HARMFULNESS OF *OSTRINIA NUBLIALIS* HBN. ON SOME NON-BT *VERSUS* GENETICALLY MODIFIED BT MAIZE (*ZEA MAYS* L.) CULTIVARS IN POLAND IN 2006–2007

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Abstract: European corn borer (ECB) (*Ostrinia nubilalis* Hbn.) is currently the most dangerous maize pest in Poland. During last five years the pest was recorded in 14 Polish voivodeships, covering over half of the country's area. The greatest harmful activity of the caterpillars was observed in warm southern regions of Poland.

Field experiments carried out in southern Poland in the 2006–2007 growing seasons demonstrated that the average plant damage of non-Bt hybrids by *O. nubilalis* caterpillars varied between 40.0% and 44.0% in 5 locations and two seasons. Under the same pest pressure a high level of resistance of genetically modified (GM) Bt maize (MON 810) cultivars, expressing Cry1Ab toxic protein to infestation by ECB was noted. The average plant infestation of transgenic hybrids was equal to 0.5–0.7% in the same locations. Only some slight symptoms of injuries, such as small number of holes in stalks and gnawing of cobs was observed. Moreover, the tested transgenic hybrids demonstrated a high suitability for climate and soil conditions in Poland, providing early crops of high yield.

Key words: *Ostrinia nubilalis* Hbn., harmfulness, Bt maize, MON 810, non-Bt maize, southern Poland

INTRODUCTION

European corn borer (ECB) was recorded as the maize pest causing economical damage in maize crop in Poland since the 1950s (Kania 1962). Currently, the pest is present in 14 voivodeships, which accounts for over half the country's area (Bereś 2008). ECB caterpillars pose the most serious threat to yield and quality of maize crops in southern Poland (Lisowicz 2001; Bereś *et al.* 2007). Numerous caterpillars of this moth could damage from 50% to 80% and locally up to 100% of plants, causing 20–30%, and sometimes up to 40% losses of grain yield (Lisowicz and Tekiela 2004). The present recommendations on ECB control include: (a) the biological method using *Trichogramma* spp., and (b) chemical treatment with registered insecticides, mainly pyretroids. However, these methods do not fully protect maize plants against caterpillars and their efficacy varies year by year (Lisowicz 2003; Bereś and Lisowicz 2005; Bereś 2006).

One of the most efficient methods to prevent maize losses by ECB under commercial production involves cultivation of genetically modified (GM) maize hybrids expressing the toxic Cry1Ab protein, transformed from *Bacillus thuringiensis* spp. *kurstaki* (Ostlie *et al*. 1997; Beaute *et al*. 2002; Kaiser-Alexnat *et al*. 2005). Recently genetically modified maize was grown in 17 countries and occupied 37.3 million ha (30% of total GM crops) in 2008 (James 2008).

The objective of the presented study was to establishe harmfulness of *O. nubilalis* larvae on some transgenic maize hybrids (containing Cry1Ab protein) as compared to infestation of their maternal non-Bt (isogenic) cultivars in five locations in Poland.

MATERIALS AND METHODS

The study was carried out in two growing seasons 2006–2007 in five locations as follows: the Podkarpackie (A), Małopolskie (B), Śląskie (C), Lower Silesia (D) and Wielkopolskie (E) voivodeships in Poland (Fig. 1). The regions were selected based on the regular abundance of ECB in the recent years. The 10 maize hybrids were chosen for the study as follows: (a) DKC3421YG, PR39F56, PR39D82, PR38F71, ES Paroli Bt as Bt-maize (containing Cry1Ab protein from the MON 810 line) and (b) DKC3420, PR39F58, PR39D81, PR38F70, ES Paroli as non-Bt conventional cultivars (maternal non-Bt). The field experiment was set up under random block design in four replications. The cultivars were chosen with respect to similar early maturation period under soil and climate conditions of Poland (FAO 250–270).

The level of damage of maize plants caused by ECB caterpillars in all locations was evaluated in two periods:

1. Between 30 August – 12 September 2006 and 20 August – 4 September 2007, when plants were at the

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ripening dough stage (BBCH 85) (Adamczewski and Matysiak 2002). The percentage of plants and cobs damaged by caterpillars was calculated by the evaluation of 135 consecutive plants on each plot.

2. Between 1–7 October 2006 and 28 September – 2 October 2007, when plants were at the stage of full kernel maturity (BBCH 87). The percentage of stalks broken below the cob was calculated. The evaluated sample included 135 consecutive plants from each plot.

The results were analysed using standard statistical methods. The correlation between the cultivar and tested parameters was evaluated using a variance analysis model for one-factor experiments carried out in randomized complete block design. The influence of location on the experiment results was tested using a mixed variance analysis design which assumed a constant result for a hybrid and a random effect for location. The statistical significance of differences between mean values was analysed using Tukey's test at the significance level $p < 0.05$.

RESULTS

In both growing seasons the weather was variable. The conditions, particularly favourable for maize growth and the ECB development were observed in the Podkarpackie (A) and the Małopolskie (B) provinces. Less favourable conditions were observed in the Wielkopolskie (E) province in 2006 and in the Śląskie province (C) in 2007, with prolonged periods of high temperatures and lack of precipitation. Plants with signs of water deficit less frequently hosted caterpillars of European corn borer.

The obtained results showed differences in the European corn borer damage of tested cultivars in regard to location and growing season. In the 2006 experiment the greatest harm caused by ECB was observed in Podkarpackie (A), Śląskie (C) and Lower Silesia (D) provinces, where the average percentage of damaged plants of all tested hybrids was equal to: 60.5%, 53.1% and 41.7%, respectively. In these three locations, the percentage of other types of infestation symptoms, such as cob injuries and stalks broken below the cob, increased along with increasing the number of overall plants damage by ECB caterpillars. In 2006 the PR39D81 conventional non-Bt hybrid was classified as the most susceptible, except for one location in the Śląskie region (C), where the PR39F58 hybrid showed the highest infestation level (Tables 1–5).

The overall infestation level of validated maize hybrids by ECB was higher in 2007 than in the 2006 growing season. The greatest infestation caused by this pest was observed in the locations of Małopolskie (B), Podkarpackie (A) and Lower Silesia (D) regions, where the average percentage of plants damaged by caterpillars was equal to: 61.6%, 57.7% and 48.3%, respectively. Additionally, in these three locations, the highest percentage of cobs damaged by caterpillars was also recorded, ranging from 18.8% to 24.8%. In the location Podkarpackie (A) and Małopolskie (B) regions the conventional non-Bt ES Paroli hybrid was classified as the most susceptible to the ECB infestation, in the locations Śląskie (C) and Lower Silesia (D) regions the PR39D81 hybrid, and only the PR38F70 cultivar in the Wielkopolska (E) location (Tables 1–5).

In 2006–2007 the studied GM hybrids demonstrated a high level of resistance to damage caused by the European corn borer in all locations. In transgenic plants only a low number of gnawed stalks and cobs was recorded, in contrast to conventional ones whose above–ground vegetative and generative organs were all damaged by ECB caterpillars. The average percentage of damaged plants of the GM hybrids ranged for five locations and two seasons from 0.5–0.7 (Table 6). This resulted from the fact that newly emerged caterpillars started feeding on maize plants but soon were dying off due to the toxic effect of Cry1Ab protein.

The analysis of influence of a location on the experiment results, based on a mixed variance analysis design, which assumed a constant result for a hybrid and a random effect for location, demonstrated no statistical difference (Table 6).

Evaluation of the GM hybrids' performance in five locations indicated their higher yields of 6.3–12.7 dt/ha in

Table 1. Comparison of infestation of some non-Bt conventional and transgenic (Bt) maize hybrids by European corn borer in location A of the Podkarpackie region in 2006–2007

Hybrid/Year	% damaged plants		% damaged cobs		% of broken stalks below cob		Yield [dt/ha]	
	2006	2007	2006	2007	2006	2007	2006	2007
DKC3420	43.5	45.4	23.2	16.3	6.9	5.7	113.5	117.3
DKC3421YG*	0.2	1.8	0.2	0.0	0.2	0.5	132.6	114.8
PR39F58	60.4	58.8	25.9	20.9	24.0	13.9	94.4	106.6
PR39F56*	0.4	0.2	0.0	0.0	0.2	0.0	108.9	110.2
PR39D81	70.9	61.8	33.1	24.1	25.7	4.4	86.1	113.6
PR39D82*	0.2	0.4	0.2	0.0	0.2	0.0	113.6	113.8
PR38F70	63.4	59.7	32.9	17.6	23.4	19.7	118.3	98.6
PR38F71*	0.4	0.4	0.2	0.0	0.0	0.0	120.1	111.5
ES Paroli	64.1	62.7	27.7	27.9	24.4	2.2	98.0	112.3
ES Paroli Bt*	1.7	0.2	0.5	0.0	0.0	0.0	112.7	116.9
Mean for isogenic hybrids	60.5	57.7	28.6	21.4	20.9	9.2	102.1	109.7
Mean for transgenic hybrids	0.6	0.6	0.2	0.0	0.1	0.1	117.6	113.4
LSD (0.05)	16.62	13.83	10.90	6.63	11.71	7.16	14.12	13.27

* transgenic hybrid

Table 2. Comparison of infestation of some non-Bt conventional and transgenic (Bt) maize hybrids by European corn borer in location B of the Małopolskie region in 2006–2007

Hybrid/Year	% damaged plants		% damaged cobs		% of broken stalks below cob		Yield [dt/ha]	
	2006	2007	2006	2007	2006	2007	2006	2007
DKC3420	31.7	56.9	4.8	18.7	5.7	8.3	123.0	110.8
DKC3421YG*	0.4	0.4	0.0	0.2	0.0	0.0	122.7	126.7
PR39F58	40.9	54.8	4.8	20.0	13.5	11.9	122.8	112.8
PR39F56*	0.2	0.5	0.0	0.0	0.0	0.0	125.3	119.8
PR39D81	44.7	57.2	4.0	24.6	14.5	7.9	110.0	111.6
PR39D82*	0.4	1.1	0.0	0.5	0.2	0.0	125.5	113.1
PR38F70	35.4	66.7	3.6	24.9	15.9	23.1	118.2	106.3
PR38F71*	0.5	0.4	0.0	0.2	0.0	0.0	138.6	118.1
ES Paroli	38.6	72.6	2.9	36.0	21.8	6.8	103.8	116.7
ES Paroli Bt*	1.1	0.7	0.0	0.0	0.2	0.0	121.9	122.5
Mean for isogenic hybrids	38.3	61.6	4.0	24.8	14.3	11.6	115.6	111.6
Mean for transgenic hybrids	0.5	0.6	0.0	0.2	0.1	0.0	126.8	120.0
LSD (0.05)	18.16	14.99	4.11	9.45	11.59	8.45	12.92	12.97

* transgenic hybrid

Table 3. Comparison of infestation of some non-Bt conventional and transgenic (Bt) maize hybrids by European corn borer in location C of the Śląskie region in 2006–2007

Hybrid/Year	% damaged plants		% damaged cobs		% of broken stalks below cob		Yield [dt/ha]	
	2006	2007	2006	2007	2006	2007	2006	2007
DKC3420	50.4	20.6	15.2	4.8	1.8	2.0	67.5	82.2
DKC3421YG*	1.7	1.5	0.4	0.5	0.0	0.0	69.9	86.1
PR39F58	55.6	21.5	20.8	5.3	3.0	4.9	70.9	80.4
PR39F56*	3.5	0.5	1.6	0.0	0.0	0.0	76.1	76.0
PR39D81	53.0	37.7	18.5	7.2	3.7	5.3	65.6	78.9
PR39D82*	2.8	0.4	0.4	0.0	0.0	0.0	88.5	82.0
PR38F70	55.1	24.8	12.5	6.6	4.1	9.2	57.8	77.0
PR38F71*	0.4	0.5	0.0	0.0	0.0	0.0	65.9	85.7
ES Paroli	51.4	32.7	16.9	7.9	4.2	2.0	48.3	79.6
ES Paroli Bt*	1.6	0.7	0.3	0.0	0.0	0.2	57.4	82.0
Mean for isogenic hybrids	53.1	27.5	16.8	6.4	3.4	4.7	62.0	79.6
Mean for transgenic hybrids	2.0	0.7	0.5	0.1	0.0	0.04	71.6	82.4
LSD (0.05)	18.55	14.74	9.62	6.84	3.36	4.62	13.74	9.48

* transgenic hybrid

Table 4. Comparison of infestation of some non-Bt conventional and transgenic (Bt) maize hybrids by European corn borer in location D of the Lower Silesia region in 2006–2007

Hybrid/Year	% damaged plants		% damaged cobs		% of broken stalks below cob		Yield [dt/ha]	
	2006	2007	2006	2007	2006	2007	2006	2007
DKC3420	43.9	38.6	9.5	11,8	9.2	9.9	85.9	109.3
DKC3421YG*	0.2	0.0	0,0	0.0	0.0	0.0	101.9	117.1
PR39F58	37.4	43.2	4,8	17.8	12.2	17.3	86.8	102.2
PR39F56*	0.0	0.7	0,0	0.0	0.0	0.0	92.9	108.3
PR39D81	49.1	56.8	10,6	21.9	23.7	12.3	99.4	98.4
PR39D82*	0.5	0.0	0,0	0.0	0.0	0.0	102.0	102.7
PR38F70	44.7	54.0	12,7	20.6	11.6	31.8	97.6	99.0
PR38F71*	0.2	0.2	0,0	0.0	0.0	0.0	95.9	110.4
ES Paroli	33.6	48.7	8,9	22.0	10.6	5.3	93.0	102.9
ES Paroli Bt*	0.6	0.7	0.0	0.2	0.0	0.0	88.3	106.2
Mean for isogenic hybrids	41.7	48.3	9,2	18.8	13.5	15.3	92.5	102.4
Mean for transgenic hybrids	0.3	0.3	0,0	0.04	0.0	0.0	96.2	108.9
LSD (0.05)	10.91	19.17	3,29	9.98	8.98	8.35	13.76	16.25

* transgenic hybrid

Table 5. Comparison of infestation of some non-Bt conventional and transgenic (Bt) maize hybrids by European corn borer in location E of the Wielkopolska region in 2006–2007

Hybrid/Year	% damaged plants		% damaged cobs		% of broken stalks below cob		Yield [dt/ha]	
	2006	2007	2006	2007	2006	2007	2006	2007
DKC3420	3.8	16.2	0.5	5.9	0.4	3.1	61.4	126.6
DKC3421YG*	0.2	0.0	0.0	0.0	0.0	0.0	59.7	128.0
PR39F58	4.7	25.5	0.5	10.5	1.9	4.6	55.7	124.3
PR39F56*	0.2	0.2	0.0	0.0	0.0	0.0	58.6	116.0
PR39D81	8.8	27.6	1.1	10.6	2.2	1.6	63.6	116.8
PR39D82*	0.0	0.4	0.0	0.0	0.0	0.0	58.4	113.3
PR38F70	5.9	28.8	0.7	9.7	0.5	6.8	44.7	113.8
PR38F71*	0.0	0.0	0.0	0.0	0.0	0.0	65.3	101.6
ES Paroli	6.8	27.0	0.2	14.5	1.6	1.8	46.3	120.5
ES Paroli Bt*	0.4	0.2	0.0	0.0	0.0	0.0	49.1	129.1
Mean for isogenic hybrids	6.0	25.0	0.6	10.2	1.3	3.6	54.3	120.4
Mean for transgenic hybrids	0.2	0.2	0.0	0.0	0.0	0.0	58.2	117.6
LSD (0.05)	4.93	11.12	1.13	7.10	2.40	3.34	12.56	10.22

* transgenic hybrid

Table 6. Summary of evaluation of harmfulness of *O. nubilalis* to some non-Bt and Bt hybrids for five locations in 2006–2007 growing seasons

Hybrid/Year	% damaged plants		% damaged cobs		% of broken stalks below cob		Yield [dt/ha]	
	2006	2007	2006	2007	2006	2007	2006	2007
DKC3420	34.6	35.5	10.7	11.5	4.8	5.8	90.3	109.2
DKC3421YG*	0.5	0.7	0.1	0.1	0.1	0.1	97.4	114.5
PR39F58	39.8	40.8	11.4	14.9	10.9	10.5	86.1	105.3
PR39F56*	0.8	0.4	0.3	0.0	0.1	0.0	92.4	106.1
PR39D81	45.3	48.2	13.5	17.8	13.9	6.3	84.9	103.9
PR39D82*	0.8	0.4	0.1	0.1	0.1	0.0	97.6	104.9
PR38F70	40.9	46.8	12.5	15.9	11.1	18.1	87.3	98.9
PR38F71*	0.3	0.3	0.1	0.1	0.0	0.0	97.2	105.5
ES Paroli	38.9	48.7	11.3	21.7	12.5	3.6	77.9	106.4
ES Paroli Bt*	1.1	0.5	0.2	0.1	0.1	0.1	85.9	111.3
Mean for isogenic hybrids	40.0	44.0	11.9	16.4	10.6	8.9	85.3	104.7
Mean for transgenic hybrids	0.7	0.5	0.2	0.1	0.1	0.04	94.1	108.5
LSD (0.05) A	6.21	6.30	2.91	3.41	3.61	2.82	5.64	5.32
LSD (0.05) L*A	ns	ns	ns	ns	ns	ns	ns	ns

* transgenic hybrid
A – hybrids
L – location
L*A – interaction
ns – insignificant influence with respect to the effect of A factor

Fig. 1. Geographical distribution of *O. nubilalis* infestation areas in 2006 according to Bereś (2008) (dark grey colour) and location of experimental sites: A – Podkarpackie province, B – Małopolskie province, C – Śląskie province, D – Lower Silesia province, E – Wielkopolskie province

2006 and 1.0–6.6 dt/ha in 2007 over the yields of non-Bt maternal cultivars (Table 6). However, the advantage of growing GM maize was not only restricted to the quantity of kernel yield. The more important differences laid in the cob infestation by ECB larvae. For five locations, the significant differences between cob damage were equal to 17.7% for GM *versus* non-Bt cultivars (Table 6). It was well established that maize cobs injured by ECB are always infected by pathogenic fungi, contaminating kernels with mycotoxins (Papst *et al*. 2005).

DISCUSSION

The results of presented above field experiments should be evaluated on four levels: (a) the efficacy of Bt cultivars in controlling ECB in southern Poland; (b) their effect in reducing mycotoxins content in kernels; (c) their advantages over long–term classical breeding for plant resistance to ECB; and (d) growing Bt resistant cultivars as ECB control methods as compared to presently recommended biological and chemical control methods in Poland.

The high efficacy in controlling ECB in studied location and demonstrated yield increase of Bt maize supports recommendation by Brooks and Anioł (2005). Both authors, considering average yields and prices of maize in Poland, estimated that Bt technology would be profitable only for farming located in the regions of moderate and high infestation by ECB in Poland. They calculated that the cost of Bt technology implementation should be covered by yield surplus of 0.2 dt/ha, equal to 3.5% increase for average yields and 2.8% for high yields. The authors also indicated that injured kernels containing mycotoxins may face the decrease of quality a rejection by markets (Brooks and Anioł 2005).

The independent experiments carried in 2005 and 2006 in southern Poland showed that Bt maize cultivars contained only traces of mycotoxins. The cobs of non-Bt maize carried higher ear root infection of kernels (Tekiela and Gabarkiewicz 2008). The total concentration of fumonisins ranged from 165 to 511 ppb in 2005 and from 18 to 847 ppb in 2006. Deoxynivalenol concentration ranged from 50 ppb to 502 ppb in non-transgenic cultivars.

Both international and national recommendation on Integrated pest management (IPM) list growing resistant/tolerant plants. Nearly 50 years' screening and breeding maize for resistance to ECB, applying classical genetic methods, in spite of heavy investment in insect mass rearing (Guthrie and Barry 1989) and human resources in breeders and entomologists (Ortega *et al*. 1980) in public institutions and commercial companies, did not finalized by releasing commercial cultivars with high resistant to two ECB generations (Dąbrowski 1989). In addition, the resistant donor lines in other regions than their original breeding station were loosing their resistance as was shown by their evaluation in 15 countries by the International Working Group on *Ostrinia*. The effect of climatic conditions and probable geographic genetic variation of ECB populations were responsible for the differences in expression of resistance (Chiang *et al*. 1973). In contrast, the Bt maize cultivars confirmed their stable resistance to ECB in the USA, Europe and Near East and in addition to other species of stem borers such as *Sesamia nonagrioides* Lef. in Spain and Egypt (James 2008).

The reported significant differences in the infestation and injury levels on Bt GM hybrids and non-Bt maize cultivars in both 2006 and 2007 seasons confirmed the earlier authors' studies (Bereś and Gabarkiewicz 2007, 2008) for southern Poland and for other locations in south-western Poland (Twardowski *et al*. 2008).

Introduction of GM cultivars to common cultivation should be verified by efficiency of presently recommended other control methods of ECB on maize in Poland. Both biological and chemical control showed their limitation when applied by farmers. Releasing *Trichogramma* spp. parasitoids reduced the level of plant infestation from 5.3 to 2.3% in 1996 and 39.0% to 16.0% in 1997 experiment but still the harvested cobs showed the average number of feeding holes equal to 0.64 per cob for *Trichogramma* spp. treatment *versus* 1.45 holes/cob for untreated control (Bereś and Lisowicz 2005).

Similar efficacy level was observed for chemical control using Karathe Zeon 050 CS (lambda-cyhalothrin) spraying. There was a reduction of caterpillars infesting control plants from 2.9–4.7 larvae/plant to 0.4–0.9 larvae/plant for the pyrethroid treatment in the 2003–2005 experiment in southern Poland (Bereś 2006). The experiment also showed that during higher pressure of ECB population, two pesticide treatment would be required to secure an economic efficacy of treatments. In addition, only very few farms have access to the specialized sprayer for maize and few others are using hand sprayer to treat maize plants of approximate 2 m height at the ECB adult emergence and oviposition period.

Comparison of three described methods of the ECB control indicated that for majority of Polish farmers growing maize in the high ECB risk regions, planting Bt maize would provide the most convenient approach eliminating the crop infestation by this pest. There is hope that new legislation on GMO coexistence would permit Polish farmers to benefit from the Bt technology.

CONCLUSIONS

1. The studied transgenic maize hybrids demonstrated a high suitability for the climate and soil conditions in Poland, providing early crop of high quality.
2. The statistical analysis proved a significant difference in the infestation level between Bt GM plants and non-Bt cultivars, indicating the high resistance of transgenic maize to damage caused by the European corn borer.
3. In the transgenic MON 810 hybrids only some slight infestation of stalks and cobs was observed, associated with the fact that young caterpillars started feeding on plants before dying due to the toxic effect of Cry1Ab protein.
4. The analysis of influence of a location on the experiment results, based on a mixed variance analysis design, demonstrated no significant statistical effect.
5. In the regions at serious risk of European corn borer outbreaks (especially southern Poland) cultivation of GM maize cultivars may provide effective method of reducing the harmfulness of this pest species.

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POLISH SUMMARY

SZKODLIWOŚĆ *OSTRINIA NUBLIALIS* HBN. DLA WYBRANYCH ODMIAN KONWENCJONALNEJ KUKURYDZY (*ZEA MAYS* L.) W PORÓWNANIU DO ICH TRANSGENICZNYCH MODYFIKACJI W POLSCE W LATACH 2006−2007

Omacnica prosowianka (*Ostrinia nubilalis* Hbn.) jest aktualnie najgroźniejszym szkodnikiem kukurydzy w Polsce. W 2007 roku szkodnik zasiedlał obszar 14 województw, co stanowi ponad połowę kraju; największą szkodliwość gąsienic notuje się w najcieplejszych rejonach południowej Polski. Jedną z metod pozwalających zabezpieczyć rośliny przed uszkodzeniami powodowanymi przez *O. nubilalis* jest dobieranie do uprawy transgenicznych odmian kukurydzy z ekspresją toksycznego białka Cry1Ab.

Badania wykonane w południowej Polsce w latach 2006–2007 wykazały wysoką efektywność odmian kukurydzy Bt zawierających białko Cry1Ab z linii MON 810, na ograniczenie uszkodzeń powodowanych przez *O. nubilalis*. W latach badań, we wszystkich lokalizacjach, gąsienice *O. nubilalis* uszkodziły średnio od 40,0 do 44,0% roślin u odmian izogenicznych, natomiast u odmian transgenicznych średnio od 0,5 do 0,7% roślin. U odmian kukurydzy Bt notowano jedynie nieliczne otwory w łodygach oraz wgryzienia do kolb. Ponadto badane odmiany transgeniczne wykazały dużą przydatność pod względem wczesności oraz plonowania do polskich warunków glebowo-klimatycznych.