

# EFFECT OF CHLOROCHOLINE CHLORIDE AND TRIAZOLES – TEBUCONAZOLE AND FLUSILAZOLE ON WINTER OILSEED RAPE (*BRASSICA NAPUS* VAR. *OLEIFERA* L.) IN RESPONSE TO THE APPLICATION TERM AND SOWING DENSITY

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**Abstract:** Winter oilseed rape is strongly responsive to changes in the plant density. Any change in plant density significantly affects the morphological characteristics and yield of the crop. In addition, plant growth habit can be modified by the use of plant growth regulators. Apart from plant growth regulators like eg. chlorocholine chloride some triazoles have dual properties (fungicide and plant growth regulator) eg. tebuconazole. The trials were carried out in the years 2006–2008 at the Institute of Plant Protection – National Research Institute in Poznan (Poland). The treatments consisted of chlorocholine chloride, tebuconazole and flusilazole applied in spring at the growth stage BBCH 30 and BBCH 50 of winter oilseed rape. The field trials were conducted with two sowing densities of winter oilseed rape: 60 seeds/m<sup>2</sup> and 120 seeds/m<sup>2</sup>. The vegetation seasons varied according to the weather conditions, and the second testing year was characterised by drought in May and June. In the experiments plant height, SPAD units, number of siliques per plant, seed number per silique, weight of thousand seeds, yield, protein and fat content in the seeds were estimated. The increase of silique numbers per plant appeared only at the lower sowing density as a result of the application of tebuconazole or a mixture of tebuconazole with CCC. Flusilazole had a positive impact on seed number per silique. At both sowing densities, changes in the weight of a thousand seeds under the influence of the test preparations, were observed only in that year which had wet weather conditions. A more favourable effect of the test substances on the weight of a thousand seeds was obtained at the lower sowing density. All the tested substances positively affected SPAD unit values at the lower sowing density. At the higher sowing density, SPAD unit values increased after the application of flusilazole, and after a mixture of CCC + tebuconazole. The tested substances had a positive impact on plant yield but they did not affect the protein and fat content in oilseed rape seeds.

**Key words:** chlorocholine chloride, chlorophyll, flusilazole, growth regulation, tebuconazole, winter oilseed rape, yield components

## INTRODUCTION

The use of plant growth regulators is an important element in crop production. In the case of plants such as cereals and oilseed rape, plant growth regulators are used primarily to prevent lodging of plants. Current research, however, has shown that plant growth regulators are not only anti-lodging agents, but also substances which to a large extent, permit full use of the plant potential even in the absence of lodging. It is estimated that plant growth regulators account for only 3–4% of all plant protection products on a global scale. This group is still dominated by relatively old substances, such as: etephon, chlorocholine chloride, and mepiquat chloride. There has been a smaller group of substances placed on the market relatively recently, including: trinexapac-ethyl or prohexadione-calcium. In recent years, triazole fungicides acting as growth regulators in oilseed rape cultivation, introduced to the market in the 1990s, have become an alternative to the previously used growth retardants (Rajala and Peltonen-Sainio 2000; Rademacher 2000; Rademacher and Bucci 2002).

Oilseed rape is highly susceptible to the occurrence of diseases such as light leaf spot, phoma and white mold. These diseases can cause a decrease in seed yield of up to 1 t/ha. Triazole fungicides are commonly used to protect oilseed rape against these fungal diseases. Some of these fungicides have the additional properties of plant growth regulators (Luster and Miller 1993; Coules *et al.* 2002). From a biochemical point of view, the properties of some triazoles are due to their dual effect on plants: inhibition of the biosynthesis of gibberellins in the plant (retardant properties), and inhibition of the biosynthesis of sterols (fungicide properties). Substances with this double action include metconazole and tebuconazole, while scientific reports have noted, for instance, that flusilazole showed only a fungicidal activity. Recent scientific reports have shown that the properties of metconazole and tebuconazole go significantly beyond having just retardation properties. It has also been shown that these substances can be used for oilseed rape plant population modelling, and such action is closely related to plant density (Henneken *et al.* 2000; Rademacher 2000; Berry and Spink 2009).

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Winter oilseed rape is highly responsive to changes in the density of plants, and any change in the density has a significant impact on the variation of morphological features and yield components. In high density, plants strongly compete with each other and do not endure winter well. In high density, plants also establish fewer branches, and are more susceptible to lodging and fungal diseases. Plant density significantly affects the growth habit of plants in the canopy. The optimum plant density of oilseed rapeseed is, depending on the growing region, from 30–60/m<sup>2</sup> or from 40–80/m<sup>2</sup>. In addition to plant density, the biological yield of winter oilseed rape is the product of the growth rate and the duration of the vegetative period. Both these factors determine the amount of light reaching into the canopy and become yield factors which may change various plant structural components (Muśnicki 1989; Budzyński and Ojczyk 1996; Kuchtova *et al.* 1996; Zając *et al.* 1999; Diepenbrock 2000; Chen *et al.* 2005; Kotecki *et al.* 2007; Pusz 2007a,b; Sincik *et al.* 2010). Siliques are the main photosynthetic apparatus for oilseed rape. The silique numbers per area unit are the component of yield that is subject of the biggest changes in relation to the density of plants. To maximize the yield potential of oilseed rape, there must be an even ripening of the siliques. Even ripening will reduce the fall of siliques and ensure that the seeds fill the siliques evenly. It is estimated, that under favourable conditions, the potential of oilseed rape can be as high as 130,000 seeds/m<sup>2</sup>. In addition, it has been proven that the best use of the yield potential of oilseed rape is reached at 5,000–10,000 siliques/m<sup>2</sup> (Wielebski 2007a; Berry and Spink 2009).

The first aim of this study was to compare the effect of chlorocholine chloride retardant, the two triazoles fungicides: tebuconazole and flusilazole, and the mixture of chlorocholine chloride with tebuconazole, and the mixture of chlorocholine chloride with flusilazole, on selected morphological and physiological properties and yield. The second aim was to compare the qualitative and quantitative characteristics of winter oilseed rape, according to the date of the application of tested substances and the sowing density.

## MATERIALS AND METHODS

The field trials were carried out in 2006–2008 at the Field Experimental Station in Winna Góra (52°12'N; 17°27'E), owned by the Institute of Plant Protection National – Research Institute in Poznań, Poland. Two separate experiments were conducted in winter oilseed rape cv. Californium with two sowing rates: 60 seeds per m<sup>2</sup> and 120 seeds per m<sup>2</sup>. The experiments were established in completely randomized block design with four replications on luvisoil soil. Depending on the year, the pH ranged from 5.7 to 5.8. The size of every plot was 16.5 m<sup>2</sup>. The row spacing was 0.25 m. Soil tillage, fertilization, and other crop management was done according to the recommendations for oilseed rape. In both of the experimental years, winter wheat had been the previous crop. Every experiment consists of two experimental factors: I – preparation, and II – term of application. The test objects in each experiment included:

A. Untreated; B. Chlorocholine chloride – CCC (550 g/ha) applied at the growth stage BBCH 30 or BBCH 50; C. Tebuconazole (250 g/ha) applied at the growth stage BBCH 30 or BBCH 50; D. Flusilazole (450 g/ha) applied at the growth stage BBCH 30 or BBCH 50; E. A mixture of CCC with Tebuconazole (337.5 + 200 g/ha) applied at the growth stage BBCH 30 or BBCH 50; F. A mixture of CCC with Flusilazole (337.5 + 450 g/ha) applied at the growth stage BBCH 30 or BBCH 50.

The spraying was performed using the Gloria field sprayer with a compressed air cylinder. The sprayer had a capacity of 4 litres. Two hundred litres of water per hectare were used, at a pressure of 3 bar. All test objects were subject to standard protection against weeds, pathogens, and pests. During vegetation, the following parameters were evaluated: plant height, SPAD units, silique numbers per plant, and seed number per silique. After harvest, seed yield, the weight of a thousand seeds as well as protein and fat content in the seeds were determined. To determine SPAD units in plants, N-tester apparatus and the SPAD method were used. On the leaf, SPAD measurement covered 2 mm x 3 mm. Two weeks after the BBCH 50 stage, SPAD units were measured. Silique number per plant, and seed number per silique were determined based on 25 randomly selected plants/siliques from each plot. Siliques were collected from different parts of the plants (base, middle, top), at the beginning of technical maturity. The weight of a thousand seeds was calculated based on 4 samples of 250 oilseed rape seeds from each test combination. Oilseed rape was harvested with the Wintersteiger plot harvester. Seed yield was determined at 12% moisture. The qualitative analysis of seeds was performed using an Inframatic 8100 analyser.

The results of the studies were analyzed statistically according to the experimental model, in the program FR – ANALWAR – 4.3. Significance of differences was determined with Tukey's test, at the significance level  $p = 0.05$ .

### Weather conditions (Table 1)

The course of the weather conditions was determined based on measurements made at a field meteorological station located at the Experimental Station in Winna Góra. Weather analysis was specified according to Sielianinov's coefficient, determined as a sum of a month's precipitation · 10/mean of monthly temperature · number of days in the month (Molga 1970). The winter oilseed rape growing seasons differed in terms of weather conditions. The autumn growing season in the first test year was characterised by a shortage of rainfall in August and September, while in the following test season there was significant rainfall in those months. Similar weather conditions in both years occurred in October. Vegetation in the spring of 2007 was characterised by very good moisture and thermal conditions in March, May, June, and July. A semi-drought period occurred only in April. In the next test year, less favorable weather conditions occurred. Although there was excess rainfall in March, there was drought during the critical period for the crop, *i.e.* in May and June.

## RESULTS

### Height of plants (Table 2, 3)

In the experiment conducted at a lower sowing rate (60 seeds/m<sup>2</sup>), in the 2006/2007 season, shorter plants were obtained only after applying the mixture of chlorocholine chloride (CCC) with tebuconazole at the BBCH 30 stage. In this test object, plants were 21 cm (15%) shorter compared to the control plants. The results obtained for this object were not, however, confirmed in the next test year. In 2007/2008, a decrease in plant height by 18 cm (13%) was observed only after the application of CCC at the early stages. But in both test years a stimulating effect of flusilazole on oilseed rape growth at the lower plant density, was observed. Depending on the treatment period and test year, plant height increased by 8–16 cm (6–11%) compared to the control plants. The significant impact of flusilazole on plant height was not recorded in the experiment with the higher plant density (120 seeds/m<sup>2</sup>).

In the first test year, there was a significant decrease in plant height at the higher plant density (120 seeds/m<sup>2</sup>) after the application of CCC with tebuconazole at the BBCH 30 stage. The plants were 16 cm (11%) shorter compared to the control plants. In the next test year, regardless of the application date, all plants treated with CCC and tebuconazole, separately or in combination, were shorter than the control plants. In 2007/2008, CCC reduced plant height by 15–20 cm (10–14%), tebuconazole reduced plant height by 18–26 cm (13–18%), and a mixture of both substances reduced plant height by 24–25 cm (16–17%). In that experimental year, the mixture of CCC with flusilazole used at the BBCH 30 stage, reduced plant height by 15 cm (10%). In contrast to the results of the experiment with a lower plant population, with a high sowing rate, there was no significant effect of flusilazole applied separately on the height of plants in any test year.

### Lodging

No lodging of the crop occurred in any test year.

### Greenness indicator – SPAD units (Table 2, 3)

Plant density had a significant impact on SPAD units in the leaves. In the first test year, at a lower plant density (60 seeds/m<sup>2</sup>), an increase of SPAD units was achieved in almost all test objects. No changes were observed after the application of CCC at the BBCH 30 stage, and after a mixture of CCC with flusilazole at the BBCH 50 stage. The highest SPAD units were found in objects treated with tebuconazole at the BBCH 30 stage, and in objects treated with CCC with flusilazole at the BBCH 30 stage (an increase of more than 20%). In the same experimental year, in high plant density (120 seeds/m<sup>2</sup>), an increase of SPAD units in leaves was confirmed only for flusilazole, and for the mixture of CCC with tebuconazole. The next test year (2007/2008), confirmed the beneficial effect of tebuconazole, and the beneficial effect of the mixture of CCC with tebuconazole on the SPAD unit values – at the lower plant density, and the beneficial effect of flusilazole (BBCH 50), and the beneficial effect of the mixture of CCC with tebuconazole – in higher plant density.

### Silique numbers per plant and seed number per silique (Table 4, 5)

In both years, significant changes in silique numbers per plant were obtained only at the lower plant density (60 seeds/m<sup>2</sup>). In 2006/2007, a higher number of siliques was found in objects treated with tebuconazole or in objects treated with a mixture of tebuconazole with CCC. Depending on the application time of the substances, silique numbers increased by 22% after the application of tebuconazole at the BBCH 30 stage, and by 19% at the BBCH 50 stage. The mixture of tebuconazole with CCC, contributed to the increase of siliques number by 17% (after application at the BBCH 30 stage) and by 11% (BBCH 50). In the second test year (2007/2008), significant changes occurred only after the application of tebuconazole. The increase in silique numbers was 20% (BBCH 30) and 13% (BBCH 50) compared to the control plants.

Significant changes in seed number per silique were obtained only in the object treated with flusilazole at the BBCH 30 stage (growing season 2006/2007). After the application of the fungicide, seed number per silique increased by 35%. When using a higher plant density (120 seeds/m<sup>2</sup>), there were no significant changes in the number of siliques per plant and in number of seeds per silique, after application of the tested substances.

### Weight of a thousand seeds (Table 6, 7)

Only in the first test year (2006/2007), was there a significant effect of the tested substances on the weight of a thousand seeds at a lower plant density (60 seeds/m<sup>2</sup>). In this case, the weight of 1,000 seeds increased after the application of CCC, tebuconazole, and flusilazole regardless of the application period, and after the application of the mixture of CCC with tebuconazole at the growing stage BBCH 30, and the mixture of CCC with flusilazole at the growing stage BBCH 30. In these test combinations, the weight of 1,000 seeds increased by 10–18% in relation to the control plants. In a higher sowing density (120 seeds/m<sup>2</sup>), the application of CCC (BBCH 50), the application of tebuconazole (BBCH 30), and the application of CCC with tebuconazole (BBCH 30) had a significant effect on this feature. The greatest increase in the weight of 1,000 seeds – 20% compared with the control plants – was achieved with a mixture of CCC with tebuconazole at the BBCH 30 stage. The results obtained in both sowing densities were not confirmed in the next test year (2007/2008).

### Yield (Table 6, 7)

In both years, the test substances increased yields at the lower sowing density. In the first test year, there was a significant increase in the yield after the application of all tested substances and mixtures, regardless of the application term, compared to the control plants. This does not concern CCC in BBCH 30, and it does not concern CCC + tebuconazole at BBCH 50. The highest yield was recorded after the application of flusilazole at BBCH 50 (12%). Flusilazole applied at the earlier growth stage increased yield about 10%. Yield increase after the application of CCC + flusilazole varied, depending on the term of application, and consequently the yield increase was 5–10% compared to the control. Tebuconazole used alone

Table 1. Sielianinov's coefficient at the Experimental Station in Winna Góra

Years	Month							
	III	IV	V	VI	VII	VIII	IX	X
2006	-	-	-	-	-	0.4	0.8	1.3
2007	2.1	0.7	1.7	1.6	2.4	1.1	1.4	1.2
2008	3.3	1.4	0.3	0.2	1.0	-	-	-

0-0.5 – drought period  
 0.51-1.0 – semi-drought period  
 1.01-2.0 – relatively moist period  
 > 2.01 – high-moist period

Table 2. Influence of chlorocholine chloride and triazols fungicides on plant height, and the greenness indicator in leaves of winter oilseed rape at the sowing density of 60 seeds/m<sup>2</sup>

Treatment	Dose per ha [g/ha]	Term of application in BBCH	Sowing density 60 seeds/m <sup>2</sup>					
			plant height [cm]			SPAD units value		
			2006/2007	2007/2008	average of years	2006/2007	2007/2008	average of years
Untreated	-	-	142	145	144	613	703	658
Chlorocholine chloride	540	30	143	127	135	668	727	698
		50	144	134	139	677	696	687
Tebuconazole	250	30	142	139	141	736	771	754
		50	139	146	143	690	761	726
Flusilazole	375	30	150	148	149	705	748	727
		50	141	161	151	702	747	725
Chlorocholine chloride + Tebuconazole	337.5+200	30	121	148	135	718	760	739
		50	138	136	137	711	782	747
Chlorocholine chloride + Flusilazole	337.5+450	30	134	150	142	738	706	722
		50	142	145	144	613	703	658
LSD 0.05			13.2	15.4	ns	60.5	56.1	67.3

ns – not significant differences

Table 3. Influence of chlorocholine chloride and triazols fungicides on plant height, and the greenness indicator in leaves of winter oilseed rape at the sowing density of 120 seeds/m<sup>2</sup>

Treatment	Dose per ha [g/ha]	Term of application in BBCH	Sowing density 120 seeds/m <sup>2</sup>					
			plant height [cm]			SPAD unit values		
			2006/2007	2007/2008	average of years	2006/2007	2007/2008	average of years
Untreated	-	-	147	146	147	650	683	667
Chlorocholine chloride	540	30	147	126	137	700	691	696
		50	152	131	142	689	743	716
Tebuconazole	250	30	150	128	139	702	748	725
		50	144	120	132	686	708	697
Flusilazole	375	30	141	134	138	755	712	734
		50	151	136	144	733	801	767
Chlorocholine chloride + Tebuconazole	337.5+200	30	131	121	126	768	776	772
		50	148	122	135	783	798	791
Chlorocholine chloride + Flusilazole	337.5+450	30	147	131	139	697	721	709
		50	147	136	142	650	683	667
LSD 0.05			15.8	12.6	14.3	65.3	68.7	70.1

Table 4. Influence of chlorocholine chloride and triazols fungicides on number of siliques/m<sup>2</sup> and number of seeds per silique of winter oilseed rape at the sowing density of 60 seeds/m<sup>2</sup>

Treatment	Dose per ha [g/ha]	Term of application in BBCH	Sowing density 60 seeds/m <sup>2</sup>					
			number of siliques/plant			number of seeds/silique		
			2006/2007	2007/2008	average of years	2006/2007	2007/2008	average of years
Untreated	–	–	119	122	121	20	22	21
Chlorocholine chloride	725	30	120	125	123	23	23	23
		50	126	131	129	21	25	23
Tebuconazole	250	30	146	148	147	24	23	24
		50	142	138	140	21	20	21
Flusilazole	450	30	127	124	126	27	24	26
		50	124	130	127	22	24	23
Chlorocholine chloride + Tebuconazole	337.5+200	30	139	129	134	21	21	21
		50	132	126	129	21	23	22
Chlorocholine chloride + Flusilazole	337.5+450	30	125	126	126	23	24	24
		50	123	130	127	21	20	21
LSD 0.05			12.7	13.1	13.7	6.7	ns	ns

ns – not significant differences

Table 5. Influence of chlorocholine chloride and triazols fungicides on number of siliques/m<sup>2</sup> and number of seeds per silique of winter oilseed rape at the sowing density of 120 seeds/m<sup>2</sup>

Treatment	Dose per ha [g/ha]	Term of application in BBCH	Sowing density 120 plants/m <sup>2</sup>					
			number of siliques/plant			number of seeds/silique		
			2006/2007	2007/2008	average of years	2006/2007	2007/2008	average of years
Untreated	–	–	86	89	88	20	22	21
Chlorocholine chloride	725	30	90	95	93	22	19	21
		50	91	99	95	22	24	23
Tebuconazole	250	30	93	86	90	24	21	23
		50	84	101	93	23	23	23
Flusilazole	450	30	93	94	94	24	22	23
		50	84	97	91	24	24	24
Chlorocholine chloride + Tebuconazole	337.5+200	30	83	90	87	25	22	24
		50	86	88	87	22	25	24
Chlorocholine chloride + Flusilazole	337.5+450	30	97	96	97	25	25	25
		50	93	85	89	22	21	22
LSD 0.05			ns	ns	ns	ns	ns	ns

ns – not significant differences

Table 6. Influence of chlorocholine chloride and triazols fungicides on weight of 1,000 seeds, and seed yield of winter oilseed rape at the sowing density of 60 seeds/m<sup>2</sup>

Treatment	Dose per ha [g/ha]	Term of application in BBCH	Sowing density 60 plants/m <sup>2</sup>					
			weight of 1,000 seeds [g]			yield [t/ha]		
			2006/2007	2007/2008	average of years	2006/2007	2007/2008	average of years
Untreated	–	–	4.17	4.59	4.38	4.24	4.25	4.25
Chlorocholine chloride	725	30	4.65	4.47	4.56	4.35	4.08	4.22
		50	4.73	4.71	4.72	4.48	4.40	4.44
Tebuconazole	250	30	4.59	4.73	4.66	4.73	4.98	4.86
		50	4.63	4.56	4.60	4.69	4.66	4.68
Flusilazole	450	30	4.58	4.68	4.63	4.68	4.59	4.64
		50	4.94	4.63	4.79	4.76	5.09	4.93
Chlorocholine chloride + Tebuconazole	337.5+200	30	4.60	4.69	4.65	4.74	4.40	4.57
		50	4.49	4.72	4.61	4.24	4.95	4.60
Chlorocholine chloride + Flusilazole	337.5+450	30	4.65	4.65	4.65	4.46	4.58	4.52
		50	4.50	4.72	4.61	4.65	5.42	5.04
LSD 0.05			0.38	ns	ns	0.215	0.196	0.397

ns – not significant differences

Table 7. Influence of chlorocholine chloride and triazols fungicides on the weight of 1,000 seeds, and seed yield of winter oilseed rape at the sowing density of 120 seeds/m<sup>2</sup>

Treatment	Dose per ha [g/ha]	Term of application in BBCH	Sowing density 120 plants/m <sup>2</sup>					
			weight of 1,000 seeds [g]			yield [t/ha]		
			2006/2007	2007/2008	average of years	2006/2007	2007/2008	average of years
Untreated	–	–	4.11	4.78	4.45	4.21	4.55	4.38
Chlorocholine chloride	725	30	4.26	4.68	4.47	4.58	4.80	4.69
		50	4.46	4.71	4.59	4.52	5.14	4.83
Tebuconazole	250	30	4.46	4.76	4.61	4.40	5.34	4.87
		50	4.20	4.74	4.47	4.39	5.50	4.95
Flusilazole	450	30	4.35	4.75	4.55	4.26	5.49	4.88
		50	4.37	4.75	4.56	4.17	5.80	4.99
Chlorocholine chloride + Tebuconazole	337.5+200	30	4.95	4.83	4.89	4.61	5.99	5.30
		50	4.18	4.76	4.47	4.49	5.40	4.95
Chlorocholine chloride + Flusilazole	337.5+450	30	4.39	4.79	4.59	4.66	5.23	4.95
		50	4.15	4.49	4.32	4.71	5.22	4.97
LSD 0.05			0.29	ns	ns	0.405	0.354	0.395

ns – not significant differences

Table 8. Influence of chlorocholine chloride and triazols fungicides on fat and protein content in seeds of oilseed rape at the sowing density of 60 seeds/m<sup>2</sup>

Treatment	Dose per ha [g/ha]	Term of application in BBCH	Sowing density 60 plants/m <sup>2</sup>					
			fat content [%]			protein content [%]		
			2006/2007	2007/2008	average of years	2006/2007	2007/2008	average of years
Untreated	–	–	45.6	44.7	45.2	22.8	20.8	21.8
Chlorocholine chloride	725	30	45.4	44.3	44.9	22.5	21.3	21.9
		50	45.3	44.8	45.1	22.6	21.1	21.8
Tebuconazole	250	30	45.8	44.9	45.3	22.4	21.4	21.9
		50	45.5	45.0	45.3	22.5	20.6	21.6
Flusilazole	450	30	45.8	44.9	45.4	22.2	20.9	21.6
		50	45.5	44.7	45.1	22.4	21.0	21.7
Chlorocholine chloride + Tebuconazole	337.5+200	30	45.4	44.9	45.1	22.6	21.1	21.9
		50	45.4	44.8	45.2	22.3	21.0	21.6
Chlorocholine chloride + Flusilazole	337.5+450	30	45.5	44.6	45.1	22.5	21.3	21.9
		50	45.6	45.0	45.3	22.4	20.9	21.6
LSD 0.05			ns	ns	ns	ns	ns	ns

ns – not significant differences

Table 9. Influence of chlorocholine chloride and triazols fungicides on fat and protein content in seeds of oilseed rape at the sowing density of 120 seeds/m<sup>2</sup>

Treatment	Dose per ha [g/ha]	Term of application in BBCH	Sowing density 120 plants/m <sup>2</sup>					
			fat content [%]			protein content [%]		
			2006/2007	2007/2008	average of years	2006/2007	2007/2008	average of years
Untreated	–	–	45.3	44.8	45.1	22.2	21.5	21.9
Chlorocholine chloride	725	30	44.5	44.7	44.6	23.2	20.9	22.1
		50	45.1	44.9	45.0	22.4	20.6	21.5
Tebuconazole	250	30	45.2	44.8	45.0	22.2	20.6	21.4
		50	44.8	44.6	44.7	22.5	20.9	21.7
Flusilazole	450	30	45.2	45.0	45.1	22.3	20.8	21.6
		50	45.2	44.2	44.7	22.4	21.5	22.0
Chlorocholine chloride + Tebuconazole	337.5+200	30	44.9	44.8	44.8	22.6	20.9	21.8
		50	45.0	44.3	44.7	22.7	21.4	22.1
Chlorocholine chloride + Flusilazole	337.5+450	30	45.3	44.4	44.8	22.4	21.4	21.9
		50	45.3	44.8	45.1	22.1	20.6	21.4
LSD 0.05			ns	ns	ns	ns	ns	ns

ns – not significant differences

enhanced the yield by 11–12%. A similar yield value was obtained after the application of CCC + tebuconazole at BBCH 30 (12%). In 2007/2008, at a sowing density of 60 seeds/m<sup>2</sup>, a significant yield increase was observed compared to the control object after the application of tebuconazole (10–17%), and after the application of flusilazole (8–20%), and after the application of CCC + tebuconazole at BBCH 50 (16%) and after the application of CCC + flusilazole (8–27%). The largest grain yield (27%) was observed in 2007/2008 after a CCC + flusilazole application at BBCH 50 stage.

Significant changes in seed yield in a sowing density of 120 seeds/m<sup>2</sup> were only observed in 2006/2007, in the object treated with a mixture of CCC with flusilazole. The increase in yield in this object was 11–12% compared to the control plants. However, the next test year was characterised by a significant increase in seed yield after application of all the used substances, except CCC at BBCH 30 stage. Chlorocholine chloride applied at BBCH 50 enhanced the yield by 13%. A higher yield increase was observed after the application of CCC + flusilazole (15%) in both growth stages of winter oilseed rape as well as tebuconazole used at BBCH 30 (17%), and after CCC + tebuconazole (19%) used at BBCH 50. A yield increase of 21% was recorded for tebuconazole applied at BBCH 50 and a yield increase of 21% was recorded for flusilazole applied at BBCH 30. The best yield enhancing actions were observed following the application of flusilazole at BBCH 50 (27%), and following the application of CCC + tebuconazole at BBCH 30 (32%).

#### Fat and protein content in seeds (Table 8, 9)

The results obtained in the experiments do not indicate any influence of the tested substances on fat and protein content in oilseed rapeseed in both sowing densities.

## DISCUSSION

Based on the results obtained in this study it can be concluded that the reduction of winter oilseed rape height under the influence of anti-gibberelin plant growth regulators, depended on the weather conditions and ranged from 12.4% in 2007/2008 to 14.8% in 2006/2007 at a sowing density of 60 seeds/m<sup>2</sup>, and from 10.9% in 2006/2007 to 17.8% in 2007/2008 at a sowing density of 120 seeds/m<sup>2</sup>. Bączkowska *et al.* (2006) report that the application of CCC significantly reduces plant height. The authors found that use of this substance at the BBCH 30 stage, contributes to the shortening of plants by 5–30%. The height reduction of oilseed rape under the influence of CCC is also confirmed by Armstrong and Nicol (1991). The results obtained in our own experiments, conducted in a higher sowing density, indicate that the effect of CCC as well as tebuconazole on the height of oilseed rape plants, strongly depends on the weather conditions, and that the treatment period does not significantly affect the final result.

The tests conducted by Berry and Spink (2009) demonstrated, however, that the period of the application of the anti-gibberelin triazole fungicide is vital to the effective reduction of plant height. These authors showed that

the fungicide applied at the BBCH 45 stage had a lower effect on the reduction of plant height compared with application in the previous stages. Similarly, Toboła *et al.* (2008) found that triazole fungicide, acting as a growth retardant, used in the late stage of development (BBCH 50 stage), can reduce the height of oilseed rape plants by 6%. By contrast, Cieśllicki and Muśnicki (2006) reported that tebuconazole applied in the spring did not affect the height of oilseed rape plants. Similarly Cieśllicki and Toboła (2007), on the basis of their research, reported that triazole fungicide acting as a regulator of plant growth and development (metconazole), does not show any growth retardant effect on oilseed rape plants. Slightly different results were showed by Mączyńska *et al.* (2007), and Gundula *et al.* (1990), indicating that triazoles significantly reduce the height of oilseed rape plants. In our study, the greatest impact on reducing the height of plants under the influence of both chlorocholine chloride and tebuconazole, was achieved in one test year at a sowing density of 120 seeds/m<sup>2</sup>. The year in which such changes occurred was characterised by a significant deficiency of rainfall in May and June.

There is a lack of scientific literature about the effect of the triazole fungicide which had no anti-gibberelin effect – flusilazole – on the height of plants. However, in our study, at a lower sowing density (60 seeds/m<sup>2</sup>), a stimulating effect of this substance on the growth of plants was obtained in 2006/2007 after application at BBCH 30 stage, and in 2007/2008 at BBCH 50 growth stage.

Reports in literature discussing the impact of plant growth regulators on the height of oilseed rape plants, are not always consistent. The results in scientific publications on plant height in higher and lower sowing densities, are different. Such authors as: Wielebski and Wójtowicz (1998, 2001), Wielebski (2007a, b), Muśnicki (1989) reported that plants growing in a higher sowing density are significantly shorter than those growing in a lower sowing density. Malarz *et al.* (2006) disagree, claiming that a doubling of the number of plants per area unit increases the height of the plants.

The greater influence of the plant growth regulators, on the greenness indicator of plants at the lower sowing density (60 seeds/m<sup>2</sup>) proven in our study, is confirmed in scientific publications. The production of chlorophyll in the leaves requires good lighting conditions, and higher plant populations strongly compete for light (Dipenbrock 2000). According to Gitelson *et al.* (2003), the content of chlorophyll in plants measured *in situ* is a reliable indicator of the physiological status of the plant. The amount of solar radiation absorbed by plants is reflected in the amount of pigment. The amount allows us to directly determine the photosynthetic potential of plants. Chlorophyll content is also a measure of the stress level in a plant. The SPAD tests conducted as part of this study, demonstrated the greater influence of the regulators on the growth and development on this feature, in the year characterised by very good moisture and thermal conditions. The relationship between chlorophyll content and the application of growth regulators is also pointed out by Matysiak (2004) and Mir *et al.* (2010). Matysiak *et al.* (2010), after the application of tebuconazole acting

as a growth regulator, obtained an 18–21% increased amount of SPAD units. A higher value for this feature occurred after application at BBCH 30 growth stage. Similar results were obtained in this study – there were higher SPAD units in plants treated with tebuconazole at BBCH 30 stage, but the effect of this substance on SPAD unit values in the leaves was observed only at the lower sowing density.

There are a lack of studies in the literature concerning the effect of flusilazole on SPAD unit values in oilseed rape leaves. The tests presented in this paper indicate that flusilazole, in wet conditions, increases the amount of SPAD units in oilseed rape leaves.

The relation between the sowing rate and silique numbers per plant is an issue extensively discussed in the scientific literature: Muśnicki (1989), Rudko and Szot (1996), Wielebski and Wójtowicz (1998), Sincik *et al.* (2010), Sylwester-Bradley *et al.* (2002), Wielebski (2007a, b). These authors agree that the reduction in the number of plants per unit area has a positive influence on the formation of siliques in the plant. The effect of plant growth regulators on this feature is clear. In the tests presented in this paper, regardless of the weather conditions during vegetation (in both years), tebuconazole showed a stimulating effect on the formation of siliques per plant, but only at the lower sowing density. At the same low sowing density, there was also an increase in silique numbers per plant under the influence of the mixture of tebuconazole with CCC, but only in one test year. These results might suggest that the effect of this mixture depends more on the weather than the effect of tebuconazole. A stimulating effect of triazole preparations on the formation of siliques is confirmed by Berry and Spink (2009), whereas no effect on this feature has been demonstrated by Toboła *et al.* (2008), Cieśllicki and Muśnicki (2006), Cieśllicki and Toboła (2007), Matysiak *et al.* (2010).

The impact of plant growth regulators and flusilazole on the increase in the weight of a thousand seeds in just one test year of our studies, demonstrated the close relation between the test substances and weather conditions. Changes in this feature occurred since the year was characterised by more favourable moisture and thermal conditions. The tests demonstrated that the increased weight of a thousand oilseed rape seeds is due to the clear relation between the two substances and the sowing rate. Greater changes to this property under the influence of plant growth regulators, were obtained at the lower sowing density. The increased weight of a thousand seeds found as a result of the application of the triazole fungicide acting as the regulator of plant growth and development, with a lower sowing rate, is confirmed by Berry *et al.* (2009). However, Pits *et al.* (2008) reported no effect of these substances on the weight of a thousand seeds of oilseed rape. Bączkowska *et al.* (2006) draw attention to the beneficial effects of CCC, and Brachaczek *et al.* (2011) found no effect of flusilazole on this yield component. Although the studies by Bączkowska *et al.* (2006) coincide with the results of our experiments, it was demonstrated that flusilazole could significantly increase the weight of a thousand seeds of winter oilseed rape.

A positive impact of CCC on the yield of crops is confirmed by Bączkowska *et al.* (2006), and the positive impact of triazoles is confirmed by Pits *et al.* (2008), Toboła *et al.* (2008), and Matysiak *et al.* (2010). Berry *et al.* (2009) reported that triazole fungicide with plant growth regulation properties, has a beneficial effect on seed yield only at the lower sowing density. Their findings were partly confirmed in our experiments. We found, that at the lower sowing density in both test years, there was an increase in the yield of oilseed rape after the application of tebuconazole, while at the higher sowing density, a significant yield increase was observed only in one test year. In the studies by Matysiak *et al.* (2010), tebuconazole increased plant yield to a greater extent than CCC. These results are confirmed by our results. The effect of a mixture of these preparations on winter oilseed rape yield differed in the test years.

The tests conducted at the Institute of Plant Protection – the National Research Institute, showed no effects of the test plant growth regulators on the percentage of fat and protein content in plants. No significant differences were obtained for flusilazole, which was in contrast to the results obtained by Brachaczek *et al.* (2011). In their studies, they demonstrated the impact of flusilazole on the quality of winter oilseed rape seeds.

## CONCLUSIONS

1. At the lower sowing density, the height of oilseed rape plants decreased after the application of CCC or a mixture of CCC with tebuconazole at the BBCH 30 growth stage (one test year). At the higher sowing density, a significant reduction of plants, regardless of the stage of development at the time of the treatment, occurred as a result of the application of CCC, or tebuconazole, or a mixture of these substances (one test year). Flusilazole increased the height of plants at the lower sowing density.
2. All the tested substances positively affected SPAD unit values at the lower sowing density. At the higher sowing density, SPAD unit values increased after the application of flusilazole, and after a mixture of CCC + tebuconazole (for both treatment periods).
3. The increase of silique numbers per plant appeared only at the lower sowing density as a result of the application of tebuconazole or a mixture of tebuconazole with CCC (for both treatment periods). In one test year, at the lower sowing density, flusilazole had a positive impact on seed number per silique.
4. At both sowing densities, changes in the weight of a thousand seeds under the influence of the test preparations, were observed only in that year which had wet weather conditions. A more favourable effect of the test substances on the weight of a thousand seeds was obtained at the lower sowing density. At the higher sowing density, an increase in the weight of a thousand seeds was obtained for all the tested mixtures except CCC + tebuconazole applied at the later stage of development in wet weather conditions, and except CCC + flusilazole applied at the later stage of development in wet weather conditions. At the higher sow-



ing density, the weight of a thousand seeds increased after the application of CCC (BBCH 50), and after the application of tebuconazole (BBCH 30), and after the application of the mixture of CCC + flusilazole (BBCH 30), but the increase after these applications took place only in wet weather conditions.

5. In general, the test substances had a positive impact on plant yield. In the first experimental year at the lower sowing density, there was a much higher yield after the application of flusilazole, and tebuconazole, and the tebuconazole mixture with CCC, while in 2007/2008 the highest yield was recorded for CCC + flusilazole (BBCH 50). At the higher sowing density, in the first test year, only the mixture of CCC+ flusilazole had a positive impact on the yield. In 2007/2008, almost all treatments positively affects this feature (except CCC applied at BBCH 30). In this year, CCC applied with tebuconazole at the growth stage BBCH 30 had the best effect on yield.
6. The tested substances did not affect the protein and fat content in oilseed rape.

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