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Impact of the variety and severity of *Cercospora beticola* infection on the qualitative and quantitative parameters of sugar beet yields

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Abstract. Cercospora leaf spot (CLS) causes significant economic losses. *Cercospora beticola*, the fungus that causes this disease, quickly acquires resistance to the active substances of the used fungicides. Therefore, the combination of chemical protection and varieties with increased resistance to CLS is currently the basic way to fight this disease. The aim of the study was to check the reaction of selected breeding materials and varieties of Kutnowska Hodowla Buraka Cukrowego (KHBC) in conditions of fungicides protection, without protection and artificial inoculation. In the years 2011–2013 field experiments were carried out in Straszków (Wielkopolskie voivodeship). The study used 2 varieties of sugar beet grown by KHBC: Finezja, Luzon and the breeding line KTA1015 with different levels of resistance to CLS. Three treatments were studied: control plots (no chemical control and no inoculation), inoculated plots and protected plots. The sugar beet crop was analyzed on yield, biological and technological sugar content and molasses-forming substances content in the pulp. The highest average yield of sugar beet of the studied varieties was obtained in the treatment with fungicide protection. The yield of the tested varieties grown on control plots and inoculated with mycelial fragments was lower by 4.6% and 11.3%, respectively. The lowest content of molasses-forming substances was found in the pulp of roots from chemically protected plots. The content of these compounds increased with the severity of CLS.

Keywords: sugar beet, cercospora leaf spot, yield of sugar beet roots, yield of technological and biological sugar, molasses content

INTRODUCTION

Cercospora leaf spot (CLS) is one of the most important diseases occurring on sugar beet leaves. The fungus *Cercospora beticola*, which causes CLS, is widespread in all areas of sugar beet cultivation. The presence of the fungus is strictly linked to the climatic conditions. The high temperature and high humidity intensify the pressure of the pathogen (Holtschulte, 2000). Counteracting the development of the fungus is extremely important because the disease causes a significant reduction in root yield. Under optimal conditions for pathogen, these losses can reach as much as 50% (Shane, Teng, 1992). A reduction in yield is not the only effect of the disease. The content of beet molasses-forming substances, especially sodium and α-amino nitrogen, is increased in the roots, which additionally leads to deterioration of their technological properties (Rossi et al., 2000). In order to protect sugar beet plantations from CLS, it is essential to apply the fungicide treatments (Piszczek, 2010). However, the inhibition of the development of infections cannot be limited to chemical treatments exclusively, since the intensive application of plant protection products increases the resistance to their active substances in pathogens (Secor et al., 2010). The severity of the disease can also be mitigated by the appropriate crop rotation, deep tillage or elimination of secondary hosts (Windels et al., 1998). An important factor in the fight against CLS infestation is the sowing of resistant varieties (Brown, 2002). The symptoms caused by *C. beticola* are observed later on the leaves of resistant varieties in comparison to deprived of such resistance ones. This is due to the longer germination period of spores (Rossi et al., 1999). Moreover, a slower development of the disease was stated for the resistant varieties (Rossi, 1995). Yet, the polygenic inheritance of resistance (Smith, Gaskill, 1970) makes breeding difficult. The multi-genous nature of resistance to *C. beticola* makes the varieties differ in it's level (Pfleiderer, Schäufele, 2000; Rossi et al., 2000).

The research hypothesis assumed that the qualitative and quantitative parameters of sugar beet yield deteriorate themselves with an increase in the degree of *C. beticola* infestation. The extent of the decrease in yield parameters depends on the level of resistance of a certain cultivar.

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The aim of the study was to verify the reaction of two cultivars and one breeding line of Kutnowska Hodowla Buraka Cukrowego (KHBC) under conditions of fungicide protection, without protection (natural pathogen pressure) and inoculation.

MATERIALS AND METHODS

In the years 2011–2013 field experiments were carried out in Straszków (Wielkopolskie voivodeship). The experiment was established by means of the randomized blocks method, in three replications. The area of the experimental plot was 10.8 m^2 and the number of plants per plot ranged from 128 to 132. The study used 2 varieties of sugar beet grown by KHBC: 'Finezja', 'Luzon' and the breeding line KTA1015 with different levels of resistance to CLS. Finezja is characterized by medium resistance to the CLS and Luzon by its absence. The line KTA1015, on the other hand, is a hybrid revealing the resistance to the CLS. Three treatments were studied: control plots (no chemical control and no inoculation), inoculated plots and protected plots. The root harvesting was done by hand. The gathered roots were washed and weighed on the dirty processing line in Straszków. The quality and quantity parameters (i.e. biological sugar content in %, potassium, sodium and α-amino nitrogen content in millimoles per 1000 grams of pulp) of the sugar beet root pulp were analyzed on the VENEMA line.

The fungicides Topsin M 500 SC (active substance thiophanate-methyl) and Orius Extra 250 EW (active substance tebuconazole) were used to protect the tested plants from the CLS. Fungicides were applied two times. In each application a combination of both fungicides was used, in doses with accordance to instructions on the preparation labels. The first spraying was performed after the first symptoms of the disease (BBCH 41-49 plant growth phase), while the second one was applied 3 weeks later (BBCH 41-49).

The parcels were inoculated in early July. The isolates from which the inoculum was prepared originated from Straszków. They were grown on the Potato-Dextrose Agar (PDA) on Petri dishes until the overgrowing of the whole plate. The plants were inoculated with *C. beticola* mycelium suspension obtained by mixing the mycelium together with the substrate with the addition of water by means of laboratory mill.

All fungicide treatments and inoculation were performed with a field plot pump sprayer. The quantity of spray liquid, both during inoculation and protective measures, was 400 l/ha.

The degree of leaf infestation by *C. beticola* was assessed by means of the EPPO scale (EPPO, 2002). EPPO is a 9-degree scale, determining the percentage of the leaf area under the symptoms of pathogen infection, where 1 is an infection of up to 0.1% of the leaf area and 9 is an infection of more than 60% of the leaf area. Three evaluations were performed: the first one before inoculation, the second one – 14 days after inoculation and third one – 30 days after inoculation.

All statistical analyses were performed using a computer program MakBet. The technological sugar content (in %) in roots and the technological sugar yield (in dt ha^{-1}) were also calculated. The LSD level was obtained from the Duncan test. The sugar loss in the molasses (S_m) was determined on the basis of the Reinefeld formula modified by VENEMA for the line located at KHBC in Straszków presented below.

$$S_m = 0.0343\ (K + Na) + 0.0094\ N + 0.29$$

where:
S_m – loss of sugar in molasses,
K – content of potassium in the pulp,
Na – content of sodium in the pulp,
N – content of α-amino nitrogen in the pulp.

RESULTS AND DISCUSSION

One of the most important concerns currently faced by plant protection is the increase in resistance of pathogens to the active substances of plant protection products. The widespread application of fungicides and the consequent selection pressure have led to the development of *C. beticola's* resistance to fungicides with different modes of action (Kiniec et al., 2019). Therefore, the fundamental way to fight against CLS is nowadays the sowing the varieties with increased resistance and adequate fungicide protection. The above approach is consistent with the integrated plant protection methodology (Piszczek et al., 2018).

The Table 1 presents the mean leaf infestation of sugar beet, depending on the variety, the date of the estimation of the prevalence of CLS and the experimental combination achieved during the three years of the study. The average

Table 1. The average beet leaf infestation by *C. beticola* depending on variety, experimental treatment and time of disease development in 2011–2013.

No.	Treatment	KTA1015	Finezja	Luzon
1	Control at inoculation	0.6 aA	0.5 aA	0.6 aA
2	Control after 14 days	0.6 aA	1.0 aB	1.2 abB
3	Protected after 14 days	0.5 aA	0.6 aA	0.6 aA
4	Inoculated after 14 days	0.8 aA	2.0 abB	2.5 bcC
5	Control after 30 days	1.0 aA	1.8 abB	1.8 abB
6	Protected after 30 days	0.6 aA	1.1 aB	1.0 abB
7	Inoculated after 30 days	1.7 aA	2.8 bB	3.6 cC

Mean values followed by the same lower case letters (in columns) do not differ significantly at p = 0.05
Mean values followed by the same upper case letters (in rows) do not differ significantly at p = 0.05

Table 2. The qualitative and quantitative yield parameters of the tested sugar beet varieties with different degrees of resistance[#] to *C. beticola* in 2011.

No.	Variety – treatment	Root yield [dt ha^{-1}]	Biological sugar content [%]	Technological sugar content [%]	Biological sugar yield [dt ha^{-1}]	Techno-logical sugar yield [dt ha^{-1}]	Potassium content	Sodium content	α-amino nitrogen content
							[mmol/1000 g pulp]		
1	FINEZJA protected	623.2 a	17.7 abc	15.9 abc	110.2 a	99.1 a	32.2 c	4.1 b	12.6 ab
2	KTA1015 protected	588.9 ab	18.3 a	16.5 a	107.4 a	97.2 a	32.8 c	4.8 ab	15.7 a
3	LUZON control	593.5 ab	17.9 ab	16.1 ab	106.2 a	95.5 a	33.0 bc	4.8 ab	12.2 ab
4	LUZON protected	586.1 ab	17.5 bc	15.7 bc	102.7 ab	91.9 abc	34.4 abc	4.6 ab	11.4 ab
5	KTA1015 control	551.9 abc	18.0 ab	16.3 ab	99.3 ab	90.2 abc	34.8 abc	4.8 ab	13.1 ab
6	FINEZJA control	557.4 abc	17.4 bc	15.7 bc	97.1 ab	87.4 abc	35.4 abc	5.8 ab	10.5 b
7	KTA1015 inoculated	514.8 abc	18.0 ab	16.3 ab	92.5 ab	83.7 abc	35.5 ab	5.9 a	14.0 ab
8	FINEZJA inoculated	500.9 bc	17.2 c	15.3 c	85.7 b	76.5 bc	35.6 ab	6.3 a	10.7 b
9	LUZON inoculated	466.7 c	17.7 abc	15.9 abc	82.4 b	73.9 c	36.7 a	4.7 ab	9.9 b

Values followed by the same letters (in columns) do not differ significantly at p = 0.05

[#] Luzon – a variety susceptible to *C. beticola;* Finezja – a variety with medium resistance to *C. beticola*; KTA1015 – breeding line resistant to *C. beticola* infection

Table 3. The qualitative and quantitative yield parameters of the tested sugar beet varieties with different degrees of resistance[#] to *C. beticola* in 2012.

No.	Variety – treatment	Root yield [dt ha^{-1}]	Biological sugar content [%]	Technological sugar content [%]	Biological sugar yield [dt ha^{-1}]	Techno-logical sugar yield [dt ha^{-1}]	Potassium content	Sodium content	α-amino nitrogen content
							[mmol/1000 g pulp]		
1	FINEZJA protected	870.4 a	16.0 a	13.4 ab	139.2 a	116.6 a	51.5 ab	5.9 a	31.5 a
2	KTA1015 protected	851.9 a	16.0 a	13.3 ab	136.0 ab	112.9 a	52.5 ab	6.1a	37.2 a
3	LUZON control	845.4 a	16.2 a	13.3 ab	136.9 ab	112.4 a	54.4 ab	4.8 a	32.2 a
4	LUZON protected	811.1 ab	16.2 a	13.7 a	131.4 abc	110.7 ab	55.8 ab	4.8 a	36.6 a
5	KTA1015 control	812.0 ab	15.6 ab	12.6 bc	126.3 bcd	102.2 bc	58.1 ab	6.0 a	40.0 a
6	FINEZJA control	801.9 ab	15.5 ab	12.6 bc	124.3 cd	100.6 c	59.0 ab	5.2 a	43.9 a
7	KTA1015 inoculated	759.5 b	15.8 a	13.2 ab	120.1 cd	99.9 c	59.4 ab	5.5 a	37.8 a
8	FINEZJA inoculated	810.2 ab	14.9 b	12.1 c	120.9 cd	97.6 c	60.5 ab	6.2 a	41.2 a
9	LUZON inoculated	761.1 b	15.5 ab	12.6 bc	117.9 d	95.8 c	60.9 a	6.4 a	37.8 a

Values followed by the same letters (in columns) do not differ significantly at p = 0.05

[#] Luzon – a variety susceptible to *C. beticola;* Finezja – a variety with medium resistance to *C. beticola*; KTA1015 – breeding line resistant to *C. beticola* infection

Table 4. The qualitative and quantitative yield parameters of the tested sugar beet varieties with different degrees of resistance[#] to *C. beticola* in 2013.

No.	Variety – treatment	Root yield [dt ha^{-1}]	Biological sugar content [%]	Technological sugar content [%]	Biological sugar yield [dt ha^{-1}]	Techno-logical sugar yield [dt ha^{-1}]	Potassium content	Sodium content	α-amino nitrogen content
							[mmol/1000 g pulp]		
1	FINEZJA protected	651.2 a*	17.5 a	14.9 a	113.7 a	96.8 a	60.5 ab	2.5 c	14.9 abc
2	KTA1015 protected	628.4 ab	17.3 ab	14.8 ab	108.6 ab	93.4 a	56.0 abc	3.5 bc	11.8 d
3	LUZON control	631.8 ab	17.3 ab	14.7 ab	109.3 ab	93.1 a	59.1 abc	2.8 c	17.0 a
4	LUZON protected	617.9 abc	17.3 ab	15.0 a	107.0 ab	92.4 a	54.1 bc	3.1 c	12.5 cd
5	KTA1015 control	621.6 abc	17.2 ab	14.5 ab	106.8 ab	90.4 a	59.4 abc	5.1 ab	13.6 bcd
6	FINEZJA control	598.2 abc	17.1 ab	14.7 ab	102.6 ab	88.1 a	55.0 abc	3.2 c	15.1 abc
7	KTA1015 inoculated	568.8 bcd	17.3 ab	14.7 ab	98.3 bc	83.6 ab	59.5 abc	3.1 c	15.8 ab
8	FINEZJA inoculated	552.8 cd	17.3 ab	15.1 a	95.7 bc	83.3 ab	50.7 c	3.1 c	13.9 bcd
9	LUZON inoculated	522.2 d	16.9 b	14.0 b	88.0 c	73.4 b	63.9 a	5.7 a	13.6 bcd

Values followed by the same letters (in columns) do not differ significantly at p = 0.05

[#] Luzon – a variety susceptible to *C. beticola;* Finezja – a variety with medium resistance to *C. beticola*; KTA1015 – breeding line resistant to *C. beticola* infection natural leaf infestation of all varieties of beet plants by *C. beticola* before inoculation of plots was similar. During the subsequent evaluations the lowest infestation was observed on the leaves of the resistant breeding line KTA1015. The observations were confirmed in all experimental treatments. In the control treatment and chemically protected one the differences in the degree of leaf infestation between the varieties Finezja and Luzon were not large. They became apparent only under intense pathogen pressure.

The Tables 2-4 show the results of the yield of beet roots in each year of the experiment. The highest root yields were observed in combination with fungicide protection in each year of the study. The average beet root yield obtained for the three-year study period (2011–2013) is shown in Figure 1. The highest average root yield (707.3 dt ha^{-1}) was obtained from chemically protected plots on which an average resistant variety Finezja was grown. The reduction in the root yield harvested from control plots compared to the yields collected from plots protected by fungicides was 4.6%. Similar results were achieved by Prośba-Białczyk and Regiec (2006) and Gummert et al. (2015). In all the years of conducted study, the lowest root yield was recorded in a combination inoculated with fragmented *C. beticola* mycelium. The Kaiser and Varrelmann studies (2009) undertaken in Germany show yield differences between control and protected treatments only under high pathogen pressure. Shane and Teng (1992) in the USA and Rossi et al. (2000) in Italy report yield losses of up to 50% caused by CLS. According to Byford (1996), in Austria root yield reductions ranged from 10% to 50% and in France from 15% to 40%. Among the varieties studied, the lowest average root yields were characteristic for Luzon one, susceptible to CLS in all variants of experiment. An interesting results were obtained during the cultivation of resistant breeding line KTA1015 and medium-resistant Finezja variety. The highest root yield on *C. beticola* inoclulated plots was observed for the resistant variety KTA 1015 (641.1 dt ha^{-1}). However, in the control and protected combinations the root yields of Finezja variety were higher. It supposedly results from the negative influence of genes conditioning resistance to *C. beticola* in variety KTA1015 on the yield obtained. The hybrids with a very high resistance to CLS free from disease may yield up to 18% lower than non-resistant hybrids (Miller et al., 1994). In conditions of strong pathogen impact, resistant varieties not chemically protected may yield worse than medium-resistant ones, effectively protected against *C. beticola* (Smith, Campbell, 1996; Gaurilčikienė et al., 2006). It is therefore recommended, both in case of cultivation and breeding, to use varieties with medium resistance, which effectively preserved chemically, provide a high yield (Windels et al., 1998).

The highest average sugar content, both biological and technological, was found in the root pulp of the variety KTA1015 in treatment with fungicide protection (17.3% and 14.9%, respectively) – Fig. 2 and 3. The average biological and technological sugar content complies with the literature data, however, when considering individual varieties the results are not so unequivocal. In respective years of the study, the highest sugar content, as biological as well as technological, was generally recorded in the protected plot (Table 2-4). Only in 2013 the highest technological sugar content was observed in the root pulp of the susceptible Luzon variety in a plot inoculated with fragmented *C. beticola* mycelium. The average biological sugar content in the roots of the Luzon variety from the treatments without chemical protection of plants (natural infection) decreased and amounted to 16.9%, and after the application of inoculation, despite higher *C. beticola* infestation, it increased to the level obtained in the treatment

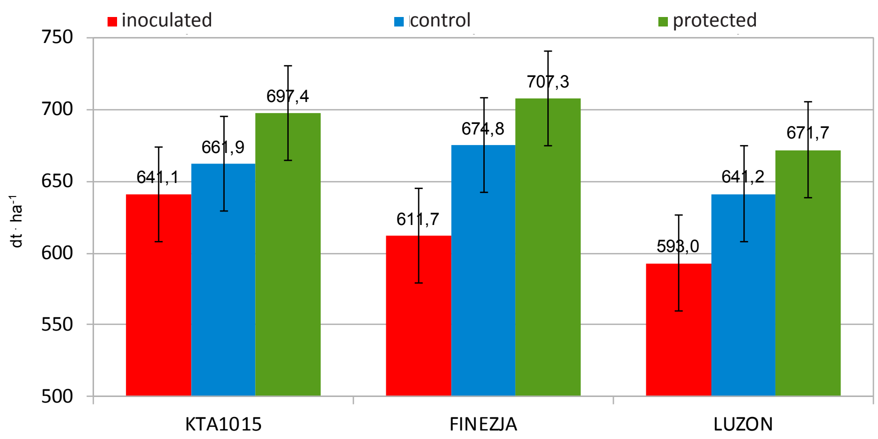

Figure 1. The average yield of sugar beet root obtained in 2011–2013 depending on the variety and variant of the experiment ($p = 0.05$, LSD = 32.78).

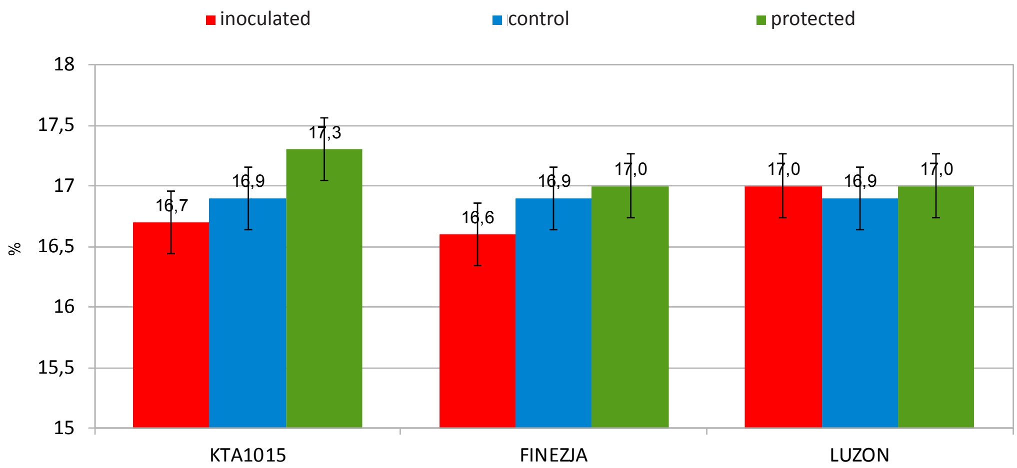

Figure 2. The average content of biological sugar obtained in 2011–2013 depending on the variety and variant of the experiment (p = 0.05, LSD = 0.26).

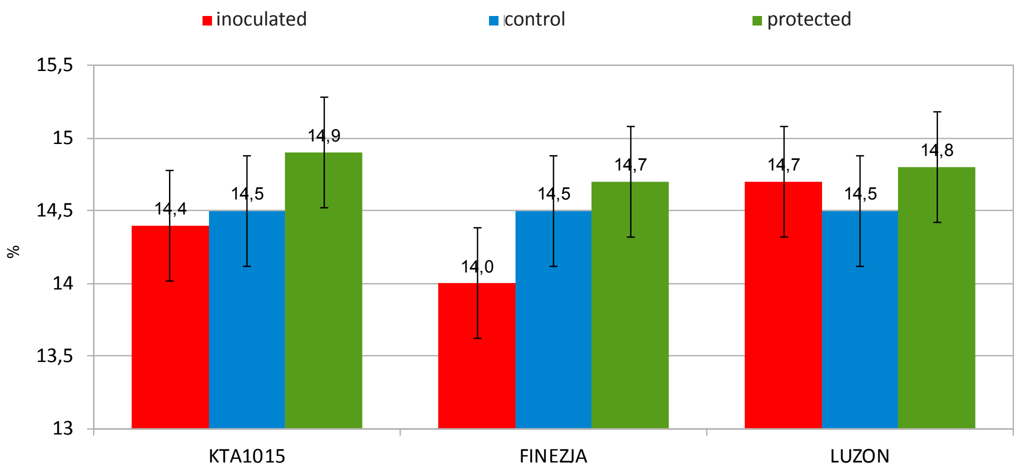

Figure 3. The average technological sugar content obtained in 2011–2013 depending on the variety and variant of the experiment (p = 0.05, LSD = 0.38).

with chemical protection (17%). A similar correlation was observed for the technological sugar content of this variety. However, the technological sugar content in the inoculated treatments was slightly lower in relation to its content obtained in the protected one. The medium-resistant variety Finezja and the resistant breeding line KTA1015 showed a decrease in biological and technological sugar content along with the intensification of the infestation. Variations in the sugar content of the roots should be considered in two respects. On the one hand, it is explained by the fact of a negative impact related to the increasing infestation by *C. beticola*, which leads to decrease in sugar content (Coe, 1967; Rossi et al., 2000). Vogel et al. (2018) revealed that with low pathogen infection the sugar yield reduction of an unprotected treatment reaches 3.3%, while if the level of infection is high the yield reduction can be as high as 9.9%. The field experiments clearly demonstrate a very important role of weather conditions in the development of the disease and the resulting losses (Kaiser, Varrelmann, 2009). On the other hand, during resistance breeding, genes from wild species are brought in and thus the economic parameters of varieties are diminished (Holtschulte, 2000). This as a consequence translates into difficulty in unambiguous evaluation of the research results. The solution to this issue would be to use near isogenic lines for testing, which would allow to test materials differing only in the presence of genes conditioning resistance to *C. beticola*. However, due to the multigenous nature of the resistance it would be very difficult and would require long-term research on gene mapping and development of such lines.

In line with the expectations, the lowest average biological and technological sugar yield was observed in treatments with inoculation (Fig. 4 and 5). In the individual years of the study the trend was similar (Table 2-4), except for 2012, when the lowest sugar yield was obtained from Luzon variety in the control treatment. The average increase in biological sugar yield noted in the control plot compared to the treatment with inoculation amounted to 9.1 dt ha^{-1} (9.0%) whilst in a chemically protected treatment was 15.7 dt ha^{-1} (15.5%). The yield of technological sugar was lower in the inoculated treatment than in the control treatment by 7.8 dt ha^{-1} (8.3%) and compared to the protected one by 14.7 dt ha^{-1} (14.5%). This is comparable with the results reported by Rossi et al. (2000). The highest average biological and technological sugar yield in the inoculated treatment was recorded for the resistant KTA1015 line. The same results were obtained for the years 2011 and 2013, whereas in 2012 the highest sugar yields in aforementioned treatment were observed in the case of Finezja variety. In the control treatment, with natural infestation by *C. beticola*, the highest average sugar yields were obtained by the medium-resistant Finezja variety. The application of fungicide protection resulted in an elevated sugar yield, although the highest yielding variety was also Finezja.

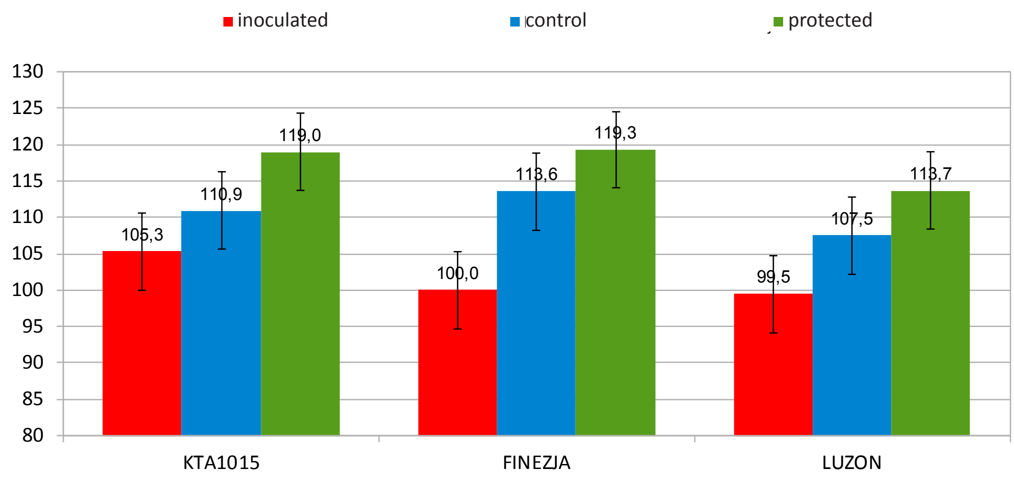

Figure 4. The average yield of biological sugar obtained in 2011–2013 depending on the variety and variant of the experiment (p = 0.05, LSD = 5.33).

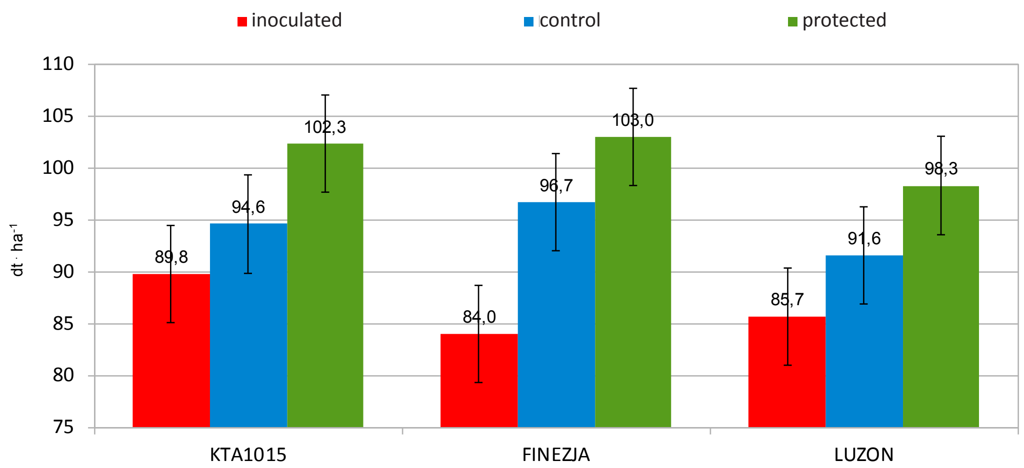

Figure 5. The average technological sugar yield obtained in 2011–2013 depending on the variety and variant of the experiment (p = 0.05, LSD = 4.67).

The lowest average potassium content was found in the pulp obtained from beet roots grown in a fungicide protected treatment (47.8 mmol/1000 grams of pulp) – Fig. 6. The results from the years 2011 and 2012 display similarities (Table 2-4). In 2013, the lowest potassium content was obtained in the root pulp of Luzon variety in the inoculated treatment. Under conditions of natural plant infection and inoculation, the average potassium content in beet roots increased by 5.9% compared to the content determined in the roots of plants grown in chemically protected treatment. In a study carried out in Italy, Rossi et al. (2000) also found an increase in the content of molasses-forming substances in sugar beet roots unprotected from CLS. The rise in potassium content in pulp was measured by above authors at 6.4%.

In all years of the experiment, the lowest sodium content in the pulp obtained from the examined roots was recorded in the protected treatment (Table 2-4). The lowest average sodium content in pulp achieved from the tested roots was also observed in treatment with fungicide preservation (4.4 mmol/1000 grams of pulp) – Fig. 7. In roots collected from unprotected plots an increase in sodium content by 0.4 mmol/1000 grams of pulp was detected (9.1%). Rossi et al. (2000) report an increase in the sodium concentration

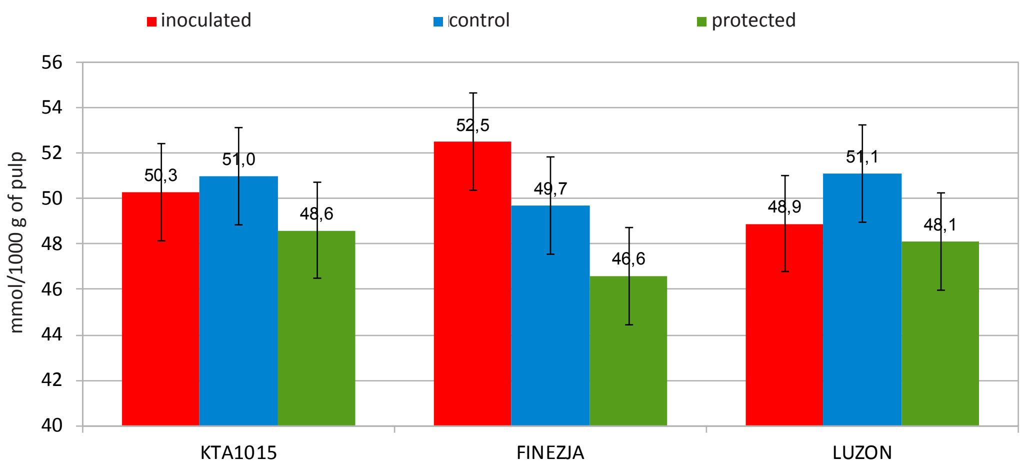

Figure 6. The average potassium content found in sugar beet pulp in 2011–2013 depending on the variety and experiment variant ($p = 0.05$, LSD = 2.13).

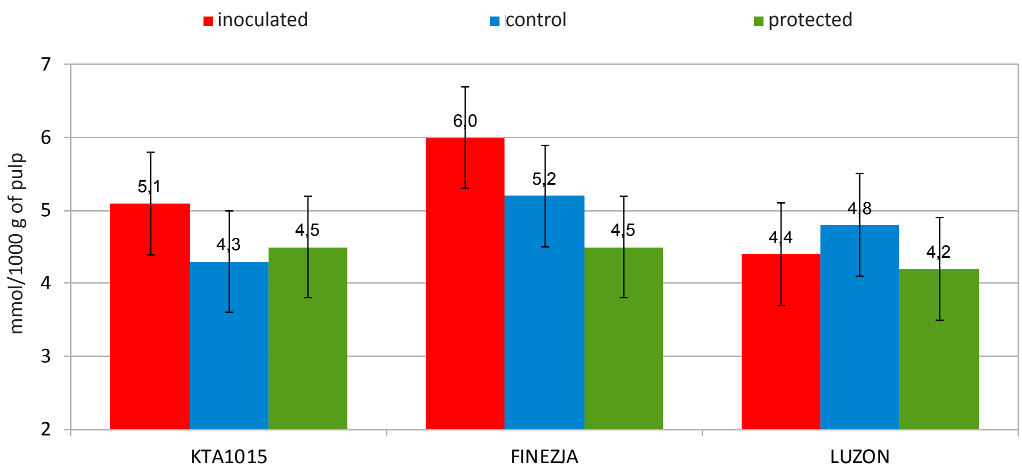

Figure 7. The average sodium content found in sugar beet pulp in 2011–2013 depending on the variety and experiment variant ($p = 0.05$, LSD = 0.74).

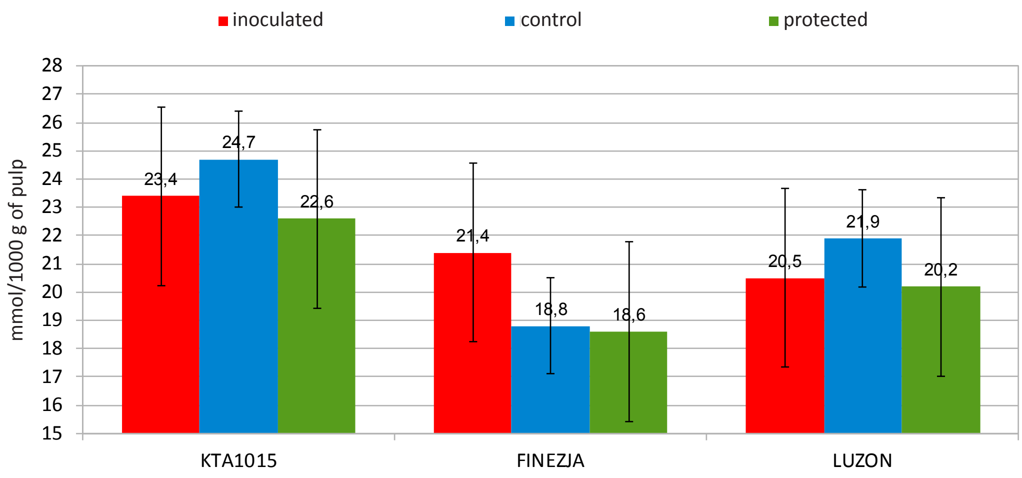

Figure 8. The average content of α-amino nitrogen found in sugar beet pulp in 2011–2013 depending on the variety and experiment variant (p = 0.05, LSD = 3.16).

of chemically unprotected sugar beet roots at 24.7% compared to plants protected by fungicides. However, after inoculation an average rise in sodium content by 0.8 mmol/1000 grams of pulp (18.2%) was recorded. The average sodium content in the pulp obtained from the roots of the tested varieties in the treatment with chemical protection was similar. Larger variability in sodium levels in pulp was recorded for plants in both treatments without chemical protection.

During the study, the differentiation in the content of α-amino nitrogen in the pulp obtained from the roots of the sugar beet, depending on the severity of the disease was also observed (Fig. 8; Table 2-4). The average content of α-amino nitrogen in the pulp of the studied roots obtained from the control treatment and with the use of inoculation was higher by 1.3 mmol/1000 grams of pulp (6.3%) in comparison with the content of mentioned compound in roots of protected plants. Baltaduonytė et al. (2013) and Borówczak et al. (2004) also noted that in the pulp of roots taken from the control plot the α-amino nitrogen content was higher than in the pulp of roots from the chemically protected treatment. In all experimental treatments and in all years of the study a particularly high content of α-amino nitrogen in the root pulp from KTA1015 breeding line plants was observed. This is presumably due to the pleiotropic effect of the genes conditioning resistance to *C. beticola* on the content of molasses-forming substances. The technological sugar produced in sugar factories depends on the content of harmful molasses-forming substances in sugar beet roots. Potassium and α-amino nitrogen have the greatest negative impact on the final sugar production, while the sodium effect is considerably lower. The content of molasses-forming substances in roots is also affected by fertilization applied on the beet production plantations (Pytlarz-Kozicka, 2005).

CONCLUSIONS

1. The application of the fungicides resulted in increased yield of sugar beet.
2. The tested breeding line, resistant to *Cercospora beticola*, under conditions of weak pathogen infestation (up to the second degree on the EPPO scale) yielded worse than the medium resistant variety.
3. In resistant to *Cercospora beticola* varieties an increased molasses-forming substances content is observed.
4. The content of molasses-forming substances in the pulp of sugar beet roots increases with the severity of CLS infection.

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