ha^{-1}, and 17-DMC 455 and 406 kg ha^{-1}, respectively (Table 4). Thus, by exposing plants to the compound 17-DMC, the lightest extra yield may be obtained in autumn, while under the effect of 3-DEC – in spring. Though the meteorological conditions were comparatively good for oilseed rape wintering and subsequent development, the average yield of control plants was rather low (2,300 kg ha^{-1}).

Our earlier data showed that the effect of 3-DEC and 17-DMC on the processes of spring oilseed rape stem growth (the inhibition of linear stem growth) and the development of stem anatomic structure manifested itself in the changes of stem hormonal balance, particularly in a decrease of the content of GA, zeatin, IAA and an increase of ABA content (Miliuvienė et al., 2003).

Table 4. Effect of the compounds 3-DEC and 17-DMC on the yield of the winter oilseed rape 'Kasimir F_1' (2000–2002) (in the phase of complete ripeness).

Test variant	1000 seed mass		Yield		Extra yield
	g	%	kg ha^{-1}	%	kg ha^{-1}
Application in autumn (at 4–5 leaf rosette stage)					
Control (H_2O)	3.9	100	2300	100	-
3-DEC (250 g ha^{-1})	4.3	110	2650	115	350
17-DMC (500g ha^{-1})	4.2	108	2755	120	455
Application in spring (at 5–6 leaf rosette stage)					
Control (H_2O)	3.9	100	2300	100	-
3-DEC (250 g ha^{-1})	4.3	110	2796	121	496
17-DMC (500g ha^{-1})	4.2	108	2706	117	406

Note: 40 plants were used per treatment

LSD_{05}/R_{05} -226

Seed quality is an important parameter. The data obtained by using the computerised NIR system demonstrated that protein and fat content did not change under the effect of the test compounds. The effect did not result in an increase of glucosynolates. Applying 3-DEC in autumn, glucosynolates decreased by about 12% as compared to the control.

CONCLUSIONS

We can state that the quaternary ammonium salt derivatives 3-DEC and 17-DMC can be used for modification of oilseed rape growth in autumn. They also influence oilseed rape growth and development, as well as the formation of productivity elements in spring. That is why the extra yield of seeds under the effect of the compounds 3-DEC (250 g ha^{-1}) and 17-DMC (500 g ha^{-1}) increases in autumn by 350 and 455 kg ha^{-1} and in spring by 496 and 406 kg ha^{-1}, respectively.

Thus, the data on the study confirm our opinion that these compounds modify the growth and development of oilseed rape plants and the formation of their productivity elements.

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