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THE PREVENTION OF TIPBURN ON CHINESE CABBAGE (*BRASSICA RAPA* L. VAR. *PEKINENSIS* (Lour.) Olson) WITH FOLIAR FERTILIZERS AND BIOSTIMULATORS

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Abstract

Investigations were carried out in 2008-2010 on Chinese cabbage (*Brassica rapa* L. var. *pekinensis* (Lour.) Olson). The main problem in cultivation of this vegetable is physiological disorder – tipburn. It is connected with low level of calcium in young leaves and with water deficiency. In 2008, seeds of Chinese cabbage were sown twice, in April and July. In July, the day temperature was high (25-30 °C) and relative air humidity was low (35-50%). In these conditions, the young leaves were injured heavily. Rotting was caused by the activity of bacteria *Pectobacterium carotovorum* subsp. *carotovorum* (Jones) Hauben et al. However, three times foliar application of 1.5% calcium nitrate or 1.5% Wapnovit significantly reduced the tipburn. Also spraying with 0.03% of Tytanit (containing ions of titanium) or with 2.5% of Biochikol 020 PC (containing chitosan) gave similar effect. In these conditions, application of 1.5% K-300 (containing potassium oxide and ammonium nitrate) exacerbated symptoms of tipburn. Application of Wapnovit or Tytanit reduced instantly rotting of heads contrary to the application of their mixture. In autumn cultivation, when the relative air humidity was 80-100%, spraying with 1.5% solution of K-300 significantly decreased injuries in comparison to control. Application of Wapnovit, K-300, Biochikol, Tytanit or the mixture of Biochikol and calcium nitrate eliminated rotting. In experiments done in the springs of 2009 and 2010, when weather conditions were less favorable for tipburn appearance, a severity of it was lower but application of K-300 increased it appearance. In these experiments, Biochikol and Wapnovit eliminated rotting of heads. The results of three years of study have shown that calcium nitrate, Wapnovit, Tytanit and Biochikol limited occurrence of tipburn and bacterial rotting of Chinese cabbage, but the weather conditions during cultivation had the greatest impact on the severity of tipburn.

Key words: stress conditions, calcium deficiency, chitosan, titanium, physiological disorder, rotting

INTRODUCTION

Chinese cabbage (*Brassica rapa* L. var. *pekinensis*) (Lour.) Olson) is a very popular vegetable all over the world. Productivity is estimated to 100 $t \cdot ha^{-1}$ in 2-3 months cycle of cultivation (Hill 1990; Yazgan 1980). Cultivation of Chinese cabbage in Poland is now twice as big as it was 25 years ago; however, there is a problem with tipburn. It is a physiological disorder caused by calcium and water deficiency or high temperature (Imaj 1990; Poovaiah 1993; Borkowski & Schwonek 1994; Kobryń 1998; Saure 1998; Barta & Tibbits 2000; Borkowski et al. 2006, 2007; Olle & Bender 2009). Hernandez et al. (2004) found that a high air humidity prevents tipburn appearance. Tipburn is the main cause of the yield loses of Chinese cabbage. It appeared mainly during head formation. Heads with tipburn symptoms are not marketable. This disorder also affects other leafy vegetables (Saure 1998). Cultivars of Chinese cabbage differ significantly in susceptibility to tipburn (Larsen 1988; Borkowski

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2000; Borkowski & Kowalczyk 2003). Besides agrotechnical treatments and adequate calcium fertilization, some other measures were undertaken to prevent tipburn. Borkowski and Kowalczyk (2003) and Borkowski et al. (2006) found that Biochikol 020 PC, a preparation containing chitosan, decreases tipburn injuries, in spite of the fact that it does not contain calcium. Chitosan can act as an elicitor, which can increase resistance of plants to various diseases (Wojdyła 2001; Orlikowski & Skrzypczak 2003; Pięta et al. 2003; Borkowski et al. 2004). According to some reports, chitosan also increased plant growth (Borkowski et al. 2003), retarded growth of mycelium of some genera of pathogenic fungi (Borkowski et al. 2004; Saniewska 2001) and inhibited cucumber infection with *Botrytis cinerea* when was applied 1 day before inoculation (Ben-Shalom et al. 2003). Susceptibility of cultivars to tipburn is not tightly connected with calcium content in leaves (Borkowski & Kowalczyk 2003). Probably different cultivars susceptibility is the result of different reaction to abiotic stress (Saure 1998). It has been found that titanium accelerates the growth of plants, increases the yield and reduces the effects of certain stressors (Pais 1983; Borkowski & Dyki 2000; Borkowski et al. 2003, 2004, 2006; Marcinek & Hetman 2007; Kuzel et al. 2009).

In the present study, the effect of foliar application of calcium, titanium and chitosan preparations on the yield and healthiness of *Chinese cabbage* grown in pots were studied.

MATERIAL AND METHODS

The pot experiments were performed in the Institute of Horticulture in Skierniewice, Poland, on Chinese cabbage cultivar 'Bilko F_1' sensitive to tipburn and resistant to club root (Borkowski 2000). The seeds were sown in multipots in glasshouse conditions. Seedlings of about 3 weeks old were transplanted singly to 5-dm^3 containers, containing peat substrate. The content of mineral nutrients in the substrates is shown in the Table 1. During vegetation, the plants received top fertilization twice: 5 g·5 dm^{-3} calcium nitrate on June 6th and 5 g·5 dm^{-3} ammonium nitrate on June 20th. In 2010, the potassium level in the substrate was very low, and for that reason, on May 14th, 5 g granulated K_2SO_4 was added to every container. Chinese cabbage also received the top fertilization with calcium nitrate 7 g·5 dm^{-3} on June 15th. For the water drain, two holes below half of the container were given.

Table 1. Chemical composition, pH and salinity of the substrate at the beginning of the experiments

Year	The content of mineral nutrients in mg·dm^{-3}					pH	Salinity (g NaCl·dm^{-3})
	$N\text{-}NO_3$	P	K	Mg	Ca		
2008	377	184	837	359	2855	7.1	2.96
2008	231	148	328	285	2850	6.9	2.60
2009	364	182	662	408	3222	6.8	3.74
2010	648	149	359	302	2789	6.1	3.91

Table 2. Weather data for 2008, 2009 and 2010 from the meteorological station in Skierniewice

Years	Mean air temperature (°C)			Total precipitation (mm)		
	May	June	July	May	June	July
2008	13.7	18.6	19.6	59.0	18.7	74.4
2009	13.6	18.1	19.8	57.2	136.6	89.7
2010	13.3	17.6	21.2	132.1	44.4	69.6
Means for years 1921-2004	13.6	16.7	18.6	55.2	63.5	80.7

Experimental plants were grown outside, in the place of high insolation, where temperatures during sunny middays were 5-10 °C higher than that measured in the meteorological station. During hot and dry days, plants were watered. After 5 days, seedlings were watered with the 0.1% Grot 250 EC to prevent the injurious insects. Snails and caterpillars were removed by hand during watering time.

Experiments were performed in the spring of 2008, 2009 and 2010 and in the autumn of 2008. Plants were sprayed with 1.5% calcium nitrate,

1.5% of Wapnovit (containing 26% CaO, 15% N-NO_3 and Mg, Cu, B, Mo, Zn) (Intermag, Olkusz, Poland), 1% K-300 (22% K_2O and 3% N-NH_4, Intermag, Olkusz, Poland), 2.5% Biochikol 020 PC (3% chitosan) (Sumitex Poly-Farm, Łowicz, Poland), 0.03% Tytanit (containing 0.85% Ti as chelate) (Intermag, Olkusz, Poland). The K-300 was chosen because there was the idea to enrich it with selenium what could increase a dietetic attractiveness of Chinese cabbage. The control plants were sprayed with tap water at the same time. The treatments in details are showed in Tables 3-6. Sprayings were carried out in the morning between 8 and 10 am. Preparation Biochikol 020 PC was mixed with $Ca(NO_3)_2$ or Wapnovit before application. Tytanit and Wapnovit were applied separately but in the same day.

There were 11 plants per treatment. For evaluating heads yield, only eight plants least disordered were taken. Completely rotten or untypical heads were rejected. Before weighing, the yellow leaves were rejected. Some rotted leaves were collected, and bacteria were isolated on microbiological agar media. With the use of biochemical and molecular methods, bacteria were identified and deposited in collection of microorganisms of Microbiology Laboratory of the Institute of Horticulture in Skierniewice. 16S rRNA sequences obtained for the identification of two collected *Pectobacterium carotovorum* subsp. *carotovorum* strains (1Kp9 and 9Kp6) are available in the GenBank (https://www.ncbi.nlm.nih.gov/genbank/) with accession numbers: KJ818354.1 and KJ818360.1, respectively. Fragments of rotting leaves were fixed with CrAF (chromic acid, acetic acid, formalin), dehydrated in ethanol and next desiccated with Critical Point Drying CO_2 and sputter – coated with gold for study in scanning electron microscope (SEM) JSM-6390LV (JEOL, USA) (Fig. 1, 2).

Plant healthiness was assessed by 4-point scale: 0 is lack of tipburn symptoms and 4 is plant completely destroyed and rotted.

The experiments were designed as a completely randomized with 11 replications. The statistical analysis of yield was done by one-way analysis of variance (ANOVA), and the means was separated by the Newman-Keuls comparison procedure at $\alpha = 0.05$. Differences in tipburn incidences were calculated on 11 plants/treatment with test χ^2 at $\alpha = 0.05$ or 0.01.

RESULTS

Results of four experiments clearly showed that the occurrence of tipburn (Figs. 1 & 2) and accompanying heads rotting depended largely on the weather during cultivation. The rotting was caused by the *P. carotovorum* subsp. *carotovorum* (Figs. 3 & 4) (Jones 1901; Hauben et al. 1999). In the spring 2008, the temperature was high (25-30 °C) and the relative humidity was low (35-50%) (Table 2), and these weather elements favored the occurrence of both sickness. In these conditions, it was found that the tipburn injuries on plants treated with calcium nitrate, Wapnovit, Biochikol 020 PC or Tytanit were significantly lower in comparison with control (Table 3). The strongest tipburn injuries and 80-100% rotting heads was recorded in the result of spraying with the mixture of Wapnovit + Tytanit, K-300 and in control. The foliar fertilizer K-300 injured leaves, and symptoms were evident as soon as 3 days after treatment as foliar wilting and bronze spots resulting in significant yield decrease. In the autumn 2008, the most severe tipburn symptoms and the highest percentage of rotting heads (27.3%) appeared within control plants (Table 4). Rotting heads were also appeared amongst plants sprayed with ammonium nitrate and K-300. The yield of heads was not affected by the application of above preparations. The lowest tipburn appearance was observed on plants treated with Wapnovit 1.5%. Good results were also received with the application of Biochikol 020 PC and calcium nitrate. June 2009 was very humid with high precipitation (Table 2) and the temperature at midday was sometimes only about 13 °C. The appearance of tipburn in these weather conditions was at low level and the treatment with calcium nitrate or Wapnovit did not influence the health of Chinese cabbage (Table 5). Spraying with the 1.0% foliar fertilizer K-300 caused significant increase in tipburn appearance in comparison with control. None of the foliar application preparations affected yield in 2009.

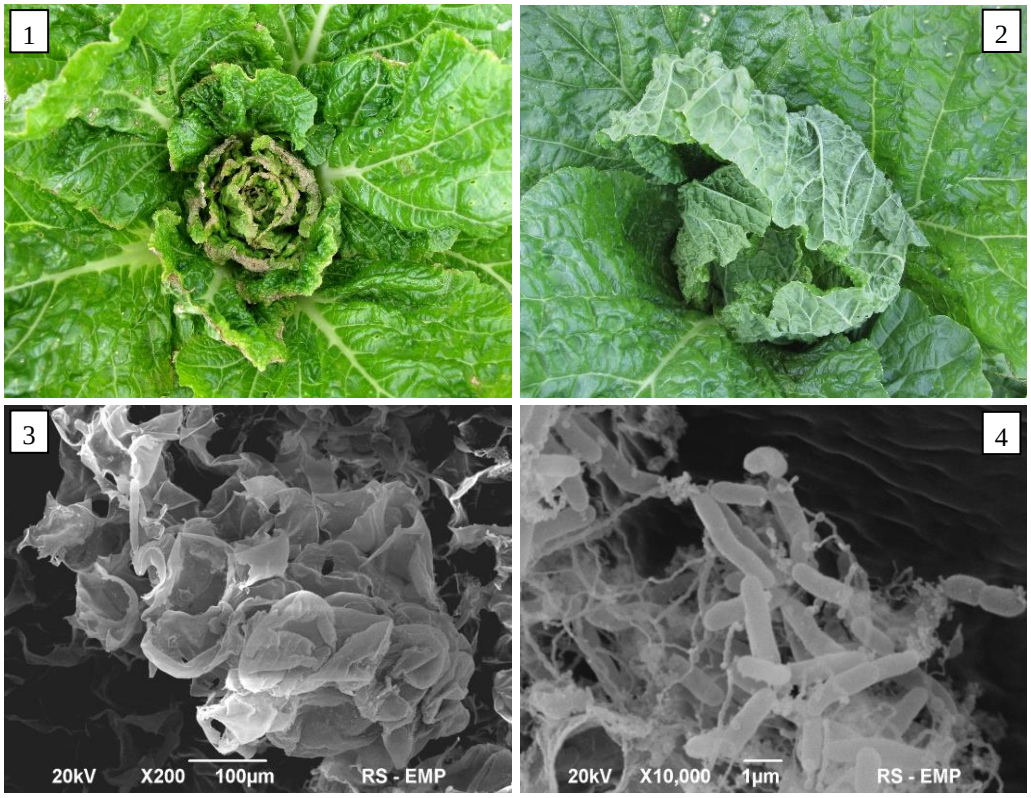

Fig. 1-4. 1. Chinese cabbage plant without tipburn nor rotting symptoms, 2. tipburn symptoms, 3. the rotting leaf tissue, 4. bacteria visible in the rotting sector.

Table 3. The effect of foliar fertilizers and Biochicol on the healthiness and weight of Chinese cabbage heads in April-June 2008 experiment

Treatment	Date of tipburn injuries observations [1)]				Rotting heads %	Weight of one head (kg)
	June 13th	June 19th	June 24th	June 30th	June 30th	
1. Control	0.64	1.00	1.64	2.86	81.9	1.35 a
2. $Ca(NO_3)_2$ 1.5% (May 28, June 4, 11 and 25)	0.36	0.36**	0.50**	1.82*	36.4	1.51 a
3. Wapnovit 1.5% (May 28, June 4, 11 and 25)	0.27	0.64	0.73*	1.55**	18.2	1.49 a
4. K-300 – (1x) 1% (June 3)	0.09*	1.14	2.45	3.14	81.9	1.00 b
5. K-300 – (3x) 1% (June 3, 10 and 23)	0.41	1.36	2.45	3.24	81.9	0.91 b
6. Biochikol 020 PC 2.5% (May 28, June 4, 11 and 25)	0.18*	0.59	0.77*	1.55**	45.5	1.32 a
7. Tytanit 0.03% (May 28, June 4, 11 and 25)	0.18*	0.50*	1.00	1.68*	27.3	1.45 a
8. $Ca(NO_3)_2$ + Biochikol 020 PC (May 28, June 4, 11 and 25)	0.32	0.55	1.41	2.50	72.8	1.43 a
9. Wapnovit + Tytanit (May 28, June 4,11 and 25)	0.36	1.00	2.45	3.36*	100.0	1.17 ab

*, ** Differences significant in comparison with control data calculated respectively with χ^2 at $\alpha = 0.05$ and $\alpha = 0.01$. The same letters indicate insignificant differences according to Newman-Keuls test at $\alpha = 0.05$

[1)] In the scale 0 = healthy plant; 4 = completely destroyed by tipburn and rotting inside of head

Table 4. The effect of foliar fertilizers and Biochicol on the healthiness and weight of Chinese cabbage heads in August-October 2008 experiment

Treatment	Data of tipburn injuries observations[1)]					Rotting heads %	Weight of one head (kg)
	Sept. 15th	Sept. 29th	Oct. 7th	Oct. 20th	Oct. 27th		
1. Control	0.36	0.91	1.23	1.14	1.05	27.3	0.83 a
2. $Ca(NO_3)_2$ 1.5% – 3x (August 28, September 2 and 9)	0.14	0.23**	0.27**	0.36*	0.45	9.1	0.88 a
3. Wapnovit 1.5% – 3x (August 28, September 2 and 9)	0.05**	0.14**	0.18**	0.27**	0.41	0	0.93 a
4. K-300 – (1x) 1% (Sept. 10)	0.27	0.36	0.50**	0.50*	0.50	9.1	0.84 a
5. K-300 – (3x) 1% (Sept. 5, 10 and 22)	0.23	0.64	0.82	0.68	0.59	0	0.93 a
6. Biochikol 020 PC 2,5% – 3x (August 28, September 2 and 9)	0.14	0.27**	0.50*	0.41*	0.45	0	0.86 a
7. Tytanit 0.03% – 3x (August 28, September 2 and 9)	0.27	0.50	0.86	0.82	0.82	0	0.92 a
8. Biochikol+ $Ca(NO_3)_2$ – 3x (August 28, September 2 and 9)	0.41	0.36	0.41**	0.55*	0.50	0	0.96 a

Note: see Table 2

Table 5. The effect of foliar fertilizers and Biochicol on the healthiness and weight of Chinese cabbage heads in April-June 2009 experiment

Treatment	Data of tipburn injuries observations [1)]					Rotting heads %	Weight of 1 head (kg)
	May 29th	June 9th	June 15th	June 25th	June 30th		
1. Control	0.41	0.86	1.09	0.77	0.82	9.1	1.25 a
2. $Ca(NO_3)_2$ 1.5% 4x (May 25, June 4, 9 and 25)	0.50	0.91	1.14	0.95	0.73	9.1	1.25 a
3. Wapnovit 1.5% – 4x (May 25, June 4, 9 and 25)	0.50	1.32	1.55**	1.00	0.86	9.1	1.23 a
4. Biochikol 2.5% – 4x (May 25, June 4, 9 and 25)	0.45	0.91	1.36	0.95	0.91	0	1.17 a
5. K-300 1% – 4x (May 25, June 4, 9 and 25)	0.41	1.45**	1.77**	1.41**	1.41**	9.1	1.23 a
6. Biochikol + $Ca(NO_3)$ – 4x (May 25, June 4, 9 and 25)$_2$	0.36	1.14	1.23	1.00*	0.91	9.1	1.27 a

Note: see Table 2

At the beginning of 2010, the lowest symptoms of tipburn were on plants treated with Wapnovit + Tytanit, but at the end of cultivation, they were similar to those on control plants. Plants treated with Tytanit, Wapnovit and Biochikol or calcium nitrate were less injured with tipburn than the control plants (Table 6). The level of tipburn injuries on June 21st in some cases was higher than that on June 28th, because tipburn commonly appeared on young leaves. New tipburn symptoms did not appear when some days were cloudy or rainy (Table 2), and old tipburn symptoms did not increase in such conditions but remained on older leaves. In such conditions, tipburn injuries on whole plant decreased. Treatments with Tytanit did not increase heads yield, but 91% plants were injured by snails in this treatment.

Table 6. The effect of foliar fertilizers and Biochikol on the healthiness and weight of Chinese cabbage heads in April-July 2010 experiment

Treatment	Data of tipburn injuries observations [1)]					Rotting heads %	Weight of one head (kg)
	June 7th	June 14th	June 21th	June 28th	July 5th		
1. Control	0.77	1.05	1.23	1.09	1.68	9.1	1.04 a
2. $Ca(NO_3)_2$ 1.5% (June 4, 11 18 and 28)	0.87	1.15	1.23	1.05	0.91*	27.3	1.01 a
3. Biochikol 2.5% – 4x (June 4, 11, 18 and 28)	0.59	1.14	1,27	0.91	0.82*	0.0	0.90 a
4. Wapnovit 1.5% – 4x (June 4, 11, 18 and 28)	1.09	1.15	1.00	0.95	0.77**	0.0	1.08 a
5. Tytanit 0.03% – 4x (May 20, June 11, 18 and 28)	0.77	1.09	0.91	0.77	0.68**	18.2	0.97 a
6. Tytanit + Wapnovit (June 4,11,18 and 28)	0.0**	0.23**	0.59**	0.86	1.27	18.2	1.00 a

Note: see Table 2

May and June 2010, as well as 2009, were rainy, which limited tipburn appearance. However, when rainy weather occurred after prolonged hot days, it could stimulate secondary changes in necrotic tissues and could also stimulate rotting of Chinese cabbage heads.

DISCUSSION

Heavy injuries in the experiment conducted in April-June 2008 were probably connected with high temperature and low air humidity. In autumn, the bacteria *P. carotovorum* and *Pseudomonas marginalis* were also present, but did not cause the rotting of whole heads. In the experiment performed in October 2008 when the temperature was close to 12 °C and the relative humidity was 80-100%, sprayings with 1% K-300 did not injure leaves.

A tipburn injury appears on young leaves that contain lower level of calcium than the oldest leaves. This fact was demonstrated by Barta and Tibbitts (2003) with lettuce and Chinese cabbage (Borkowski & Kowalczyk 2003; Hernandez et al. 2004). Borkowski and Kowalczyk (2003) have found that the youngest leaves inside the head contain 7.1-10.9 g $Ca \cdot kg^{-1}$ of dry matter and the old leaves 29.9-44.7 g $Ca \cdot kg^{-1}$ of dry matter. These authors demonstrated that cultivars sensitive and cultivars tolerant to tipburn contained similar calcium content in leaves. Therefore, the reason of tipburn symptoms is probably connected with different tolerance to stress caused by high temperature or water deficit (Saure 1998). Very important is high and stable air humidity that retards plant transpiration. It can be assured, for instance, by covering row of plants by polypropylene sheets (Hernandez et al. 2004). In these conditions, calcium content in inner leaves is increased and tipburn symptoms are decreased. In the recent paper and in earlier papers (Borkowski et al. 2006, 2007), it was demonstrated that foliar spraying with calcium salts solution can limit tipburn. Similar results were reported by Corriveau et al. (2012) with lettuce in which problem with tipburn also occurs. They demonstrated that irrigation of plants in warm days strongly decreased air temperature and increased air humidity. In these conditions, tipburn incidence was low. Also Lee et al. (2013) found that low temperature (18 °C) prevented tipburn appearance in leaf lettuce. Similar effect can be obtained when stable horizontal air flow is applied, as in this condition calcium level increased in inner leaves. Inthichack et al. (2012) found that intensification of tipburn symptoms in lettuce may occur as a the result of increasing salinity caused by fertilization with potassium salts. High potassium level also increased tipburn injuries in Chinese cabbage and decreased calcium uptake (Borkowski & Szwonek 1994). In our experiments,

mass tipburn on Chinese cabbage appeared in condition of strong solar irradiation and low air humidity. Such weather may cause plant stress, which in turn favors tipburn appearance (Saure 1998). Our investigation confirmed that favorable meteorological conditions are the most important factors in order to avoid tipburn of Chinese cabbage, and in such conditions, foliar application of calcium salts is unnecessary. The effect of spraying with calcium nitrate or Wapnovit was similar for tipburn incidence, but rotting process was more intensive when the plants were sprayed with calcium nitrate. Wapnovit contains copper and probably this microelement reduces the growth of rotting bacteria.

Treatment with 1% K-300 injured leaves and increased tipburn appearance, because vegetables are tolerant only to low concentration of this foliar fertilizer, whereas agricultural crops are tolerant to its high concentrations (Czuba 1988). Injured plants had probably more intensive transpiration and this could cause stress for Chinese cabbage plants. A high temperature and low humidity that prevailed in June 2008 caused very high tipburn injury and as a consequence, rotting of heads. The best results in all sequential investigations were obtained with foliar application with 1.5% calcium nitrate or 1.5% Wapnovit containing calcium, which is indispensable for tipburn prevention (Borkowski & Szwonek 1994; Saure 1998; Olle & Bender 2009). It is interesting that Tytanit and Wapnovit used separately diminished markedly tipburn appearance, but applicated as the mixture in the same term and conditions strongly augmented the symptoms of this disorder. It is possible that using two preparations mixture caused stress in plants. This should be clarified in a future experiment. The calcium is a macroelement, and in a high concentration, it decreases the activity of different physiological processes. For example, spraying every week tomato fruits with calcium compounds or dipping them in calcium solution caused retardation of their growth, ripening respiration and ethylene production (Leonard & Hepler 1990; Labawith & Mignami 1993; Ben Arie et al. 1995; Pandrangi & Barringer 1999). Also a high concentration of calcium delayed ripening of the musk melons (Li TianLai et al. 2009).

Titanium, a component of Tytanit is considered as a trace element important for plants (Pais 1983; Carvajal et al. 1994a, b; Carvajal & Alearaz 1998; Kuzel et al. 2009). Borkowski and Dyki (2000) demonstrated that titanium intensifies the process of photosynthesis, increases the activity of several enzymes, stimulates pollen germination and increases seed yield. In the present experiments, Tytanit did not increase the yield of Chinese cabbage as in the former reports (Borkowski et al. 2003, 2006; Borkowski & Dyki 2000). The best results with Tytanit on *Sparaxis tricolor* were received at dry and hot summers (Marcinek & Hetman 2007). High humidity is also a very important factor in the cultivation of Chinese cabbage in glasshouse or under row covers (Kobryń 1998; Hernandez et al. 2004). When Chinese cabbage is cultivated in the open air in low air humidity, the marketable yield can amount to only 25% of total yield (Hernandez et al. 2004). In the experiment performed in April-June 2008, the strong incidence of tipburn injury was accompanied with heads rotting caused by *P. carotovorum* subsp. *carotovorum* (Jones 1901; Hauben et al. 1999). This bacterium multiplies rapidly in the temperature 25-30 °C (Waleron et al. 2004; Rimmer et al. 2006), and for this reason, it is mainly dangerous in summer.

Advantageous effect of calcium nitrate on Chinese cabbage has been known for many years (Borkowski & Szwonek 1994; Borkowski et al. 2006). When the day temperature is 30 °C or more, the best results were received with foliar application of calcium nitrate mixed with Biochikol 020 PC (Borkowski et al. 2007). June 2009 was very humid with high rainfall (Table 1) and for that reason spraying with Biochikol 020 PC + calcium nitrate was not effective. Similar effect was found when spraying the Chinese cabbage first with Biochikol 020 PC and later with calcium nitrate (Borkowski et al. 2007). Biochikol is a gel that probably retards drying of the used solution. Spraying Chinese cabbage with 1.5% calcium nitrate or 1.5% Wapnovit gave good results, but the same concentration of foliar fertilizers used on tomato to control blossom-end rot burned leaves severely (Borkowski 1983).

CONCLUSIONS

1. Tipburn of Chinese cabbage appearance depends mainly on the weather conditions during cultivation but some foliar application preparations used can reduce the severity of damage to some extent.
2. Tipburn was commonly accompanied with rotting of heads caused by *P. carotovorum* subsp. *carotovorum*.
3. Foliar application of 1.5% calcium nitrate or 1.5% Wapnovit are the measures most limiting tipburn incidence on Chinese cabbage.
4. It is also possible to prevent tipburn using 0.03% Tytanit 5-7 days earlier than other foliar fertilizers.
5. Spraying with Tytanit in combination with Wapnovit or calcium nitrate in 1 day could be harmful for Chinese cabbage.

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