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The maturation rate of the generative stage of *Leptosphaeria maculans* and *Leptosphaeria biglobosa* in central and eastern Poland

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Abstract. Two pathogens from the genus *Leptosphaeria*: *Leptosphaeria maculans* and *Leptosphaeria biglobosa* are the cause of stem canker – a damaging disease of oilseed rape in Poland. The fungi belong to *Ascomycetes,* with both sexual and vegetative stages in their life cycle. The fruiting bodies of the teleomorph, called pseudothecia produce ascospores, which are the primary inoculum infecting young plants. The formation of pseudothecia takes place on infected stubble from a previous season's crop. Conducive weather conditions lead to ascospore release and development of disease symptoms. The main goal of the experiments was to evaluate the uniformity of pseudothecia maturation rate within and between the climatic regions as well as the relation between the development of pseudothecia and ascospore release of *L. maculans* and *L. biglobosa*. The study was done for three years (2005–2007) at 7 experiment sites located in three climatic regions of central and eastern Poland, with Puławy located centrally on a borderline between these regions. The maturation of pseudothecia was based on the morphology of asci and ascospores. The monitoring of ascospore density in the air was done using a seven-day volumetric spore trap. We have found that at early maturation stages the differences in pseudothecia maturation were small and did not exceed 2 weeks, but at advanced maturation stages the highest differences exceeded a month. The temperature profiles in studied locations were highly correlated, but the correlations between the rainfall data were sometimes very low or non existent. The differences in pseudothecial maturation were mainly associated with the quantity of precipitation both before and during the maturation process. Ascospore release greatly depended on the rate of pseudothecial maturation of *L. maculans* and *L. biglobosa.*

key words: *Leptosphaeria maculans, Leptosphaeria biglobosa,* stem canker, pseudothecial maturation, ascospore release, volumetric spore trap

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INTRODUCTION

Oilseed rape (*Brassica napus* L.) is currently one of the fastest expanding crops worldwide. According to the prognosis of OPEC, in 2008/2009 the world production of this crop will increase by ca. 9% and it shall soon reach 53 M tons (World Oil Outlook, 2007). European Union is at present the biggest producer of oilseed rape seeds and oil. In the season 2007/2008 it produced 18.2 Mt, which constituted 37.8% of the overall production of rapeseed. Such great increase of oilseed rape production and its common use in crop rotation brings the necessity to introduce new agronomic systems and to solve numerous problems connected with crop protection against different pests, including weeds, insects and plant pathogens. The most damaging pathogens of oilseed rape are fungi, with different importance of various species depending on the area of the world. From among 2.5 million farms in Poland about 278 thousands are situated in Lublin voivodship. The acreage and yield of oilseed rape in this region in 2006 were respectively 28.6 thousands hectares and 66 thousand tons (Dmochowska, 2007).

The increasing profitability of oilseed rape in agricultural production implies constant search of methods allowing to increase plant yield and quality. One of the most important conditions of high yield is the protection against fungal diseases. The most damaging disease of oilseed rape is stem canker of brassicas caused by the species *Leptosphaeria maculans* and *L. biglobosa.* Serious crop losses were reported in Europe (Fitt et al., 1997; Allard et al., 2002; West, 2001, 2004; Jędryczka, 2007), Canada (Gugel, Petrie, 1992) and Australia (Salisbury et al., 1995; Khangura, Barbetti, 2001).

Leptosphaeria is an ascomycete with both sexual and vegetative stages in its life cycle. The fungus produces fruiting bodies of the teleomorph (pseudothecia) which contain ascospores. In Poland, ascospores are the primary inoculum, which infects young rapeseed plants in the autumn (Kaczmarek et al., 2006). The formation of pseudothecia takes place on infected stubble from a previous season's crop (Petrie, 1994, 1995). Pseudothecium must reach a complete maturity to produce and release fully developed eight ascospores, each consisting of six cells. Released ascospores can be transferred by wind to several kilometres and infect plants far away from the source of disease. The maturation rate of pseudothecia and subsequent spore release depend on weather conditions, principally wetness (rain or dew) and temperature (Salam et al., 2003; Toscano-Underwood et al., 2003; Huang et al., 2005). Conducive weather conditions in summer and autumn lead to ascospore release and development of disease symptoms. Severity of the disease and symptoms of infection highly depend on date of infection and growth rate of the fungus inside plant tissues.

The aim of this study was to evaluate differences between pseudothecia maturation rates within and between the climatic regions of central and eastern Poland. We have also studied the relation between the development of pseudothecia and ascospore release of *L. maculans* and *L. biglobosa* at the same experiment site.

MATERIALS AND METHODS

Location of experiment sites

The area of study was composed of three climatic zones located in central and eastern Poland, as proposed by Wiszniewski and Chełchowski (1987): Region 1) Lublin and Zamość; Region 2) Mazovia Plain, and Region 3) Łódź-Masłowice Highland (Fig. 1). The samples of oilseed rape stubble originated from 7 experiment sites located in: Region 1) Kościerzyn (N 51°62'36.23", E 18°64'61.60"), Masłowice (N 51°22'19.31", E 18°56'57.88"), Radom (N 51°39'92.35", E 21°15'13.48"); Region 2) Siedlce (N 52°16'77.89", E 22°27'29.32"); Region 3) Bezek (N 51°19'60.37", E 23°16'48.65"), Leśniowice (N 50°98' 37.30", E 23°49'30.36"), with Puławy (N 51° 42'75.47", E 21° 95'40.41") located in the middle of the three regions, exactly on a borderline. The highest distance between Puławy and the experiment site (Masłowice) was 240 km westwards and Leśniowice located 115 km eastwards of Puławy. The highest distance to the north was the town of Siedlce, located 85 km from Puławy (Figure 1).

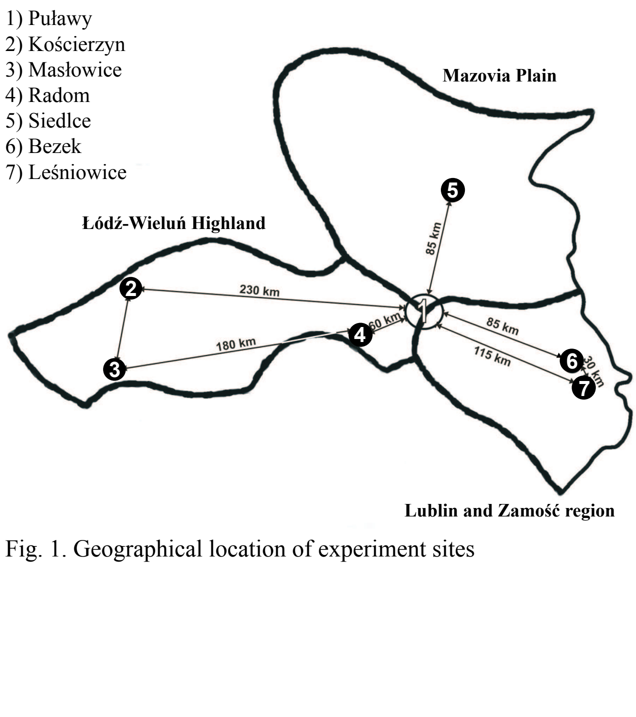

Fig. 1. Geographical location of experiment sites

Assessment of *Leptosphaeria maculans* and *Leptosphaeria biglobosa* pseudothecia maturation

The percent of *L. maculans* and *L. biglobosa* pseudothecia maturation was assessed on base of asci development stage and presence of ascospores. From five randomly chosen parts of infected oilseed rape stubble from the previous season's crop 10 fruiting bodies were isolated and microscope slides were prepared. Test samples were collected every seven days from 1 September until 30 November. The choice of this sampling period was selected on a base of previous results on pseudothecia maturation and ascospore release of *L. maculans* and *L. biglobosa* in the other regions of Poland. Pseudothecia maturation stages were classified to one of five categories (Toscano-Underwood et al., 2003):

- class A: pseudothecia without asci;
- class B: pseudothecia with asci, but without ascospores;
- class C: pseudothecia with asci, ascospores with 3–4 cells;
- class D: pseudothecia with asci, ascospores fully developed;
- class E: pseudothecia with empty asci.

Ascospore sampling

The *L. maculans* – *L. biglobosa* ascospore sampling was done at the same autumn period as the sampling of pseudothecia from oilseed rape stubble. The monitoring of ascospore concentration in the air was done using a Hirst type seven-day volumetric spore sampler (Burkard Manufacturing Co., Rickmansworth, UK). The trap was located at the grounds of the Institute of Soil Science and Plant Cultivation – National Research Institute in Puławy. The spore trap was surrounded by oilseed rape stubble infected with stem canker. The stubble was collected in the previous season at the Agricultural Experimental Station in Grabów and the Experimental Station in Osiny. The spore sampler was operated according to the instructions of Lacey and West (2006). The Melinex tape from the spore trap was cut into 7 pieces, each 48 mm long. Each piece was mounted onto a microscope slide, stained with 0.1% (w/v) trypan blue in lactophenol and examined with a light microscope under 400x magnification (Zeiss Axiostar, Germany). The numbers of spores were re-calculated to daily ascospore numbers per 1 m^3 of air.

Table 1. Rainfall data at seven experiment sites and the correlation coefficients of mean daily rainfall in Puławy and the other experiment sites in July–November, 2005–2007.

Year	Month	Experiment site						
		Puławy	Radom	Siedlce	Leśniowice	Bezek	Masłowice	Kościerzyn
				Mean daily rainfall (mm)				
	July	4.8	5.3	1.5	2.5	2.5	n.d.	n.d.
	August	4.4	2.2	2.1	9.7	9.7	n.d.	n.d.
2005	September	6.6	1.9	1.3	5.4	5.4	n.d.	n.d.
	October	0.7	0.5	0.8	0.9	0.9	n.d.	n.d.
	November	2.0	1.3	3.6	1.2	1.2	n.d.	n.d.
	July	4.7	2.3	2.2	5.2	5.2	4.1	5.8
	August	10.9	6.9	6.4	12.7	12.7	3.8	3.8
2006	September	3.3	4.9	2.6	1.7	1.7	4.5	2.1
	October	3.8	3.6	2.3	1.7	1.7	5.3	4.1
	November	2.9	2.3	8.6	2.2	2.2	3.6	4.3
	July	4.7	2.6	1.7	5.2	2.4	2.9	2.9
	August	12.0	2.6	1.2	12.7	1.6	1.9	1.9
2007	September	4.3	4.3	2.7	1.7	3.2	2.7	2.7
	October	3.5	0.9	1.9	1.7	2.6	3.6	3.6
	November	2.9	1.8	2.5	2.2	7.2	7.1	7.1
				Number of rainy days				
	July	14	13	17	10	10	n.d.	n.d.
	August	9	12	16	9	9	n.d.	n.d.
2005	September	5	10	12	4	4	n.d.	n.d.
	October	5	11	15	7	7	n.d.	n.d.
	November	14	16	20	14	14	n.d.	n.d.
	July	4	5	6	5	5	4	12
	August	21	20	31	19	19	22	27
2006	September	3	7	19	4	4	5	25
	October	9	11	20	8	8	8	29
	November	12	14	22	13	13	14	29
	July	5	21	20	6	26	28	31
	August	20	11	12	19	24	23	27
2007	September	4	15	13	4	27	26	26
	October	10	14	12	8	29	31	31
	November	13	18	16	13	23	27	27
				Correlation coefficients between rainfall in Puławy and the other experiment sites				
	July	1.000	0.290	0.260	0.040	0.040	n.d.	n.d.
	August	1.000	0.740	0.430	0.790	0.790	n.d.	n.d.
2005	September	1.000	-1.000	0.400	0.500	0.500	n.d.	n.d.
	October	1.000	0.400	0.400	0.800	0.800	n.d.	n.d.
	November	1.000	0.090	0.090	-0.870	-0.870	n.d.	n.d.
	July	1.000	0.400	0.500	-0.400	-0.400	-0.400	-0.400
	August	1.000	0.060	0.050	0.550	0.550	0.280	-0.440
2006	September	1.000	0.400	0.500	0.500	0.500	-0.400	-0.400
	October	1.000	0.400	-0.400	0.740	0.740	0.890	0.700
	November	1.000	0.590	0.280	0.720	0.720	0.710	0.390
	July	1.000	-0.400	-0.500	0.400	0.280	-0.740	-0.740
	August	1.000	0.440	-0.060	0.550	0.550	-0.010	-0.010
2007	September	1.000	0.500	-0.500	0.400	0.280	0.500	0.500
	October	1.000	-0.300	0.500	0.740	0.740	-0.140	-0.140
	November	1.000	0.360	0.180	0.720	0.720	-0.070	-0.070

n.d. – no data

All correlation coefficients are significant at $P < 0.05$

Meteorological analysis

The rainfall (mm) and temperature (°C) data were gathered from 1 July until 30 November, except experiment sites at Kościerzyn and Masłowice in 2005, where failure of a meteorological station deprived us from the rainfall data. The mean distance between experiment point and meteorological station was 13 kilometres. Spearmann's correlation coefficient was used to calculate relationships between the temperature and between the rainfall at all experiment sites, with $P<0.05$. To check normality of used data Shapiro-Wilk test was used. All calculations were performed with the use of STATISTICA version 7.0 (Hill, Lewicki, 2007)

RESULTS

The fully mature pseudothecia of *L. maculans* and *L. biglobosa* were observed at all experiment sites over the whole monitoring period. At the earliest they occurred in 2007, when pseudothecia belonging to class D were found already at the first sampling on 5 September (Fig. 2c). In 2006 the same phenomenon was observed one week later (Fig. 2b). The latest production of fully mature pseudothecia was found in the autumn 2005, when class D pseudothecia were not observed before the third week of study, *i.e.* the second half of September (Fig. 2a). In most cases a rapid increase of maturation took place, but in general it coincided with the beginning of maturation; the earlier it was observed, the earlier increase of class D pseudothecia was found. The maturation pattern was similar for the most of experiment sites, although each location experienced the maturation rate specific for the place. In 2005 and 2006 the data found for Puławy greatly differed from the results found in the remaining experiment sites (Fig. 2ab), whereas in 2007 this phenomenon was reversed and class D pseudothecia in Puławy were found early and in big numbers (Fig. 2c). This coincided with the mean daily rainfall and the number of rainy days; in 2005 this parameter was 3.7 mm of rain per day and there were 47 rainy days in total from July to November. In 2006 these values were 5.1 mm and 49 days respectively, but in 2007 the rainfall parameters were the highest, with 5.5 mm of mean rainfall per day and as many as 52 rainy days (Table 1). The mean data for the first two months of the study were comparable – the lowest in 2005 (4.6 mm, 23 days), higher in 2006 (7.8 mm, 25 days) and the highest in 2007 (8.4 mm and 25 days).

The experiment site in Puławy, although located centrally to the remaining sampling sites (Fig. 1) showed different rate of *L. maculans* and *L. biglobosa* pseudothecia maturation process. In the first two years of this study most class D pseudothecia developed later (Fig. 3ab) and in 2007 they matured one to four weeks later at most experiment sites and thresholds. Moreover, the sampling place located in Leśniowice, namely *ca*. 115 km south-eastwards from Puławy (Fig. 1) was the place of the most similar data in 2005 and 2006, and greatly different from 2007. In all

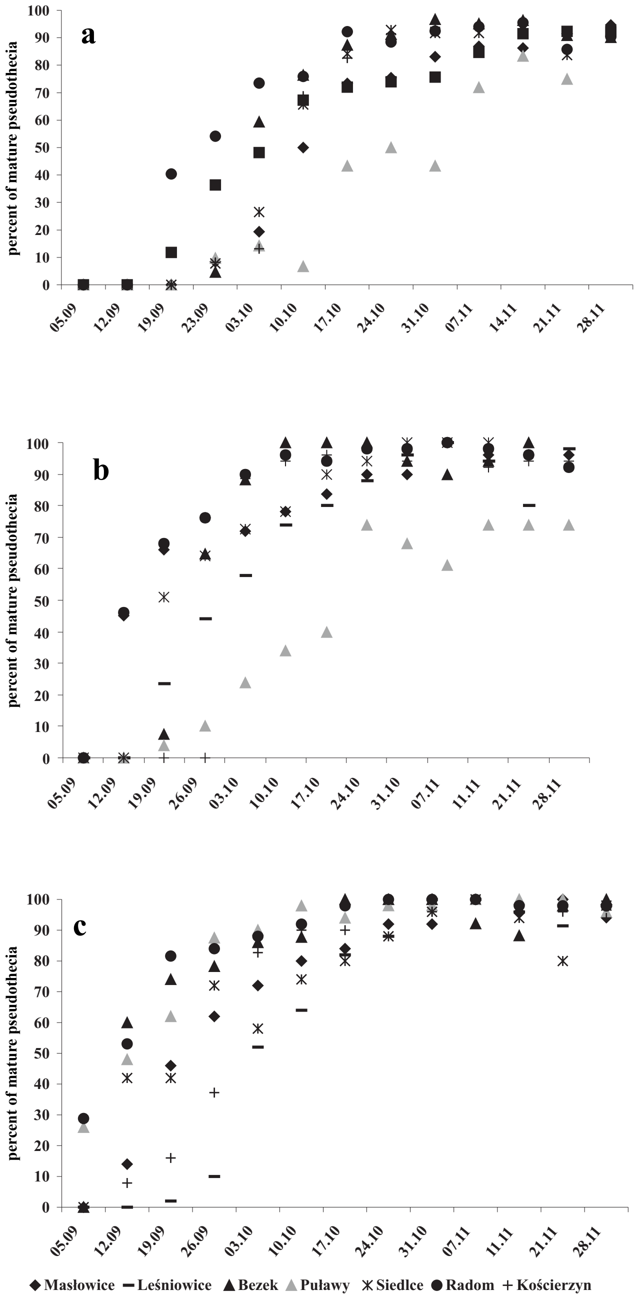

Fig. 2. The percent of mature pseudothecia of *L. maculans* and *L. biglobosa* on the stubble of oilseed rape at seven experiment sites in central and east Poland in: a) 2005, b) 2006, c) 2007.

cases the correlation coefficients between the temperature profiles of Puławy and of the other experiment sites were very high, they ranged from 0.728 to 0.984 (Table 2). It is however possible that subtle differences of daily rainfall and temperatures could affect the maturation process of generative stage fruiting bodies.

The differences between autumn periods were most clearly visible for the *L. maculans* and *L. biglobosa* as-

Table 2. The correlation coefficients of temperature profiles between Puławy and six experiment sites located in central and east Poland (July–November, 2005–2007).

Year	Month	Experiment site					
		Leśniowice	Radom	Siedlce	Masłowice	Kościerzyn	Bezek
2005	July	0.864	0.743	0.784	0.868	0.868	0.864
	August	0.920	0.845	0.814	0.784	0.784	0.920
	September	0.967	0.894	0.931	0.905	0.905	0.967
	October	0.982	0.811	0.877	0.915	0.915	0.982
	November	0.966	0.893	0.886	0.825	0.825	0.966
2006	July	0.948	0.795	0.775	0.780	0.870	0.948
	August	0.957	0.841	0.728	0.756	0.881	0.957
	September	0.924	0.831	0.887	0.875	0.809	0.924
	October	0.984	0.748	0.757	0.829	0.934	0.984
	November	0.938	0.732	0.731	0.759	0.747	0.938
2007	July	0.957	0.841	0.870	0.756	0.881	0.957
	August	0.841	0.881	0.881	0.957	0.957	0.957
	September	0.984	0.748	0.809	0.829	0.934	0.984
	October	0.748	0.934	0.934	0.984	0.984	0.984
	November	0.773	0.747	0.747	0.938	0.938	0.938

All correlation coefficients are significant at $P < 0.05$

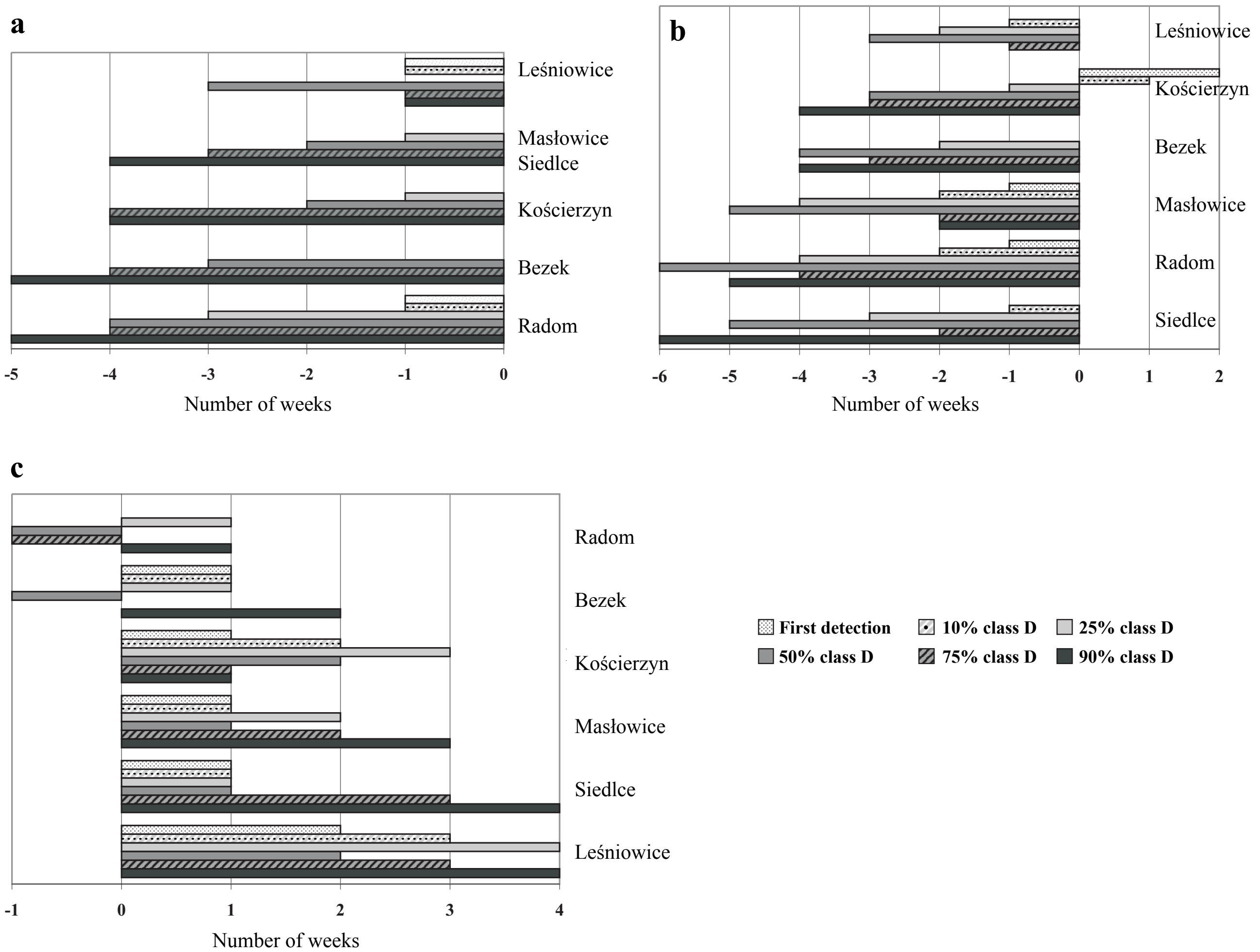

Fig. 3. The difference in maturation rate of class D pseudothecia between Puławy and the remaining experiment sites of study in autumn: a) 2005, b) 2006, c) 2007.

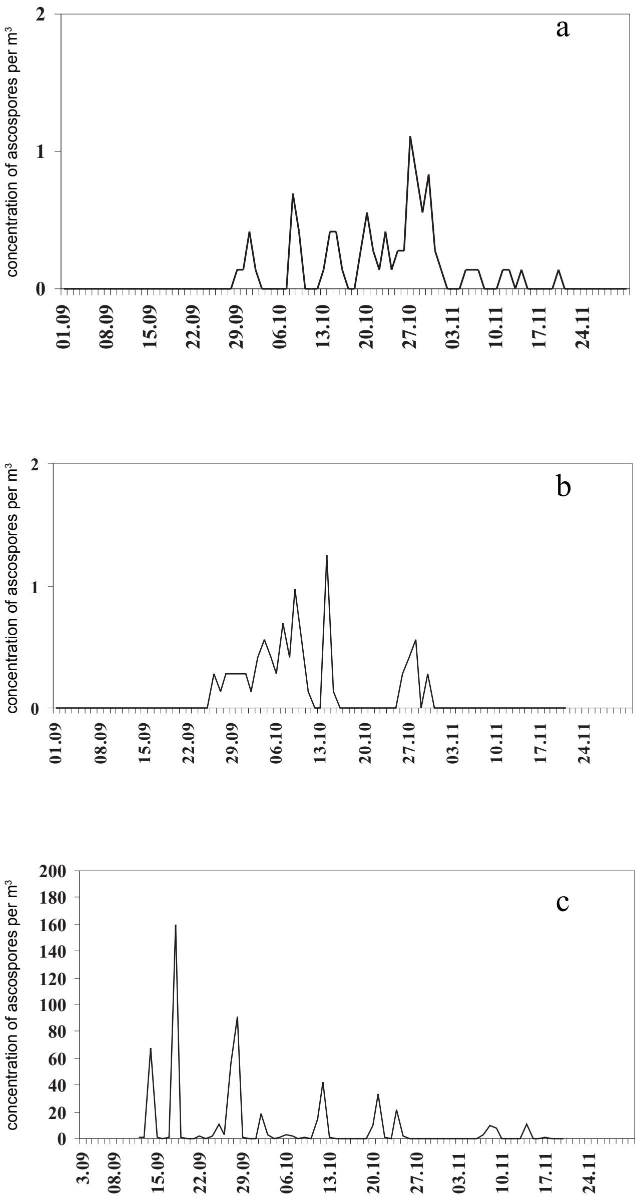

Fig. 4. The concentration of *L. maculans* and *L. biglobosa* ascospores in Puławy in: a) 2005, b) 2006, c) 2007

cospore density numbers. In 2005 and 2006 the daily concentrations of ascospores in the air was below 1, and in 2007 the peak ascospore day was *ca.* 160 ascospores per 1 m^3 (Fig. 4a-c).

In contrast to small differences of the temperatures and great similarities in temperature profiles (Table 2), great differences were observed in summary precipitation noted at different locations (Table 1). The lowest number of rainy days in the studied period was 44 days, found in Leśniowice and Bezek in 2005. The highest summary number of rainy days was noted in Kościerzyn, both in 2006 (122 days) and 2007 (142 days). The data cannot be compared to the same experiment site in 2005; the failure of the weather station did not allow us to calculate the results. Leśniowice and Puławy had the lowest numbers of rainy days.

DISCUSSION

Detailed data about *L. maculans* and *L. biglobosa* life cycles in different climatic regions allow to improve stem canker management through optimization of fungicide treatments. The ascospores of the studied species form primary inoculum infecting young plants of numerous Brassica crops, including oilseed rape. The formation of pseudothecia takes place on infected stubble from a previous season's crop (Petrie, 1995). In our hemisphere ascospores are mainly produced and dispersed in the autumn (Huang et al., 2005; Kaczmarek et al., 2006).

Suitable weather conditions in summer and autumn cause fast maturation of the fruiting bodies of *L. maculans* and *L. biglobosa*, leading to ascospore release and subsequent infections of host plants. Intensity of pseudothecial maturation of *L. maculans* and *L. biglobosa* depend on weather conditions, e.g. higher temperature within 5–20°C increases pseudothecia maturation (Toscano-Underwood et al., 2003). In susceptible plants the infections cause leaf spots. The fungus spreads from a leaf via a petiole to a stem (Hammond et al., 1985). The intensity of symptoms and severity of the disease depend on the date of infection and fungal growth rate in tissues of the host-plant, related to air temperature (Huang et al., 2003).

The experiments performed in the central and eastern part of Poland showed that pseudothecia maturation rate strongly depended on a cropping season. High and statistically significant correlation coefficients were obtained between temperature profiles originating from different monitoring sites. Even the lowest archived values were statistically significant. It is therefore postulated that – within an optimal temperature range – rainfall is the main factor responsible for pseudothecia maturation and subsequent ascospore showers. In all three years of this study the weather conditions were suitable to complete the maturation process of *L. maculans* and *L. biglobosa*. In most situations the pseudothecia of class D were observed in September. A rapid increase of fully matured pseudothecia was observed soon after their first detection.

The release of ascospores from pseudothecia is humidity and rainfall dependent (Huang et al., 2007; Oliveira et al., 2009). In this study, the amount of rainfall was doubled in 2006 and tripled in 2007, as compared to 2005. High rainfall coincided with the high number of *L. maculans* and *L. biglobosa* ascospores. This phenomenon was mainly observed in 2007 when much higher rainfall triggered abundant maturation of class D pseudothecia, followed by high concentrations of the ascospores, greatly outnumbering the negligible amounts of fungal spores in air samples, observed in 2005 and 2006. The results of this study confirm the finding of Toscano-Underwood et al. (2003), that an influence of wetness is more crucial than temperature.

Nevertheless, simple dependency of pseudothecia maturation and ascospore release on rainfall was not always found. In some cases the weather conditions were similar between years or locations, but the pseudothecia and spore numbers were different. The complicated nature of the *Leptosphaeria* spp. fungi life cycles and conditions for their growth and development, leading to plant infections were also demonstrated by Thürwächter et al. (1999). The authors studied the relations between ascospore discharge, oilseed rape plant infestation and pathogenicity of fungal strains trying to relate the impact of *L. maculans* and the healthiness, as well as the yield of oilseed rape. The relations between these characters were not straightforward, but they could be mathematically modelled (Salam et al., 2007) and subsequently – used to support decisions on chemical protection of agricultural crops against stem canker of oilseed rape (Jędryczka et al., 2008).

Based on the results of this study it can be concluded, that weather data observed at different experiment sites and years had a strong, positive effect on *L. maculans* and *L. biglobosa* maturation process. We have proved that each vegetative season differed in pseudothecia maturation rate and intensity as well as timing of ascospore release. As a consequence, a fixed term of fungicide treatment against stem canker of crucifers is not justified.

CONCLUSIONS

1. There are big differences in pseudothecia maturation speed of *L. maculans* and *L. biglobosa* in different vegetative seasons and locations in Poland.
2. There is a high relationship between pseudothecia maturation rate of *L. maculans* and *L. biglobosa* and meteorological conditions, especially rainfall.
3. The weather data observed at different experiment sites and years had a strong, positive effect on *L. maculans* and *L. biglobosa* maturation process.
4. A fixed period of fungicide treatment against stem canker of brassicas is not effective, when using chemical sprays on oilseed rape.

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The assessment of grain quality of maize cultivars depending on the way and term of herbicides application

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Abstract. Accessibility of stably-yielding maize cultivars with different degrees of earliness, as classified in the FAO system, has created the possibility of maize production across the whole area of Poland. An extensive area under maize cultivation is possible due to a favourable economic situation and wide utilization of grain both in the production of fodder mixtures and by many branches of industry. Among cereals, maize is characterized by the highest energy value and high digestibility of nutrients resulting from high content of starch and fat, as well as low content of protein and crude fibre. These properties are influenced by environmental conditions, accessibility of water and nitrogen and also by plant protection chemicals, including herbicides. The herbicides applied are not always selective for particular cultivars and, therefore, they can negatively affect the course of plant growth and development (inhibition of seedling sprouting, discoloration and deformations of leaves, or retardation of plant growth), as well as disturb assimilate production in the course of photosynthesis which led to reduction in yield and to deterioration of yield components and crop quality.

The study demonstrated that from among investigated cultivars Lg 3225 and Anjou 249 gave the highest yields. However, from among the herbicides used only Mustang 306 SE applied in BBCH 16 had significantly lowered grain yields.

Although no phytotoxicity effect was recorded, application of Maister in divided doses brought about a significantly lower content of starch and fat in the cultivar Salgado.

In the cultivars Lg 3225 and Anjou 249 treated with the herbicide Mustang 306 SE phytotoxicity symptoms of injuries and decrease of grain and dry matter yield, and reduction in the thousand grain weight were observed.

The cultivar Anjou 249 treated with the herbicide Mustang 306 SE in the 5^{th} leaf stage, contained significantly decreased amounts of protein, fat, starch and fibre in comparison to the non-treated control, due to a strong phytotoxic effect.

Tolerant cultivars, like: Ronaldinio, Hexxer, PR 39T45 did not show any morphological and qualitative changes, regardless of the application date and the manner in which the herbicide was applied.

key words: herbicides, phytotoxicity, cultivar of maize, qualitative parameters

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INTRODUCTION

Among cereals, maize is characterized by the highest energy value and high digestibility of nutrients as it contains considerable amounts of starch and fat (Normy..., 1993; Normy..., 2005). Maize grain is not known for providing protein, as it features disadvantageous balance of exogenic amino-acids composition, yet it constitutes an important nutrient in total fodder balance because of its high yielding potential (Podkówka, Podkówka, 2002). Qualitative properties, depending on such parameters as genotype (Rothkaehl, 2000), type of grain – glassy or floury form, as well as grain structure – well developed endosperm, easy separation of seed embryo and the kind of starch (amylopectin, amylose), make maize grain a marketable commodity. These properties can be modified by climate, soil and cultivation technology factors as well as by the use of plant protection preparations, including herbicides (Gołębiowska, Rola, 2008). A long-term study undertaken in the 1990s by the scientists of the Ecology and Weed Control Department in Wrocław on the response of maize cultivars to herbicides proved that reduced selectivity of herbicides often results from interaction between genotype properties of a particular cultivar, weather and environmental conditions, as well as the mode of herbicide activity (Rola, Gołębiowska, 2003; Praczyk, 2002). Thus herbicides introduced in the course of the plant growing period can disturb a number of plant vital activities which results in morphological changes (e.g. necrosis, coloration, growth retardation), or irregular course of photosynthesis, blocking of amino-acids synthesis (Rola, Gołębiowska, 2003). However, it has not been known so far if and to what extent herbicides affect grain technology value and the content of nutrients. Therefore, there does exist the necessity of doing complex qualitative examination, especially justified in the case of new cultivars and new-generation preparations being introduced into practice.

The aim of this study was the assessment of the effect of Maister OD and Mustang 306 SE herbicides on the qualitative properties of grain of six maize cultivars as affected by the date and manner of herbicide application.

MATERIAL AND METHODS

The study was conducted in the years 2005–2007. Field experiments were done in private farms in the region of Wrocław. Full description of environmental conditions prevailing during the experiments with maize cultivars was shown in Table 1.

Field experiments were established according to split-plot balanced blocks design in three replications, at sites free from weeds, with a herbicide as a primary factor and a cultivar as a secondary one. The whole field was provided with full range fertilization to meet current requirements of the plant cultivated. The experiments involved herbicides of different mode of activity and causing characteristic injury symptoms described in Table 2.

Maize was seeded in conformance with cultivation technology recommendations for the region of Lower Silesia. Herbicides were applied across the seeded maize plots using Unimog sprayer with a boom span of 6 m carried on a Mercedes chassis. In the experiment hybrids of single--cross type, featuring similar FAO earliness and utilization-related traits: Ronaldinio, Hexxer, PR 39T45, Lg 3225, Anjou 249, Salgado, were used. Their description was shown in Table 3. The assessment of the phytotoxic effect of the herbicide preparations on selected maize cultivars was done 1, 2 and 3 weeks after herbicide application, according to scale 1:9. The methods used were in accordance with EPPO Standards – PP 1/135, PP 1/152, PP1/181, PP 1/214 oraz 1/50(2) (EPPO 1995).

Grain yield and thousand grain weight (TGW) values were measured at 15% moisture content. Yield components were also subjected to evaluation. Grain of the examined maize cultivars, collected from control treatment, as well as that originating from herbicide-treated plots were analyzed for the following parameters: thousand grain weight, dry matter at 105°C – according to gravimetric method, contents of protein, starch, fat and fibre – using INSTALAB 600 device making use of near infra-red technique LSD.

The data were subjected to analysis of variance for randomized blocks design. Significance of differences was tested using Tukey`s confidence semi-interval and the smallest significant difference was estimated for confidence level of 0.05.

Calculations were done using AWAR 2.0 computer software.

Table. 1. Characteristics of environmental conditions.

Preceding crop	Winter wheat
Type of soil	Black earth formed from medium loam belonging to very good and good wheat complex
FAO classification	Haplic Phaeozems
Class	I–II, IIIa
pH	6.3–6.8
Organic matter content	3.3–3.6%
Fertilisation [kg ha^{-1}]	
N	157–173
P_2O_5	63–71
K_2O	65–85
Type of cultivation	Based on ploughing

Table 2. Mode of action of active substances of herbicides used in the experiments and symptoms of phytotoxicity injuries in maize cultivars.

Mode of action	Herbicides	Active substance	Term of application BBCH	Symptoms of injuries
Inhibitors of amino-acid synthesis	Mustang 306 SE	florasulam + 2,4-D triazolopirymidyny +growth regulator	*Postemergence* BBCH 12 BBCH 16	Sp spotting GR growth retardation Df deformation of leaves and young plants Ld lodging
	Maister OD	foramsulfuron + jodosulfuron methyl sodium + izoksadifen ethyl + adjuvant	*Postemergence* BBCH 12 + BBCH 16	Ch leaves chlorosis Sp spotting Gr growth retardation

BBCH 12 – 2–3-leaf stage of maize development
BBCH 16 – 5–6-leaf stage of maize development

Table 3. Characteristics of maize hybrids in field experiments.

Maize hyrbrids/cultivar	Type of hybrid	Year of hybrid registration	Utilization trend#			Breeder	Earliness of maize hybrids FAO
			G	CCM	S		
Ronaldinio	SC Single hybris	2003	X	X	-	KWS	FAO 260
PR 39T45		2004	X	X	X	PIONEER	FAO 240
Hexxer		2002	X	X	-	RAGT	FAO 240
Lg 3225		2002	X	X	-	LIMAGRAIN	FAO 230
Anjou 249		2002	X	X	-	MAIS ANGEVIN	FAO 250
Salgado		2003	X	X	X	KWS	FAO 240

G – grain, CCM – corn-cob mix, S – maize silage

RESULTS AND DISCUSSION

Phytotoxic effect of herbicides on maize hybrids

Mode of action of the herbicide Maister OD active ingredient consists in inhibition of the synthesis of enzymes which are necessary for forming amino-acids in plant cells. Among maize hybrids treated with this herbicide only Salgado responded by showing symptoms of phytotoxicity typical for these compounds – coloration of leaves and growth retardation (Table 4), as well as growth retardation, which occurred to be temporary and disappeared three weeks later. The symptoms of those injuries were observed in each plant growing season, yet with different intensity, depending on weather conditions.

A similar mode of action is shown by florasulam, one of the active ingredients in the herbicide Mustang 306 SE, while another component, 2,4-D in some hybrids can cause leaf curling which makes tasseling difficult, delays dusting and retards plant growth (Table 4). As a result tiny grains of decreased TGW can occur in hybrids that show low tolerance of these substances. In our study characteristic symptoms of injuries were recorded in Lg 3225 and Anjou 249. Mustang 306 SE applied in early stages of plant development, showed a mild effect and caused temporary, 3-week-lasting injuries in those cultivars. The same preparation, however, when applied in older plants resulted in considerable retardation of plant growth, leaf deformation and lodging. Unfavorable weather conditions in the plant growing season of 2005 intensified the occurrence of the symptoms.

The effect on grain yield, thousand seed weight and dry matter

From among the investigated cultivars the lowest yields were recorded in Lg 3225 and Anjou 249. However, out of the herbicides tested only Mustang 306 SE applied in BBCH 16 had a significant negative effect on yields. All cultivars responded with an increase in dry matter after application of herbicides (Table 5).

No significant differences were observed in growth and development of the five cultivars resulting from introduction of Maister OD herbicide, nor were any marked differences recorded for maize yields, dry matter and thousand grain weight since the values of those parameters resembled those of the control (non-sprayed treatment).

Phytotoxic activity of the herbicide Mustang 306 SE on Lg 3225 and Anjou 249 (BBCH 16) had a significant negative impact on grain yield when compared to the yields in the control treatment. Differences in the yields between those cultivars and the control treatment were statistically proved. Lg 3225 and Anjou 249 treated with that herbicide also responded by considerably diminishing grain dry matter whereas in Lg 3225 only the thousand seed weight was affected (Tables 6 and 7).

The effect of herbicides on the contents of protein, fat, starch and fibre

Among the investigated cultivars Hexxer was noted for the lowest crude protein content, PR 39T45 was lowest in crude fat and Anjou 249 had the lowest starch content (Tables 8, 9, and 10). Crude fibre was the lowest in Ronaldinio and PR 39T45. From among the herbicides used none had a negative effect on the value of those qualitative parameters.

With regard to the qualitative properties of the grain of the cultivars treated with the herbicide Maister OD in BBCH 12 + BBCH 16 (split-split doses), significantly decreased content of starch was found only in Salgado and of fat – in Anjou 249. The remaining cultivars did not show any reduction in the values of the examined parameters when treated with this herbicide.

Low selectivity of the herbicide Mustang 306 SE towards the cultivar Anjou 249 had a negative impact on the qualitative properties of the grain: protein, starch and fibre content as compared to the control treatment (Table 11). The preparation tested was safe in relation to the remaining cultivars and differences in the content of protein, starch, fat and fibre were not statistically proved.

Among cereals, maize features the highest energy value and high digestibility of nutrients, as a result of its high

Table 4. Phytotoxic effect of herbicides on growth and development of maize hybrids – symptoms of injuries.

Maize hyrbrids/cultivar	Term of application BBCH[$]	F – phytotoxicity – plants sensitivity to herbicides in 1:9 scale[#]					
		Ronaldinio	Hexxer	PR 39T45	Anjou 249	Salgado	Lg 3225
Untreated object	-	1 no injuries	1 no injuries	1 no injuries	1 no injuries	1 no injuries	1 no injuries
Mustang 306 SE 0,6 l ha^{-1}	BBCH 12	1 no injuries	1 no injuries	1 no injuries	3 Gr, Df	1 no injuries	4 Gr, Df, Sp
Mustang 306 SE 0,6 l ha^{-1}	BBCH 16	1 no injuries	1 no injuries	2 Gr, Df	5 Gr, Df	1 no injuries	5-6 Gr, Df, Ld, Sp
Maister OD + Maister OD 0,5 + 0,5 l ha^{-1}	BBCH 12 + BBCH 16	1 no injuries	1 no injuries	1 no injuries	1 no injuries	2 Gr, Sp	1 no injuries

1 – no reaction, 9 – crop damage
Df – deformation of leaves and young plants; Sp – spotting; Gr – growth retardation; Ld – lodging

Table 5. Effect of herbicides on grain yield of maize hybrids [t ha^{-1}].

Treatment	Term of application BBCH[$]	Maize hybrids/cultivar						Mean values for herbicides
		Ronaldinio	Hexxer	PR 39T45	Anjou 249	Salgado	Lg 3225	
Control Untreated object	-	12.16	11.74	11.9	10.27	10.24	11.08	11.23
Mustang 306 SE 0.6 l ha^{-1}	BBCH 12	12.56	12.12	11.89	10.69	11.68	10.00	11.49
Mustang 306 SE 0.6 l ha^{-1}	BBCH 16	12.74	11.89	11.72	9.09	11.74	8.63	10.97
Maister OD + Maister OD 0.5 + 0.5 l ha^{-1}	BBCH 12 + BBCH 16	12.43	12.26	11.86	11.2	11.4	10.92	11.68
Mean values for cultivars		12.47	12.00	11.84	10.31	11.27	10.16	11.34
LSD (0.05) for:								
cultivars		1.22						
herbicides								1.05
interaction cultivars × herbicides					0.986			

[$] see Table 2

Table 6. Effect of herbicides on dry matter of maize hybrids grain [%].

Treatment	Term of application BBCH[$]	Maize hybrids/cultivar						Mean values for herbicides
		Ronaldinio	Hexxer	PR 39T45	Anjou 249	Salgado	Lg 3225	
Control Untreated object	-	80.95	77.17	76.31	79.92	79.21	79.21	78.13
Mustang 306 SE 0.6 l ha^{-1}	BBCH 12	82.55	82.44	80.12	82.56	79.59	79.11	81.06
Mustang 306 SE 0.6 l ha^{-1}	BBCH 16	83.51	82.24	80.13	63.92	76.17	61.22	74.53
Maister OD + Maister OD 0.5 + 0.5 l ha^{-1}	BBCH 12 + BBCH 16	80.6	80.2	80.26	82.06	81.74	81.74	81.10
Mean values for cultivars		80.90	80.51	79.21	77.12	79.18	75.32	
LSD (0.05) for:								
cultivars		3.75						
herbicides								78.71
interaction cultivars × herbicides					4.22			

[$] see Table 2

Table 7. Effect of herbicides on weight of thousand grains (TGW) of maize hybrids [g].

Treatment	Term of application BBCH$	Maize hybrids/cultivar						Mean values for herbicides
		Ronaldinio	Hexxer	PR 39T45	Anjou 249	Salgado	Lg 3225	
Control Untreated object	-	362.7	380.6	389.6	331.7	418.2	354.4	372.87
Mustang 306 SE 0.6 l ha^{-1}	BBCH 12	365	390.3	399.6	332.1	401.2	335.6	370.63
Mustang 306 SE 0.6 l ha^{-1}	BBCH 16	367.6	384.8	407.2	326.2	406.8	315.6	368.03
Maister OD + Maister OD 0.5 + 0.5 l ha^{-1}	BBCH 12 + BBCH 16	374.5	383.3	403.9	331.1	407.9	345.6	374.38
Mean values for cultivars		367.45	384.75	400.08	330.28	408.53	337.80	371.48
LSD (0.05) for:								
cultivars		28.56						
herbicides								15.67
interaction cultivars × herbicides					17.367			

$ see Table 2

Table 8. Effect of herbicides on crude protein content [%] in grain.

Treatment	Term of application BBCH$	Maize hybrids/cultivar						Mean values for herbicides
		Ronaldinio	Hexxer	PR 39T45	Anjou 249	Salgado	Lg 3225	
Control Untreated object	-	8.6	8.6	9.9	9.7	9.3	8.2	9.05
Mustang 306 SE 0.6 l ha^{-1}	BBCH 12	8.6	8.5	9.6	9.6	9.5	9.4	9.20
Mustang 306 SE 0.6 l ha^{-1}	BBCH 16	8.6	8.5	9.8	8.1	9.6	8.7	9.22
Maister OD + Maister OD 0.5 + 0.5 l ha^{-1}	BBCH 12 + BBCH 16	8.7	8.7	9.5	9.7	9.2	8.1	8.98
Mean values for cultivars		8.63	8.58	9.70	9.27	9.40	8.60	9.11
LSD (0.05) for:								
cultivars		1.32						
herbicides								0.894
interaction cultivars × herbicides					0.977			

$ see Table 2

Table 9. Effect of herbicides on crude fat content [%] in grain.

Treatment	Term of application BBCH$	Maize hybrids/cultivar						Mean values for herbicides
		Ronaldinio	Hexxer	PR 39T45	Anjou 249	Salgado	Lg 3225	
Control Untreated object	-	3.4	4.0	2.6	4.1	4.5	3.2	3.80
Mustang 306 SE 0.6 l ha^{-1}	BBCH 12	3.3	3.8	2.9	4.5	4.8	3.3	3.62
Mustang 306 SE 0.6 l ha^{-1}	BBCH 16	3.2	3.6	3.3	4.2	4.0	3.3	3.60
Maister OD + Maister OD 0.5 + 0.5 l ha^{-1}	BBCH 12 + BBCH 16	3.6	3.8	3.5	3.0	3.9	3.5	3.87
Mean values for cultivars		3.38	3.80	3.08	4.20	4.30	3.58	3.72
LSD (0.05) for:								
cultivars		0.958						
herbicides								0.659
interaction cultivars × herbicides		0.672						

$ see Table 2

Table 10. Effect of herbicides on starch content [%] in grain.

Treatment	Term of application BBCH[$]	Maize hybrids/cultivar						Mean values for herbicides
		Ronaldinio	Hexxer	PR 39T45	Anjou 249	Salgado	Lg 3225	
Control Untreatment object	-	73.4	72.1	71.9	69.6	71.6	70.7	71.55
Mustang 306 SE 0.6 l ha^{-1}	BBCH 12	73.1	71.2	71.5	70.2	72.2	70.25	71.41
Mustang 306 SE 0.6 l ha^{-1}	BBCH 16	73.4	71.9	71.1	62.1	71.2	70.9	71.12
Maister OD + Maister OD 0.5 + 0.5 l ha^{-1}	BBCH 12 + BBCH 16	72.2	71.2	71.3	68.2	69.2	70.6	71.77
Mean values for cultivars		73.03	71.60	71.45	70.03	71.05	70.61	71.46
LSD (0.05) for:								
cultivars		1.069						
herbicides								5.69
interaction cultivars × herbicides		1.47						

[$] see Table 2

Table 11. Effect of herbicides on crude fibre content [%] in grain.

Treatment	Term of application BBCH[$]	Maize hybrids/cultivar						Mean values for herbicides
		Ronaldinio	Hexxer	PR 39T45	Anjou 249	Salgado	Lg 3225	
Control Untreated object	-	2.30	3.20	2.10	2.90	3.50	3.20	2.87
Mustang 306 SE 0.6 l ha^{-1}	BBCH 12	2.40	2.90	2.10	2.90	3.40	3.00	2.63
Mustang 306 SE 0.6 l ha^{-1}	BBCH 16	2.20	3.20	2.40	2.50	3.20	3.10	2.75
Maister OD + Maister OD 0.5 + 0.5 l ha^{-1}	BBCH 12 + BBCH 16	2.30	3.60	2.60	3.30	3.20	3.20	2.87
Mean values for cultivars		2.30	3.23	2.30	2.63	3.32	3.12	2.78
LSD (0.05) for:								
cultivars		1.01						
herbicides								0.196
interaction cultivars × herbicides		1.0539						

[$] see Table 2

content of starch and fat, and low content of crude protein and crude fibre. The most important source of energy in maize grain is starch whose contribution reaches 70%. The most concentrated source of energy is fat and it is in maize that it occurs in the highest quantity. Maize fat is characterized by high content of essential unsaturated acids and linolic acid.

Maize protein, since it is deficient in exogenic amino-acids, has low biological value. Amino-acids composition of maize protein is not balanced due to lysine and tryptophan deficit. Total protein content in maize grain, as well as its amino-acids composition, are not constant values and they can be affected by variable climate conditions in the course of plant growing period, cultivation technique, fertilization, as well as application of pesticides and genetic factors, which was stressed by Panamarioviene and Tamulis (1997) in their studies. So far neither the herbicides of the 2,4-D and MCPA group nor those of the triazine group have been shown to have a negative impact on photochemical activity of chloroplasts, as well as on breathing processes in some maize cultivars (Hwang et al., 1996). Similarly, the mode of action of the sulfonylurea herbicides, with their typical blocking of synthesis of amino-acids chain, can disturb regular plant development in sensitive cultivars, which was demonstrated in this study.

CONCLUSIONS

1. From among investigated maize cultivars the lowest yields were recorded in Lg 3225 and Anjou 249, however from among used herbicides only Mustang 306 SE significantly depressed yields of cultivars applied in BBCH 16.
2. All investigated cultivars responded with an increase of dry mass after application of herbicides.
3. In spite of the lack of phytotoxic effect of the herbicide Maister OD, the cultivar Salgado showed a significantly lower content of starch and fat.
4. In the cultivars Lg 3225 and Anjou 249 treated with the herbicide Mustang 306 SE phytotoxicity symptoms of injuries and decrease of grain yield and dry matter were observed. In the cultivar Lg 3225 a significant decrease of the weight of thousand grains was observed
5. Low selectivity of Mustang 306 SE herbicide towards the cultivar Anjou 249 significantly lowered values of grain qualitative properties – protein, fat, starch and fibre content as compared to the control treatment.
6. Tolerant cultivars Ronaldinio, Hexxer, PR 39T45 did not show any morphological and qualitative changes, regardless of the herbicide applied.

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Assessment of the effect of sulphur supplied to the soil with mineral fertilizers and waste from magnesium sulphate production on its content in spring wheat (*Triticum aestivum* L.) and in soil effluents

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Abstract. The assessment of the effect of fertilization on the plant, soil and sulphur losses as a result of leaching was conducted in a pot experiment.

The experiment was conducted in three replications and two series: without liming (0 Ca) and limed (+ Ca) on 6 treatments: 0 – soil without fertilizers, NPK – soil fertilized with nitrogen, phosphorus and potassium, NPK + S1 a.s. – soil fertilized with nitrogen, phosphorus, potassium and sulphur supplied as ammonium sulphate, NPK + S1 w. – soil fertilized with nitrogen, phosphorus, potassium and sulphur supplied with the waste from magnesium sulphate production and NPK + S3 a.s. – soil fertilized with nitrogen, phosphorus, potassium and sulphur supplied as ammonium sulphate in a dose three times bigger than introduced to the soil on NPK + S1 a.s. and NPK + S1 w. treatments. Spring wheat was cultivated in each year of the experiment. The sulphur content in the prepared experimental material (plant, soil and effluent) was assessed by means of ICP-AES method on JY 238 Ultrace apparatus.

An average (over three years) total yield of spring wheat (grains, straw and roots) at comparable values of standard error of arithmetic mean for individual treatments was the highest after sulphur application in the form of ammonium sulphate. In comparison with biomass yields from the treatments where a lower sulphur dose was used, either as ammonium sulphate or the waste from magnesium sulphate production, smaller biomass yield was obtained in the treatment where sulphur was applied in a dose which was thrice as high. Weighed arithmetic mean of the sulphur content in grains, straw and roots of wheat fertilized with sulphur was significantly higher than the content assessed in wheat biomass not fertilized with this element. Increasing sulphur dose did not cause any significant differences in this element content in wheat biomass. A single soil fertilization with smaller sulphur doses either as ammonium sulphate or as waste from magnesium sulphate production did not cause any lasting fertilizer effect, a result of removal of sulphur with the crop and its leaching from soil. Sulphur fertilization, either as ammonium sulphate or as waste from magnesium sulphate production led to an increased sulphur content in soil effluents. The amounts of this element in leaching waters were affected mainly by the quantity of sulphur supplied to the soil with fertilization and, to a lesser degree, by the plant yields.

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INTRODUCTION

Sulphur like nitrogen, phosphorus, potassium, magnesium and calcium belongs to nutrient macroelements absorbed by plants in the greatest amounts. Its physiological role is specific and impossible to be replaced by any other element. The unique role of sulphur results from its function in plant metabolism, mainly in the transformation of nitrogen compounds (Schnug, 1998; Ostrowska et al., 2008).

Sulphate anion (SO_4^{2-}) is the basic source of sulphur for plants. The quantity and rate of its uptake depend on many factors, among others on soil reaction, temperature, sulphate content in the soil solution, the content of other ions and also on the soil biological activity (Kertesz and Mirleau, 2004).

High requirements of some plants for sulphur, decreased use of fertilizers containing this element, reduction in emission of sulphur compounds into the atmosphere and considerable sulphate leaching lead to a negative balance of this element in many soils (Schnug, 1998; Zhao et al., 2003; Mathot et al., 2008). Sulphur deficiency in plants may limit utilization of other elements, including nitrogen, which in consequence leads to a decline in yield and worsens crop quality (Luo et al., 2000; Wieser et al., 2004).

Identification of the effect of fertilization with these substances of waste origin on the sulphur content in plant and soil, and determining losses of this element through leaching are important to ensure the optimal level of

Table 1. Selected chemical properties of soil and waste used in experiment (average ± standard error, n = 3).

Property	Soil	Property	Waste
pH H_2O	6.33 ± 0.01	pH H_2O	9.53 ± 0.09
pH KCl	5.70 ± 0.02	Dry matter [g kg^{-1}]	630 ± 31
Hydrolitic acidity [mmol(+) kg^{-1} DM]	23.9 ± 1.2	Ash [g kg^{-1} DM]	726 ± 37
Organic C [g kg^{-1} DM]	19.3 ± 1.1	Total forms [g kg^{-1} DM]	
Total N [g kg^{-1} DM]	1.60 ± 0.12	N	0.09 ± < 0.01
Total S [g kg^{-1} DM]	0.28 ± 0.02	P	0.35 ± 0.01
P available [mg kg^{-1} DM]	48.6 ± 1.5	K	0.28 ± 0.01
K available [mg kg^{-1} DM]	158.8 ± 5.6	S	67.1 ± 2.4
Mg available [mg kg^{-1} DM]	129.1 ± 4.7	Mg	9.28 ± 0.46
Ca exchangeable available [g kg^{-1} DM]	2.44 ± 0.08	Ca	1.68 ± 0.07

plant nutrition with sulphur and to recognize the burden to the natural environment, particularly to water resources (Schnug, 1998). Presented experiments aimed at determining the effect of applied mineral fertilizers, waste from magnesium sulphate production and liming on the sulphur content in spring wheat, in soil and in soil effluents.

MATERIAL AND METHODS

The assessment of applied fertilization effect on the plant, soil and sulphur losses due to leaching was conducted in a pot experiment in a wire netting-protected unheated greenhouse, in the pots equipped with the effluent draining systems. The soil material used for the experiments (medium silt loam with 44% granulometric fraction of Ø < 0.02 mm) was collected from the 0–30 cm of the arable layer. The characteristics of selected chemical properties of the soil material were given in Table 1.

The investigations were conducted for three years (2004–2006) in polyethylene pots, 28 cm in diameter and 38 cm high, containing 22.0 kg of air-dried soil material. The experiment, conducted in three replications and in two series: without liming (0 Ca) and limed (+ Ca), comprised 6 treatments: 0 – soil without fertilizers, NPK – soil fertilized with nitrogen, phosphorus and potassium, NPK + S1 a.s. – soil fertilized with nitrogen, phosphorus, potassium and sulphur supplied as ammonium sulphate, NPK + S1 w. – soil fertilized with nitrogen, phosphorus, potassium and sulphur supplied with the waste from magnesium sulphate production and NPK + S3 a.s. – soil fertilized with nitrogen, phosphorus, potassium and sulphur supplied as ammonium sulphate in a dose three times as high as that introduced to the soil on NPK + S1 a.s. and NPK + S1 w.

Prior to the experiment outset the soil was gradually moistened until 30% of the maximum water capacity. After moistening a part of the soil material was limed, separately in each pot, in order to increase pH value. Liming was applied with chemically pure CaO, in the dose established on the basis of the soil total hydrolytic acidity. Subsequently, soil material without liming as well as limed was left for 4 weeks and water losses were supplemented occasionally. After that time, mineral fertilizers and the waste from magnesium sulphate production were mixed with the soil. The content of dry matter in the analyzed waste was 630 g kg^{-1} and total nitrogen 0.09 g kg^{-1} DM. Determined conductivity value was 14.9 mS cm^{-1}. The other chemical properties of the waste were presented in Table 1. The doses of nitrogen, phosphorus and potassium, equal for all treatments, were respectively: 0.14 g N, 0.10 g P and 0.15 g K kg^{-1} soil DM. Sulphur was used once in the first year of the experiment. The sulphur dose on NPK + S1 a.s. treatments and NPK + S1 w. was 0.04 g S and in the treatment with NPK + S3 a.s. was 0.12 g S kg^{-1} soil DM. Basic fertilization in the first year of the research on NPK; NPK + S1 a.s.; NPK + S3 a.s. and supplementary on NPK+S1 w. was applied in the form of chemically pure salt solutions, respectively: nitrogen as NH_4NO_3, phosphorus as $Ca(H_2PO_4)_2 \cdot H_2O$ and potassium as KCl, whereas sulphur was used as $(NH_4)_2SO_4$. In the second and third year of the research supplementary doses of nitrogen, phosphorus and potassium (identical on all treatments) were applied (0.10 g N; 0.02 g P and 0.14 g K kg^{-1} soil DM). The elements, like in the first year, were supplied as solutions of chemically pure salts.

Spring wheat, Nawra cv. was cultivated in each year of the experiment. The plant density was 28 pieces per pot. Wheat was harvested at full grain maturity. The length of the growth period was 109 days in the first year, 104 days in the second and 96 days in the third. During the experiment the plants were watered with distilled water to maintain 50% of the soil water capacity.

In order to determine the changes of chemical properties, soil samples for analyses were collected from each pot separately after the completion of vegetation season.

After the harvest, wheat plants were divided into roots, straw and ears. The ears were threshed mechanically to obtain grain biomass. In order to determine dry mass yield, the individual fractions of wheat yield were dried (at 70°C) in a dryer with hot air flow to constant weight. The plant material (separately grains, straw and roots) were crushed in a laboratory mill. After crushing, the plant material was wet mineralized in the concentrated HNO_3 (d = 1.40 g mol^{-1}). After evaporation on a sand bath, the samples were mineralized in a muffle furnace, initially at 300°C (for two hours), and subsequently at 450°C (for three hours). The remains were dissolved in diluted HNO_3 25% (v/v) (Ostrowska et al., 1991).

In the soil material collected from the pots and sifted through a sieve with 1 mm mesh, sulphate sulphur content was assessed after the extraction (30 minutes on a rotor mixer) with 0.03 mol dm^{-3} CH_3COOH solution maintaining the soil to solution ratio 1:10 (Ostrowska et al., 1991). In soil material samples dried at room temperature (c.a. 25°C), ground in a porcelain mortar and sifted through a 1 mm mesh, pH was additionally assessed by potentiometer in the suspension of soil and 1 mol dm^{-3} KCl solution, maintaining the soil to solution ratio of 1:2.5 (Ostrowska et al., 1991).

During the vegetation period, soil lump in a pot was washed with distilled water at 30-day intervals simulating the 36 mm high rainfall. The obtained soil effluent was collected after each washing and kept in a refrigerator at 4°C. Total sulphur was determined after evaporating 100 cm^3 of the soil effluent and dissolving the remains in a diluted nitric acid 1:2 (v/v) (Elbanowska et al., 1999).

The sulphur content in the prepared experimental material (plant, soil and effluent) was assessed by means of ICP-AES method on JY 238 Ultrace apparatus.

The chemical analysis of the plant and soil material, and soil effluents was conducted in three replications. In order to verify the assessment results obtained for the plant material, the initial soil and waste material, plant reference material – NCS DC73348 (China National Analysis Center for Iron & Steel) and soil reference – EnviroMAT, SS-2 (SCP Science) was added to each analyzed series. The result was considered reliable if the relative standard error did not exceed 5%.

The obtained results were elaborated statistically according to the constant model where the factor was fertilization and liming. Two-way ANOVA was used for statistical computations and the significance of differences was estimated by t-Tukey test at significance level $\alpha<0.01$. The value of correlation coefficient (r) was computed using nonparametric Spearman's rank test for sulphur content in the soil effluents, for plant yields and for sulphate sulphur content in soil. All statistical computations and graphic presentations were conducted using Statistica PL package (Stanisz, 2007).

RESULTS AND DISCUSSION

The greatest diversification in wheat grain yield among the treatments and experimental series was found in the first year of the experiment (Table 2). Despite the fact that the differences were not confirmed statistically, greater yields of wheat grain biomass were harvested in the non-limed series, irrespective of applied fertilization. Introduction of the sulphur dose to the soil which was three times as great as that in the NPK + S1 w. treatments, caused a significant decrease in wheat grain yield, but only in the limed series. In the second and third year of the research, wheat grain yields, independently of the experimental series, were far less diversified among treatments, at levels of yields comparable with those obtained in the first year.

Table 2. Yield of dry matter (average ± standard error, n = 3) of grain, straw and biomass of roots of spring wheat.

Treatment	Grain [g DM pot^{-1}]		Straw [g DM pot^{-1}]		Roots [g DM pot^{-1}]	
	0 Ca	+ Ca	0 Ca	+ Ca	0 Ca	+ Ca
1st year						
0 (without fertilization)	46.1 ± 0.96^{ab}	39.4 ± 1.52^{a}	45.3 ± 1.06^{a}	47.6 ± 2.23^{a}	3.23 ± 0.23^{abc}	2.32 ± 0.14^{a}
NPK	62.8 ± 1.32^{cde}	50.1 ± 2.15^{ab}	70.1 ± 1.88^{d}	57.7 ± 1.79^{b}	4.66 ± 0.17^{c}	2.97 ± 0.22^{ab}
NPK + S1 a.s.	65.6 ± 1.74^{de}	55.3 ± 2.14^{bcd}	69.9 ± 1.64^{d}	62.6 ± 2.14^{bcd}	4.58 ± 0.34^{c}	3.42 ± 0.25^{abc}
NPK + S1 w.	66.4 ± 1.78^{de}	67.5 ± 2.55^{e}	65.0 ± 1.97^{bcd}	61.8 ± 2.86^{bcd}	4.46 ± 0.34^{c}	3.80 ± 0.36^{abc}
NPK + S3 a.s.	63.6 ± 2.46^{cde}	52.2 ± 2.94^{bc}	67.8 ± 2.14^{cd}	59.8 ± 1.19^{bc}	4.62 ± 0.31^{c}	2.89 ± 0.18^{a}
2nd year						
0 (without fertilization)	45.2 ± 4.26^{a}	46.7 ± 1.15^{ab}	35.7 ± 0.70^{a}	36.3 ± 1.06^{a}	2.28 ± 0.44^{a}	2.25 ± 0.19^{a}
NPK	63.0 ± 1.84^{c}	60.6 ± 2.74^{c}	51.9 ± 1.74^{b}	50.2 ± 1.67^{b}	4.27 ± 0.37^{bc}	4.16 ± 0.25^{bc}
NPK + S1 a.s.	67.3 ± 2.86^{c}	63.6 ± 1.81^{c}	55.2 ± 1.22^{b}	51.0 ± 0.93^{b}	4.89 ± 0.12^{c}	3.21 ± 0.18^{ab}
NPK + S1 w.	63.3 ± 0.90^{c}	61.1 ± 2.74^{c}	54.7 ± 1.15^{b}	53.0 ± 2.03^{b}	3.82 ± 0.13^{bc}	3.41 ± 0.18^{ab}
NPK + S3 a.s.	65.3 ± 0.97^{c}	60.1 ± 2.30^{bc}	55.5 ± 1.10^{b}	51.3 ± 2.20^{b}	4.16 ± 0.18^{b}	3.75 ± 0.25^{bc}
3rd year						
0 (without fertilization)	37.0 ± 0.63^{a}	39.2 ± 0.17^{ab}	35.2 ± 1.15^{a}	37.3 ± 1.04^{a}	2.23 ± 0.22^{a}	2.46 ± 0.10^{ab}
NPK	49.2 ± 0.86^{b}	62.7 ± 1.18^{c}	54.7 ± 1.86^{b}	61.2 ± 0.92^{bc}	4.26 ± 0.43^{c}	4.60 ± 0.35^{c}
NPK + S1 a.s.	67.9 ± 1.30^{cd}	74.4 ± 2.53^{d}	69.6 ± 1.29^{c}	69.0 ± 1.30^{c}	4.34 ± 0.19^{c}	4.86 ± 0.25^{c}
NPK + S1 w.	74.1 ± 1.57^{d}	68.3 ± 2.75^{cd}	67.6 ± 2.25^{c}	63.5 ± 2.60^{bc}	4.20 ± 0.35^{c}	3.82 ± 0.22^{abc}
NPK + S3 a.s.	70.4 ± 1.99^{d}	69.6 ± 2.51^{cd}	63.2 ± 2.22^{bc}	67.7 ± 2.69^{c}	3.82 ± 0.15^{abc}	4.08 ± 0.31^{bc}

Values followed by the same letters in columns did not differ significantly at $\alpha < 0.01$ according to the t-Tukey test; factors: fertilization x liming; a.s. – ammonium sulphate, w. – waste.

Straw biomass yield revealed similar dependencies as wheat grain yield. The greatest yields of straw dry mass were harvested from the non-limed series (0 Ca) in the first year of research, whereas in the limed series (+ Ca) the highest yields were obtained in the third year (Table 2). No apparent decline in yield of straw dry mass was registered on the treatment where sulphur was used in a dose three times as high as that in NPK + S1 a.s. and NPK + S1 w. treatments.

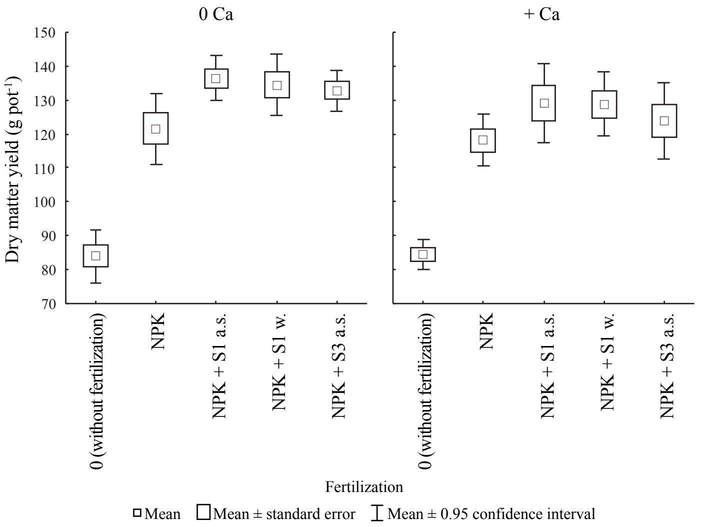
The quantity of wheat root biomass from individual treatments did not differ significantly within the experimental series (0 Ca, + Ca), irrespective of the year (Table 2). Slightly smaller amounts of biomass were obtained from this plant part in the first and second year of the experiment in the limed series treatments (+ Ca) as compared to the analogously fertilized treatments in the non-limed series (0 Ca). Like in the case of wheat grain and straw, but only in the first year of research, markedly smaller amounts of those plant parts were produced in the treatment where a three times higher dose of sulphur was used.

The average (for three years) total yield of spring wheat biomass (grain, straw and roots) at comparable values of the arithmetic mean standard error for individual treatments, was the greatest after the application of sulphur as ammonium sulphate against the background of nitrogen, phosphorus and potassium fertilization (NPK + S1 a.s.) (Figure 1). In comparison with the biomass yield from the treatments where smaller sulphur dose was applied, both as ammonium sulphate (NPK + S1 a.s.) and as the waste from magnesium sulphate production (NPK + S1 w.), a smaller biomass yield was produced in the treatment where sulphur was used in a dose which was three times bigger (NPK + S3 a.s.).

Attempts to increase crop yields are based on the activity of three factor groups: advances in plant breeding (obtaining highly productive cultivars); efficient plant protection and increased fertilizer consumption (Delin et al., 2008). Under the soil and climatic conditions of Poland nitrogen is the fertilizer component which determines the yield and crop quality (Ciećko et al., 2006). However, soil fertility and therefore its fecundity are determined also by the content of bioavailable forms of other nutrients, including sulphur, whereas unbalanced fertilization with this element may prove a burden to the natural environment. Proper plant supply with sulphur influences favourably the photosynthesis process, protein biosynthesis and the content of nucleic acids which results in a suitable technological value of the crop (Luo et al., 2000; Wieser et al., 2004). Despite the fact that wheat belongs to the group of plants with relatively small requirements for this element, at sulphur deficiencies and at high nitrogen doses N:S ratio may be disturbed, which, as a result, may decrease nitrogen utilisation and lead to a decline in yield. Spring wheat fertilization conducted in this experiment both in the form of ammonium sulphate and as the waste from magnesium sulphate production caused a greater increase in grain biomass in comparison with the amount of this part yield harvested from the treatment where no fertilization with this element was conducted. However, it should be emphasized that such visible plant reaction to fertilization with this element was observed only in the first year of research. It shows that a single application of sulphur fertilization during three years is insufficient to meet the plant nutritional requirements. According to Schnug et al. (1993) the increase in yield in the treatments where sulphur fertilization was applied might have been caused by a better utilisation of mineral nitrogen by spring wheat as compared with this element utilisation in the treatments where sulphur was not supplied. The assumed "residual" effect of the applied fertilization with the waste from magnesium sulphate production on plant yielding was not confirmed, either. In comparison with the NPK + S1 a.s. and NPK + S1 w. treatments, an addition of a thrice larger sulphur dose to the soil on NPK + S3 a.s. treatment caused a decline in wheat grain yield, mainly in the first year of the experiment, however, it indicates the necessity of plant fertilization with sulphur strictly following the requirement for this element. Skwierawska et al. (2008) also demonstrated that larger sulphur doses, particularly used in the sulphate form caused a reduction of biomass yield, among others in spring barley by additionally limiting the amounts of absorbed potassium. According to Brodowska (2003), apart from sulphur fertilization, also soil liming has a major influence on growth and development of wheat plants. In the experiments of this study smaller wheat biomass yields were obtained from limed soil. It might have been due to too short a period of time which elapsed from the liming to seed sowing. In consequence it caused negative wheat response to this measure, mainly in the first year of the research.

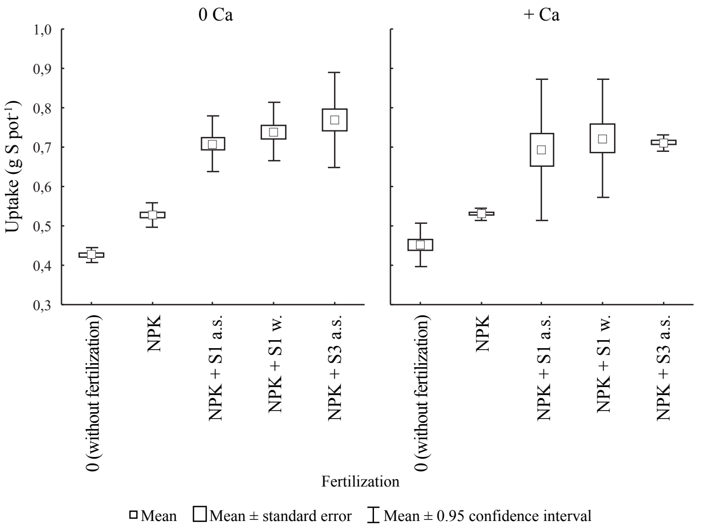
The average weighed content of sulphur in grain, straw and roots of wheat from the treatment where sulphur was supplied was significantly greater than the content assessed in wheat biomass which was not fertilized with this element (Table 3). In comparison with the sulphur content determined in wheat aboveground parts in the treatment unfertilized with sulphur (NPK) the increase in this element content, regardless of the experimental series, was for grain: 9% in the NPK + S1 a.s. treatment, 14% in NPK + S1 w. and 13% in NPK + S3 a.s., whereas for straw respectively 30%, 38% and 48%. On the unfertilized treatments the mean weighed sulphur content was significantly higher than that determined in the treatments without sulphur supplement (NPK), which in this case resulted from the cumulation of this element in a smaller crop.

The amounts of sulphur uptake with spring wheat biomass yield were over 30% higher (averaged over years, treatments and series) in the treatments where sulphur fertilization was applied in comparison with quantities of this element taken up by plants in the treatments where wheat



a.s. – ammonium sulphate, w. – waste

Fig. 1. Average (from three years) biomass yield (Σ yields of grain, straw and roots) of spring wheat.



a.s. – ammonium sulphate, w. – waste

Fig. 2. Sulphur uptake (Σ from three years) with biomass yield of spring wheat.

was fertilized only with nitrogen, phosphorus and potassium (NPK) (Figure 2). Analyzing the effect of liming on the amounts of sulphur taken up with wheat biomass yield, it was found that slightly bigger quantities of this element were absorbed from the non-limed soil (0 Ca), irrespectively of the applied fertilization.

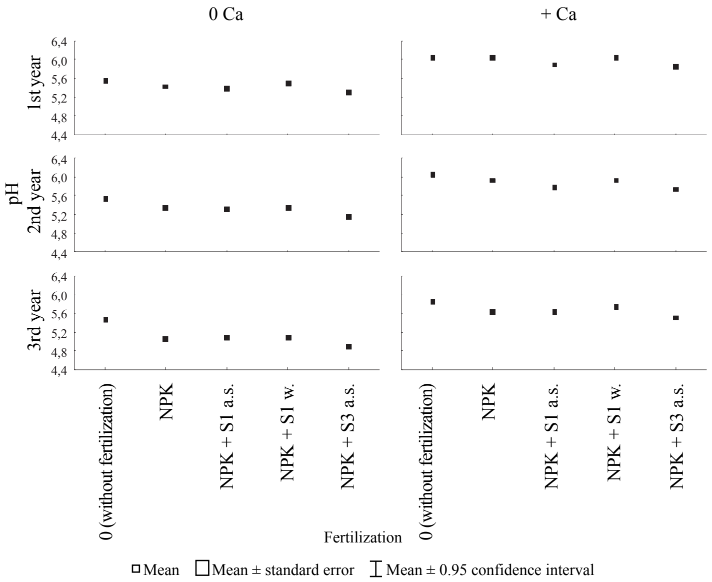
According to Kaczor et al. (2004) sulphur concentrations in plants are conditioned, beside fertilization, by plant development stage and plant organ, but also depend on soil liming. In the conducted experiment the weighed mean-based sulphur content in grains, straw and roots of wheat fertilized with sulphur was significantly higher than that assessed in biomass of wheat non-fertilized with this element. An increase in the sulphur content in plant biomass as a result of fertilization was also noted by McGrath

Table 3. Average weighted content from three years (average ± standard error, n = 9) of sulphur in dry matter of grain, straw and roots of spring wheat.

Treatment	0 Ca	+ Ca
	Grain [g S kg^{-1} DM]	
0 (without fertilization)	1.22 ± 0.03^{a}	1.31 ± 0.03^{ab}
NPK	1.32 ± 0.03^{ab}	1.45 ± 0.06^{bc}
NPK + S1 a.s.	1.44 ± 0.04^{c}	1.57 ± 0.07^{c}
NPK + S1 w.	1.56 ± 0.01^{c}	1.60 ± 0.02^{c}
NPK + S3 a.s.	1.55 ± 0.07^{c}	1.58 ± 0.04^{c}
	Straw [g S kg^{-1} DM]	
0 (without fertilization)	2.24 ± 0.10^{bcd}	2.31 ± 0.06^{bcd}
NPK	1.61 ± 0.03^{a}	1.57 ± 0.04^{a}
NPK + S1 a.s.	2.08 ± 0.06^{bc}	2.06 ± 0.01^{b}
NPK + S1 w.	2.18 ± 0.07^{bcd}	2.21 ± 0.02^{bcd}
NPK + S3 a.s.	2.38 ± 0.07^{d}	2.32 ± 0.05^{cd}
	Roots [g S kg^{-1} DM]	
0 (without fertilization)	1.58 ± 0.19^{b}	1.42 ± 0.05^{b}
NPK	1.22 ± 0.11^{a}	1.42 ± 0.21^{b}
NPK + S1 a.s.	1.43 ± 0.11^{b}	1.43 ± 0.17^{b}
NPK + S1 w.	1.30 ± 0.03^{a}	1.51 ± 0.19^{b}
NPK + S3 a.s.	1.52 ± 0.24^{b}	2.01 ± 0.11^{b}

Values followed by the same letters in columns did not differ significantly at $\alpha < 0.01$ according to the t-Tukey test; factors: fertilization x liming; a.s. – ammonium sulphate, w. – waste.

et al. (1996), Zhao et al. 1996 and by Haneklaus and Schnug (1992). Also Shahsavani and Gholami (2008) observed an increase in sulphur content in various spring wheat cultivars as a result of applied fertilization with this element, moreover, they demonstrated highly significant relationship between the sulphur content and protein concentrations in grain. In term of crop quality, the content and quality of protein are more important than the sulphur content. At proper plant supply with sulphur, changes occur in the initial period of seed development, which results in the increase in the level of protein accumulation. It has been reported for some time that in wheat exposed to sulphur deficiencies, protein nitrogen constituted less than 25% of the total nitrogen content in plant, whereas under conditions of optimal plant nutrition with this element about 75% of nitrogen was built in the protein. Sulphur supplement to the soil causes lowering the value of the ratio of total nitrogen to total sulphur, at the same time increasing the value of the ratio of protein nitrogen to sulphur contained in protein compounds. Despite thrice larger sulphur dose supplied to the soil in the NPK + S3 a.s. treatment in comparison with NPK + S1 a.s. and NPK + S1 w. treatments concentrations of this element in wheat biomass did not differ significantly from the content found in the plant biomass from other treatments



a.s. – ammonium sulphate, w. – waste

Fig. 3. Value pH_{KCl} of soil.

fertilized with sulphur. It shows that despite considerable content of bioavailable forms of this element in soil, wheat plants were not taking it up in excess. It might be supposed that choosing a more sulphur-demanding plant for cultivation might lead to greater accumulation of this element in plant biomass (McGrath and Zhao, 1996; Kaczor et al., 2004). According to Ashok and Kumar (2008) the content of sulphur and the amount thereof absorbed by plants are most strongly affected by the soil abundance in this element. According to Nesheim et al. (1997) the form in which sulphur was supplied to the soil is equally important.

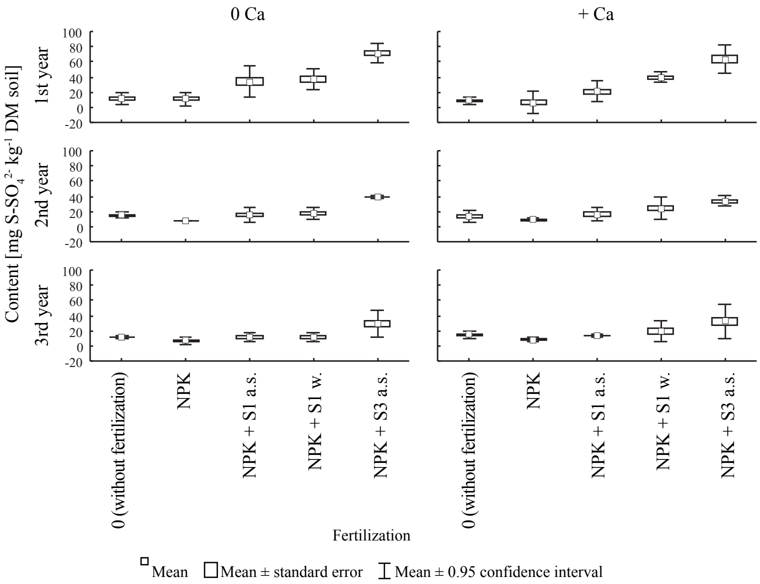
Regardless of the experimental series (0 Ca or + Ca) or the year of research the lowest pH values were assessed in the soil from treatments fertilized with nitrogen, phosphorus, potassium and a triple dose of sulphur (NPK + S3 a.s.) (Fig. 3). Statistical analysis of the results confirmed a significantly better effect of mineral fertilization with nitrogen, phosphorus and potassium and with the waste from magnesium sulphate production (NPK + S1 w.) on this parameter value in comparison with the soil from the other fertilizer treatments, regardless of the experimental series (0 Ca, + Ca). However, it should be emphasized that liming obviously decreased the rate of soil acidification process.

Fertilization with S markedly affected the sulphate sulphur content in soil (Fig. 4). The greatest amounts of sulphate sulphur, regardless of the experiment series (0 Ca or + Ca) were determined in the soil from the treatment where thrice bigger dose of this element was used (NPK + S3 a.s.). Much smaller sulphur quantities were assessed in the soil from both experimental series in the treatments where smaller sulphur dose was supplied both as ammonium sulphate (NPK + S1 a.s.) and as the waste from magnesium sulphate production (NPK + S1 w.). The contents of these sulphur forms in soil decreased in both limed and non-limed soil in the second and third year of the research in all treatments where this element was supplied with fertilizers. In the soil from the treatments where lower sulphur dose was used (NPK + S1 a.s. and NPK + S1 w.) and irrespectively of the experimental series, after three years of the experiment the content of sulphate sulphur was on the level found in the soil of treatments where solely nitrogen, phosphorus and potassium (NPK) fertilization was applied.

According to Deubel et al. (2007) big doses of sulphur do not worsen the conditions of plant growth, however, fertilization despite its favourable effect, is an intrusion in the naturally shaped relationship, which once disturbed creates disadvantageous conditions for growth and development of plants, particularly at non-balanced component doses. The obtained results concerning the soil pH most clearly point to unfavourable effect of mineral fertilization on this indicator of soil fertility (Gondek, Kopeć, 2008).

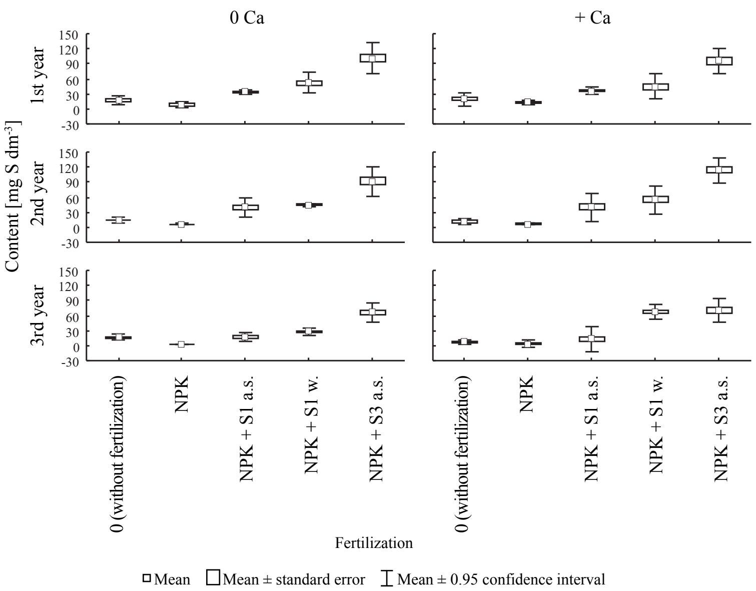
In authors' investigations an increase in the sulphate sulphur concentration in soil resulted from introducing this element with fertilizers. A significant effect of fertilization with these organic materials on the content of bioavailable sulphur was demonstrated by the results of research conducted by Kanal (2001). A single soil fertilization with smaller doses of sulphur, either as ammonium sulphate or as waste from magnesium sulphate production did not produce any durable fertilizer effect, which resulted from sulphur being taken up with crop yield and leached from soil. According to Kozłowska-Strawska and Kaczor (2004) the sulphate sulphur content in soil is not conditioned only by this element dose or the form in which sulphur was supplied to the soil. The content of sulphur mineral forms in soil is among others affected by the plant species but particularly by nutritional requirements of plants concerning sulphur and quantities of sulphur originating from mineralization of soil organic matter (Hu et al., 2002). Ashok and Kumar (2008) demonstrated that sulphur fertilization improved the content of bioavailable forms of this element in soil. Also Wołoszyk (2003) at joint application of compost and industrial waste as phosphogypsum and a mixture of iron (II) sulphate (VI) 7-hydrate for soil fertilization obtained an increase not only in sulphur total forms but also in its sulphate forms in soil.

The content of total sulphur in soil effluents was the highest (regardless of the experimental series 0 Ca or + Ca) in the treatments fertilized with a triple dose of this element in the form of ammonium sulphate (NPK + S3 a.s.) (Fig. 5). Beside the effluents from the non-fertilized treatments, the smallest amounts of sulphur were



a.s. – ammonium sulphate, w. – waste

Fig. 4. Content of sulphate sulphur in soil.



a.s. – ammonium sulphate, w. – waste

Fig. 5. Content of total sulphur in soil effluents.

found in the effluents from treatments receiving mineral fertilization with nitrogen, phosphorus and potassium (NPK). Liming generally led to a lesser sulphur leaching in the first year of the research. In the subsequent two years greater quantities of sulphur were assessed in soil effluents from NPK + S1 w. and NPK + S3 a.s. treatments than in the effluents from the analogously fertilized objects in the non-limed series (0 Ca). In comparison with the identical objects of the non-limed series (0 Ca) sulphur losses through leaching in the limed series (+ Ca) were higher as follows: from the NPK + S1 a.s. and NPK + S1 w. on average 25% in the second and 139% in the third year whereas from the NPK + S3 a.s. 22% in the second and 12% in the third year of the experiment. Total sulphur content in soil effluents was most strongly correlated with sulphate form of this element in soil ($r = + 0.816$; $\alpha < 0.001$). Much weaker, though significant relationship was registered between sulphur content in soil effluents and spring wheat biomass yield ($r = + 0.313$; $\alpha < 0.01$).

Apart from unfavourable effect concerning the biological value of the crop biomass, unbalanced fertilization has also a significant influence on the environment. Too large acumulation of fertilizer components in soil not utilized by plants, often leads to their loss through leaching. Therefore, big yields of biomass should not be the only indicator of efficient management of biogenic components. Sulphates are relatively easily leached from soil (Kopeć et al., 1991). Introduction of this element into the soil either as a mineral fertilizer or in the form of waste from magnesium production, caused an increase in the sulphur content in soil effluents. Quantities of this element in the effluent waters were determined mainly by the amount of sulphur supplied to the soil with fertilizers and to a lesser degree with crop yields. Investigations conducted by Kopeć and Gondek (2002) demonstrated that sulphur leaching from soil is determined by the crop yield and therefore by the quantities of absorbed sulphur depending on the applied fertilization. The research results presented in this paper also point to a significant dependence between wheat biomass yield and sulphur concentrations in soil effluents. On the basis of obtained results no apparent effect of soil liming on changes in sulphur concentrations in soil effluents was noted. According to Kopeć and Gondek (2002) an increase in soil pH caused a release of adsorbed sulphates, thus in conditions of limed soil sulphur bioavailability for plants may increase. Such relationship was not proved in conditions of the presented experiment. On the other hand, this process favours leaching of sulphate ions. Liming also causes changes of soil biological activity, which at increased numbers of microorganisms may determine the rate of organic matter mineralization leading to a release of a greater number of sulphur compounds soluble in the soil solution. According to Guzy and Aksomaitiene (2004), liming causes a decrease in sulphur concentration in soil solution and diminishes the quantity of this element leached from the soil profile.

CONCLUSION

1. The average (for three years) total yield of spring wheat biomass (grains, straw and roots), at comparable values of statistical mean standard error for years and for individual treatments, was the greatest after application of sulphur in the form of ammonium sulphate against the background of nitrogen, phosphorus and potassium fertilization.

2. In comparison with biomass yield from the treatments where a lower sulphur dose was used, both as ammonium sulphate and as the waste from magnesium sulphate production, a smaller biomass yield was obtained in the treatment where sulphur was applied in a thrice bigger dose.

3. The mean weighed average sulphur content in grains, straw and roots of wheat fertilized with sulphur was significantly greater than the content determined in the wheat biomass non-fertilized with this element. Increasing sulphur doses did not cause any significant differences in this element concentrations in wheat biomass.

4. A single soil fertilization with smaller doses of sulphur, either as ammonium sulphate or the waste from magnesium sulphate production did not cause any durable fertilizer effect which resulted from sulphur uptake with yield and its leaching from soil.

5. Sulphur fertilization, both in the form of ammonium sulphate and as the waste from magnesium sulphate production, led to increased sulphur concentrations in soil effluents. Amounts of this element in the effluent waters were conditioned mainly by the amount of sulphur supplied to the soil with fertilization but to a lesser degree by crop yields.

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Cost evaluation of the application of the differentiated nitrogen doses on winter wheat field

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Abstract. The materials derived from the studies of the spatial within-field variability were used in this research. The experiment was carried out in the Experimental Station Baborówko (wielkopolskie voivodship) belonging to the Institute of Soil Science and Plant Cultivation – National Research Institute (IUNG-PIB), in Puławy. The map of the variation of the nitrogen doses is based on detailed analysis of the spatial variability of field and the vegetation indices. Three types of nitrogen fertilization were used to assess the economic effect of the systems – an intensive – heavy fertilization system (according to which, the dose of 100 kg N ha^{-1} was applied on the whole field), a highly intensive – very heavy fertilization system (where 160 kg N ha^{-1} was applied), and a varied system, where a varied (within the field) dose of N was implemented and was defined on the basis of the maps of the potential productivity of the fields. The analysis showed that the unit cost of the nitrogen fertilizer per hectare was 233 PLN in the heavy fertilization system, 373 PLN very heavy fertilization system and 311 PLN in the system with the differentiated doses of N. The use of the varied nitrogen rates within the same field, allowed the costs to be reduced by about 16.7% compared to the scenario of a highly intensive use of nitrogen fertilizers. However, compared to the intensive scenario, these costs were 33.3% higher.

key words: economic assessment, map of the differentiated nitrogen rates, intense (heavy) fertilization system

INTRODUCTION

In the concept of the sustainable development, the increasingly important issues are related to protection of natural resources. The impact of agriculture on the environment is largely determined by fertilization. The fertilization treatment is an essential element of the agricultural practices, which allows high yields to be obtained and a high level of soil fertility to be maintained. The use of excessively high rates of fertilizers (particularly those of nitrogen) can affect the dispersion of the nutrients into the environment, especially into water and air (Igras, 2006). Too low rates of nitrogen, which do not fully cover the fertilizer needs of plants can reduce the size and quality of crop yield and deteriorate soil fertility. Fertilizer cost has a significant share in the structure of the direct costs and ranges from 30 to 50% depending on the crop (Skarżyńska, 2007). Therefore, both for the economic and environmental reasons, the optimal method of application of fertilizers are sought (Ostergaard, 1997; Begiebing et al., 2007; Griffin, 2007). One of the method involves adjusting the rates of nutrients to the potential productivity of the field resulting primarily from the chemical properties of the soil. Economic effects resulting from the differentiation of the fertilizers rates of are not clearly proven, and the discrepancy in the assessment of the economic effects is tremendous (Jadczyszyn, 2004b; Begiebing et al., 2007; Griffin, 2007).

The aim of this study was to assess the cost of varied fertilizer nitrogen rate depending on the potential productivity of the field.

MATERIAL AND METHODS

The material used in the study came from the research of spatial variability of cultivated wheat fields, conducted in the Experimental Station IUNG-PIB in Baborówko (wielkopolskie voivodship). The station conducts large--area experiments on an area of 53.6 ha in crop-rotation with winter wheat, spring barley and winter rape with applying the principles of the precision farming.

The standardized agrotechnology adopted in the integrated agriculture is used according to the recommendations of IUNG-PIB for integrated farming.

The soil-agricultural map 1:1000 was applied to determine the spatial variation of the soil and the nutrients

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abundance (Oczoś, 1994) as well as the results of soil tests carried out in a grid with a resolution of 24 m. The aerial photographs taken between 2005–2007 were used to estimate the vegetation index maps. The photographs were taken at the shooting, early anthesis and milk-ripe stages of winter wheat (Pudełko et al., 2006).

The spatial data from the soil maps, aerial photographs and maps of the electromagnetic resistance of the soil were used to assess the yield potential of the experimental field. The measurement of soil electromagnetic resistance was performed using EM 38, which is currently the world's most popular remote device for assessing of the variability of physico-chemical properties of the soil.

The quality assessment and classification of aerial photographs were made based on the measurement of electromagnetic conductivity of the soil, which significantly depends on the granulometric composition of the soil. The spatial data were converted to digital form in a geographic information system (GIS) and were the basis for compiling the map for the optimization of nitrogen fertilization rates (Pudełko, 2005, 2006).

The evaluation of the costs of nitrogen application on 48.83 hectares field of winter wheat, was made on the basis of actual costs of fertilizer which would be incurred in the precision farming system, assuming that the dose is varied on the basis of the map of potential field productivity. This result was compared to the cost of fertilizer application in intensive and highly intensive system of fertilization. In the intensive system a nitrogen rate of 100 kg N ha^{-1} was assumed, whereas in a highly intensive (very heavy fertilization) system of 160 kg N ha^{-1}. In a system of the different nitrogen rate, the rate was variable and ranged from 100 to 160 kg N ha^{-1}. The cost estimate assumed a unit price of nitrogen in ammonium nitrate of the spring of 2007.

RESULTS AND DISCUSSION

The large mosaic of soil was observed both in the region of Wielkopolska and the fields of the experimental station Baborówko. The research conducted under the LOTON project enabled the diagnosis and analysis of the variability range of the soil conditions of the experimental fields. The analysis of the collected spatial data showed the significant differences in soil conditions within the analyzed experimental field. A large range of the soil conditions variability was also confirmed by the aviation pictures and measurements of the electromagnetic conductivity of the soil.

The research conducted in the LOTON project demonstrated the need for diversification of the nitrogen rates in the separate zones of the field because of the soil conditions. It was also confirmed by aerial photographs and measurements of the electromagnetic conductivity of the soil. In this connection, a vector map of nitrogen rates was compiled, producing four different zones (Fig. 1). Maps were prepared on the basis of the spatial data obtained by tele-detection methods (Kozyra and Pudełko, 2006; Pudełko et al. 2007). The map of the nitrogen rates additionally took into account the agronomic possibilities (distance of technological paths) and the differentiation capacity of the fertilizer rates of the fertilizer spreader Bogbal EX-Trend type.

Table 1. The amount of nitrogen in each zone.

Zone	Rate of nitrogen [kg ha^{-1}]	Area [ha]	Amount of nitrogen [kg]
1	100	2.17	217.0
2	120	18.05	2166.0
3	140	22.65	3171.0
4	160	5.96	953.6
1–4		48.83	6507.6

Figure 1 shows the spatial extent of each zone with different nitrogen rates. The information about the surface of each zone and the amount of nitrogen applied is given in Table 1. The total amount of nitrogen needed in the field of winter wheat was calculated on that basis.

In earlier studies Jadczyszyn et al. (2004) suggest that the intake of nitrogen by winter wheat in the experimental field ranged from 110 to 180 kg N ha^{-1} for the range of variability in yield from 4.6 to 7.6 t ha^{-1}. Nitrogen rates were determined for the above range of yield variability using the fertilizer advice software NAWSALD (Jadczyszyn, 2004a). The recommendations show that the nitrogen rates should range between 100 for the lowest yield to 160 kg N ha^{-1} for the highest yield. For the comparison the cost of nitrogen fertilizer application a following assumptions were made: the lower limit of the range was taken as the intensive system of nitrogen fertilization, while the upper limit was assumed as the highly intensive fertilization regime, and the rates of nitrogen were chosen accordingly. The comparison

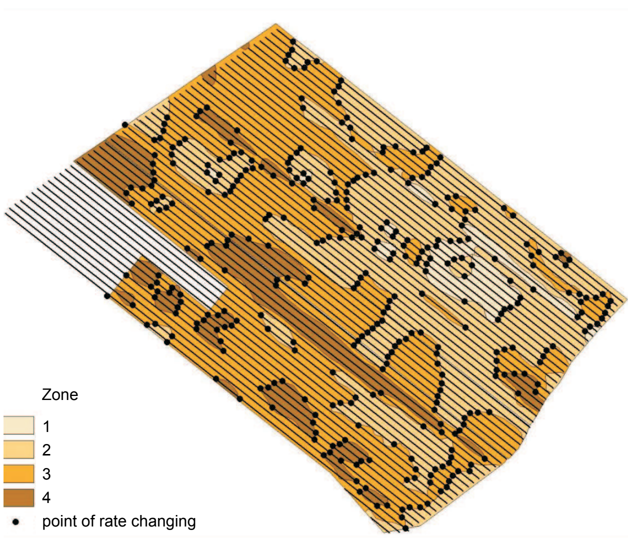

Fig. 1. The map of the differentiation of the nitrogen rates.

Table 2. The amount of nitrogen and the fertilizing costs in each fertilization system.

Fertilization system	Nitrogen rate [kg ha^{-1}]	Amount of nitrogen [kg]	Fertilizer costs [PLN]	Unit fertilizer cost [PLN ha^{-1}]
Intensive	100	4883.0	11 377.39	233.0
Highly intensive	160	7812.8	18 203.82	372.8
Based on the map of potential productivity of the field	differentiated	6507.6	15 162.71	310.5

of the different systems is presented in Table 2 assuming for calculations a unit price of nitrogen in the ammonium nitrate as 2.33 PLN per 1 kg N.

In a Danish study the direct benefit from the use of different rates of nitrogen fertilization was estimated at 15–50 \$ per hectare (Griffin, 2007). In a study conducted by Begiebing et al. (2007) the differentiation of nitrogen rates in the cultivation of winter wheat allowed the reduction of the total rate from 19 to 43 kg N ha^{-1}.

The study carried out by the authors showed that the unit costs of nitrogen fertilizer in different systems are diversified and were around 233 PLN ha^{-1} in the intensive system, and around 373 PLN ha^{-1} in the highly intensive system and about 311 PLN ha^{-1} in system with the differentiated rates of nitrogen. The use of varied nitrogen rates allowed the cost of fertilizers application to be reduced by 16.7% (an average of 27 kg N ha^{-1}) in relation to the highly intensive system. However, compared to the intensive system, these costs were higher by around 33.3%. It should be noted that, in accordance with the fertilizers recommendations that take into consideration the soil conditions of the experimental field the use of the lowest dose of nitrogen should be limited only to the surface of 2.17 ha. The use of nitrogen in the whole field under intensive fertilization system may not meet the nutritional requirements of plants and result in lower yields. The research conducted by Podolska et al. (2005) on the economic and qualitative assessment of winter wheat crops, depending on the level of the nitrogen fertilization showed that to achieve maximum grain yield of wheat, the rate of 140.6 kg N ha^{-1} was necessary. The application of this rate on the surface of the entire field would result in the cost of buying fertilizer at about 15 997 PLN, i.e. higher by 5.5% than estimated in the investigations in the variant with differentiate N doses.

The results gained in the study are representative for the Wielkopolska region, characterized by a large mosaic of the soils as well as the weather conditions unfavourable for yields, mainly due to the drought stress. The presented study shows only a general picture of possible use of techniques that involve variable dosing of nutrients in plant fertilization

CONCLUSIONS

1. The study shows that a large range of variability in soil conditions of the experimental field gives rise to the differentiation of nutrients within the same experimental field.
2. The use of varied rates of nitrogen in the production of winter wheat gave the chance to reduce the cost of application of fertilizers by 16.7% (an average of 27 kg N ha^{-1}) in relation to the application of uniform nitrogen rates
3. The maps of the differentiation of fertilizer rates should be developed for each field individually. Other factors such as weather conditions during the growing season will impact the expected economic effect

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The effect of Atonik AL application on growth and development of motherwort (*Leonurus cardiaca* L.) depending on age of plant

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Abstract. In the paper there are described the effects of foliar application of Atonik AL (0.2% solution) on the growth and development of motherwort (*Leonurus cardiaca* L.) plants in the first, second and third year of vegetation. The experiment was performed in years 2001–2004 on brown sandy loam soil. 1-year-old plants were sprayed twice (at seeding and three weeks later); 2-year and 3-year-old – three times: at rosette stage, three weeks later and after first harvest. In the experiment there were stated beneficial effects of the preparation on plants morphological features, especially in the first and second year of vegetation (stems were higher thicker, more branched and they produced longer inflorescences). Beneficial influence of Atonik on the growth and development plants resulted in significant increase of air dry mass of 1- and 2-year-old plants. In case of 3-year-old plants positive effect of Atonik regarded only height of plants and length of inflorescence.

key words: *Leonurus cardiaca*, Atonik, plant growth and development

INTRODUCTION

According to the producer, Atonik AL is an universal plant growth stimulator; active substances are sodium orto- and para-nitro-phenolate and sodium 5-nitro-guaiacolates. The preparation increases absorption of mineral compounds and regulates concentration of Ca^{2+} ions in plants cells, speeding up synthesis of organic compounds and accelerates plant growth and development.

Motherwort (*Leonurus cardiaca* L.) is a perennial medicinal plant, demonstrating sedative and soporific properties (Mścisz, Gorecki, 1997). Research on the effects of Atonik on other medicinal plants indicate usefulness of this preparation (Berbeć et al., 2003; Kołodziej, Berbeć, 2005).

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The aim of this investigation was to determine the effect of Atonik AL on the growth and development of motherwort plants during three successive years of vegetation.

MATERIAL AND METHODS

The experiment was carried out in 2001–2004 on the brown loamy-sand soil. Every year new plantation was established and effects for one- and two-year-old plants were received during 3 years, while results concerning three-year-old plants refer to two year lasting experiment. Seedlings were produced in greenhouse (seeds were sown at the end of March in multi-cell trays filled with peat origin substrate). Seedlings were planted on field at phase of 6–8 leaves (beginning of May) in distance of 50 x 30 cm (plots area – 16 m^2 in 4 replications). Soil fertilization was performed as recommended for this species (N – 80 kg, P – 21.8 kg, K – 66.4 kg per hectare) (Załęcki et al., 1994). Atonik AL was applied in 0.2% solution in quantity of 400 litres per hectare (each spraying). 1-year-old plants were sprayed twice: at the end of May and in 16^{th}–20^{th} of June. 2- and 3-year-old plants were sprayed 3 times: at rosette stage (April/May), three weeks later and after first harvest (on regrowth). Plants were harvested in blooming phase (in first year – one harvest, in following years – two harvests: half of June and late August). Before harvest, height of plants, stem thickness, number of branches and inflorescence length were measured. For 2- and 3-year-old plants additionally number of stems was calculated. After harvest, air dry mass of plants was determined. Numerical data were statistically worked out and the least significant differences were calculated with Tukey's test at 5% error margin.

RESULTS AND DISCUSSION

Beneficial effect of Atonik on plant growth and development was proved irrespectively of plant age, although effectiveness of the preparation declined along with age of

Table 1. Effect of Atonik on features of motherwort plants (first harvest)

Objects	Height of plants [cm]	Number of stems (per plant)	Number of branches (per stem)	Thickness of stem [mm]	Length of inflorescence [cm]	Air dry mass of plant [g]
			1-year-old plants			
Control	82.4	-	20.2	6.5	22.9	35.4
Atonik	97.2	-	23.2	6.9	28.6	47.7
Mean	**89.9**	**-**	**21.7**	**6.7**	**25.3**	**41.6**
LSD (0.05)	5.27	-	0.70	0.14	1.71	8.46
			2-year-old plants			
Control	125.9	9.8	12.1	6.6	12.1	84.2
Atonik	143.3	11.5	11.1	6.9	14.9	115.6
Mean	**134.6**	**10.7**	**11.6**	**6.8**	**13.5**	**99.9**
LSD (0.05)	4.34	0.88	0.55	0.10	1.53	7.94
			3-year-old plants			
Control	97.1	11.8	9.2	5.5	9.7	63.2
Atonik	105.9	10.6	9.8	5.4	10.9	58.8
Mean	**101.5**	**11.2**	**9.5**	**5.5**	**10.3**	**61.0**
LSD (0.05)	6.28	1.10	ns	ns	0.44	ns

ns – not significant

Table 2. Effect of Atonik on features of motherwort plants (second harvest)

Objects	Height of plants [cm]	Number of stems (per plant)	Number of branches (per stem)	Thickness of stem [mm]	Length of inflorescence [cm]	Air dry mass of plant [g]
			2-year-old plants			
Control	56.4	18.8	6.2	2.9	8.7	27.8
Atonik	60.6	28.0	7.2	2.9	11.2	41.6
Mean	**58.5**	**23.4**	**6.7**	**2.9**	**9.9**	**34.7**
LSD (0.05)	4.07	2.17	0.48	ns.	2.01	5.07
			3-year-old plants			
Control	56.1	19.3	5.8	3.0	8.5	31.7
Atonik	57.1	27.9	7.2	2.8	9.6	31.1
Mean	**56.6**	**23.6**	**6.5**	**2.9**	**9.1**	**31.4**
LSD (0.05)	ns	2.23	0.49	ns	ns	ns

ns – not significant

the plants. In the first year of vegetation Atonik showed the biggest influence on all examined morphological features: plant height, thickness of stems and their branching as well as length of inflorescence (Table 1). In the second year positive effect of the preparation concerned almost all examined features (exception was number of branches during first harvest and thickness of stems during second one) (Table 1 and 2).

In the third year of vegetation (3-year-old plants) spraying with Atonik had the smallest effect, resulted however in an increased height of plants and length of inflorescence (only during first harvest) and also in increased number of stems and branches (during second harvest) (Table 1 and 2). As regards stem thickness – Atonik resulted in an increase of 1- and 2-year-old plants (only the first harvest). In general, thickness of stems during first harvest was almost twice bigger than those of second one.

Advantageous effects of Atonik in culture of medicinal plants were recorded in experiments of other authors. Kołodziej (2001) got increase of stem number in curlycup gumweed, Gruszczyk and Berbeć (2004) found increased height of feverfew plants, Berbeć et al. (2003) – common thyme.

As regards the effect of Atonik on mass of particular plants, it was significant in case of 1- and 2-year-old plants (increase respectively by 35% and 37–50%). From among features examined, height of plants and number of branches (in the first year of vegetation) and also number of stems (in case of 2-year-old plants) chiefly contributed to the increased mass of plants. In the third year of vegetation (3-year-old plants) Atonik did not affect markedly air dry mass of plants. In experiments with others perennial medicinal plants: feverfew and goldenrod, Atonik brought about a significant increase of mass of plants irrespectively of plant age (Gruszczyk, Berbeć, 2004; Kołodziej, 2008).

CONCLUSIONS

1. The effect of spraying motherwort plants with 0.2% Atonik AL solution, on plant growth and development was especially marked in the first and second year of vegetation. It resulted in the increase of stem height and their thickness, length of inflorescence and number of stems (in second year of vegetation).
2. Along with plant age, the effect of spraying declined and was more apparent during the first harvest than in second one. Nevertheless it was still effective for such characters as height of plants, inflorescence length and number of branches.
3. Positive influence of Atonik on plant growth resulted in significant increase of air dry mass of plants in the first (about 35%) and second (37–49%) year of vegetation.

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Assessment of maize yields as affected by seedbed preparation method

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Abstract. The aim of this study was to compare the yields of maize grown continuously under two seedbed management systems vs. the conventional rotation-based system and to compare the changes in the soil environment brought about by those tillage methods.

Results for the study were derived from maize investigations performed at two experiment sites: at the Agricultural Experiment Station at Grabów, province of Mazovia, Poland, at an experiment station at Baborówko, province of Great Poland. It was a stationary field trial established in 2004 which involved maize cropped continuously and rotated with other crops. The layout of the experiment involved the following treatments: maize cropped continuously – zero tillage (direct seeding), maize cropped continuously – plough-based tillage with complementary cultivation practices, maize in crop rotation (spring barley – winter wheat – maize) – conventional plough-based tillage.

The highest grain yields, regardless of site area, were obtained from maize grown under a three-year rotation scheme with organic fertilization. Under such a management the yields were higher by 10% compared to those from a treatment which combined maize monoculture and direct seeding. The grain yields recorded in Wielkopolska (Greater Poland) were higher than those in the mid-eastern region. In terms of cereal units, lower yields were obtained from the rotation scheme – spring barley, winter wheat – maize than from maize grown under monoculture, regardless of tillage method and experiment site. Direct seeding of continuously cropped maize increased grain moisture at harvest by ca. 2%, did not affect number of grains per cob, but decreased weight of 1000 grains. The contents of humus and P_2O_5 in the 0–30 cm soil layer were higher and that of K_2O was lower at the termination of the trial that at its commencement, but the content of MgO remained little changed. The contents of humus and K_2O in the topsoil (0–10 cm) under continuously cropped and directly seeded maize were higher that in the 10–30 cm soil layer. Total N varied but little among seedbed preparation methods and also showed little variation across the soil profile.

key words: maize, seedbed preparation, monoculture, zero-tillage

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INTRODUCTION

Simplified cropping schemes and reduced tillage methods are increasingly used in contemporary farming. Reduced tillage usually consists in the reduction of tilling depth or in the reduction of tillage passes down to stopping tillage completely (zero-tillage). A reduction in the number of crops to be grown in a field can vary from short crop sequences to continuous cropping. Maize is a crop that tolerates continuous cropping well and in many countries it is grown from direct seeding (Cox et al., 1990; Pudełko et al., 1994; Radecki, Opic, 1991; Szymankiewicz, 1988). According to Dubas (1980) and to Dubas and Menzel (1999) the yields of maize seeded directly and grown under monoculture undergo reduction as compared to the yields of maize grown under the conventional plough-based tillage system. Pabin et al. (2003) share a similar opinion. They report the reduction in yields of maize grown under such management to occur in the first 3–5 years. In another study Dubas and Szulc (2006) found the yields to decrease over the successive years regardless of tillage system but the yield reduction was greater for direct seeding as compared to conventional tillage. The results obtained by other investigators (Dzienia, Sosnowski, 1991; Dubas, Menzel, 1999; Dubas et al., 2002) also point to yield reduction attributable to simplified tillage and direct seeding. Those researchers point out that the scale of yield reduction varied with the environment in which maize was grown. By contrast, Griffith et al. (1988) found yields of maize seeded directly to level off vs. those obtained under regular tillage. The investigators also emphasize the fact that direct seeding, when applied for some time, causes some physical, chemical, and biological properties of the soil to become stabilized and thus favours high yields in maize.

In Poland, since the year 2000 there has been a dynamic growth in the area under maize. In 2000 the crop occupied 314 000 ha whereas in 2008 the area cropped to maize increased to 733 000 ha. In the years 2001–2004 maize for grain was the leading crop but beginning with 2006 there has been a significant increase in the area cropped to maize grown for silage (60% of the total area in 2008). On many farms, especially on large-size farms, maize is not only grown in short maize-after-maize sequences but also under continuous cropping schemes of several years' duration. In addition, once the cobs have been harvested the remaining maize straw is not harvested for fodder but, rather, the residue is shredded and turned under. Nearly half of the produced biomass is returned to the soil thereby increasing the soil content of organic substance and of nutrients. By contrast, whole of the aboveground biomass of maize for silage is removed from field and has no positive effect on organic matter content in soil. In Poland, due to a substantial proportion of light and acidified soils such an approach is of considerable significance. The soils most frequently cropped to maize are rated as classes IIIa–IVb or as very good and good rye-growing complexes. Furthermore, the EU soil strategy of 2002 covers many hazards to soil fertility, and one of its major tenets is the prevention of soil degradation by restricting the reduction of soil organic matter. Poland, like the remaining EU member states, will be under obligation to develop soil degradation prevention programmes.

The aim of this study was to compare the yields of maize grown continuously under two seedbed management systems vs. the conventional rotation-based system and to compare the changes in the soil environment brought about by those tillage methods.

It was assumed that maize grown under monoculture would give yields similar to those of maize grown after cereals and that the adverse changes to physico-chemical and biological properties of the soil related to continuous cropping would be efficiently offset by the incorporation of maize straw residues.

METHODS

Results for the study were derived from maize investigations performed at two experiment sites: at the Agricultural Experiment Station at Grabów, province of Mazovia, Poland, located on lessive soil developed from a light loam and rated as very good rye-growing complex and at an Experiment Station at Baborówko, province of Great Poland, on a lessive soil with a granulometric composition characteristic of loamy sand and rated as good rye-growing complex. At both sites the arable layer of the soil was high in phosphorus, medium-high in potassium and low in magnesium. It was a stationary field trial established in 2004 which involved maize cropped continuously and rotated with other crops. The layout of the experiment involved the following treatments:

1. maize cropped continuously – zero tillage (direct seeding)
2. maize cropped continuously – plough-based tillage with complementary cultivation practices
3. maize in crop rotation (spring barley – winter wheat – maize) – conventional plough-based tillage

Maize grown under monoculture involved two seedbed preparation methods: plough-based tillage and zero tillage (direct seeding). In the conventional tillage treatment straw residues left after the cob harvest were shredded and turned under. By contrast, in the non-tilled treatment the straw was shredded but left on the soil surface. Under the crop rotation management all crop species involved were grown each year and full FYM dose was applied to maize. On dairy farms it is frequently the only species to which FYM can be applied. The experiment was set up as a long strip design with the mirror image of treatments.

Maize cv. Delitop was seeded using a precision maize plantler. Cv. Antek of spring barley and cv. Turnia of wheat were seeded at Grabów whereas cv. Weneda of spring barley and cv. Ludwiq of wheat were grown at Baborówko. Nitrogen was applied to maize at a rate of 140 kg N ha^{-1} (70 + 70), phosphorus and potassium rates (kg ha^{-1}) were P_2O_5 – 80 and K_2O – 125. Annual fertilizer rates supplied to barley were: N – 60, P_2O_5 – 35 and K_2O – 50 kg ha^{-1}, and to wheat: N – 120, P_2O_5 – 40 i K_2O – 70 kg ha^{-1}.

In order to assess the effect of maize tillage prior to the onset and after the termination of the trial pH in KCl and the soil contents (mg per 100 g of soil) of P_2O_5 (CFA method), K_2O (FES method), MgO (AAS method), percentage of total nitrogen (CFA method) and humus content (Tiurin's method) were assayed.

The maize was grown for grain. The yielding performance for the individual approaches to managing maize production were determined as total cereal units obtained from the continuous cropping of maize over three successive years and, for maize grown under crop rotation management, as total cereal units obtained from the combined crops of maize, wheat and barley over the full rotation cycle. In addition, records were also taken of grain yield and yield components. During plant growth biometrical measurements were taken. The crop stands were also evaluated for pest and disease infestation and, in maize, for lodging.

ANOVA was used to test the significance of treatment-to-treatment differences, confidence half-intervals being determined using Tukey's test at a significance level of $\alpha = 0.05$.

RESULTS AND DISCUSSION

Weather pattern had a significant effect on the yields of maize and cereals. In 2007 at Grabów small amounts of rainfall were recorded in the second and the third decade of

Table 1. Meterological conditions during the growing season (experiment stations at Grabów and at Baborówko, 2007–2009).

Years	April	May	June	July	August	September	April–September
	Average air temperatures [°C]						
	Grabów						
2007	7.8	15.2	18.7	19.2	19.1	12.8	15.5
2008	9.0	13.1	17.6	18.9	18.9	12.5	15.0
2009	10.7	13.0	16.4	19.7	18.1	14.9	15.5
Long-term mean	7.7	13.4	16.7	18.3	17.3	13.2	14.4
	Baborówko						
2007	9.8	16.0	19.8	19.5	19.7	14.0	16.5
2008	8.2	15.1	19.4	20.7	19.1	13.6	16.0
2009	12.8	14.1	16.2	20.3	20.2	15.8	16.6
Long-term mean	7.8	13.4	16.3	18.2	17.9	13.3	14.5
	Sum of precipitation [mm]						
	Grabów						
2007	13.3	74.6	99.9	75.5	151.7	77.4	492.4
2008	71.8	87.6	51.1	85.4	54.5	19.7	370.1
2009	0.6	57.5	117.9	117.8	74.6	32.3	400.7
Long-term mean	39	57	71	84	75	50	376
	Baborówko						
2007	9.7	119.4	59.0	94.7	42.4	27.1	352.3
2008	99.7	14.9	18.0	69.5	91.2	25.9	319.2
2009	17.5	76.8	91.4	75.8	27.3	45.50	334.3
Long-term mean	30.8	49.7	60.5	78.7	54.2	42.9	316.8

June and in the first decade of July whereas in 2008 there was no rainfall at all in the third decade of May and in the first decade of June and also no rainfall in April of 2009. Accompanying high air temperature and high insolation intensified water deficit. At Baborówko, drought occurred at the end of May and in the beginning of June resulting in restricted plant growth and development (Table 1). In an earlier study by Machul and Księżak (2007) the authors found that in July with the temperatures higher by 4 degrees C and with a lower rainfall (by ca. 10 mm) maize plants set ears a part of which was partly or completely void of seeds, a development which had a negative effect on yields.

Over the three-year period of the study, total cereal units recorded at Baborówko were substantially higher than those in Grabów (Table 2). It was chiefly due to much higher maize yields at the former location. Relatively low cereal yields, especially those of spring barley, caused the yields of cereal units at Grabów and at Baborówko to be lower under crop rotation than under maize monoculture both when conventional plough-based tillage or direct seeding were applied. At Grabów, the differences were ca. 6%, but at Baborówko they were substantially higher and reached ca. 23%. Total cereal units obtained over three years were much higher than those obtained by Machul and Księżak (2007). Those investigators also report that, in that period, in the central-eastern region the lowest yields in terms of cereal units were recorded under the management which combined conventional tillage and rotation whereas in the Great Poland region the lowest cereal units were obtained from the combination of continuous cropping and zero tillage.

Yields were substantially affected by region, weather pattern during the growing season and by the seedbed preparation methods applied. At Baborówko, yields higher

Table 2. Yields of cereal units obtained in the years 2007–2009.

Management system	RZD Grabów				SD Baborówko			
	2007	2008	2009	sum	2007	2008	2009	sum
Monoculture – zero tillage	75.10	73.30	83.80	232.2	97.70	88.90	95.80	282.3
Monoculture – conventional tillage	70.50	69.10	91.90	231.5	101.00	98.10	98.40	289.9
Crop rotation – conventional tillage	61.80	61.20	94.70	217.7	75.06	71.83	74.83	221.7
LSD ($\alpha = 0.05$)	4.62	3.93	3.61		4.93	5.23	3.96	

by about 25% were recorded for the study period (Figures 1, 2). At Baborówko, the highest yields were obtained in 2007 whereas at Grabów maize gave the best yields in 2009. Averaged over the three years, in both maize-growing regions the lowest yields of maize were obtained from the treatment that combined maize monoculture with direct seeding. In that treatment at Baborówko, the yields were 10% lower than those in the treatment involving a three-year rotation scheme. At Grabów, those differences were a little smaller. A survey of the literature on the effect of reduced tillage on maize yields reveals that the reported results show substantial discrepancies. There is a prevailing opinion that reduced tillage, and direct seeding in particular, leads to significant reductions in the yields of maize grain and straw (Burgess et al., 1996; Drury et al., 1999; Dubas, Menzel, 1999; Gregorich et al., 1993; Griffith et al., 1988; Ismail et al., 1994; Kapusta et al., 1996; Kaspar et al., 1987; Machul, 1993; Machul, 1995; Szymankiewicz, 1988). As reported by Dzienia and Sosnowski (1991) weather has an important impact since in warm years maize from direct seeding gave superior yields whereas in the years with spells of cool and moist weather maize grown under conventional tillage showed a better yielding performance. According to Machul (1995), an adverse impact of monoculture on maize yields becomes apparent as early as in the second year. According to Griffith et al. (1988) and to Machul (1995) yield reduction from direct seeding vs. conventional tillage is several percentage points whereas according to Menzel et Dubas (2003) it amounts to 16%. According to Sekutowski and Sadowski (2008) the application of reduced tillage in the preparation of seedbed that involved no weed control resulted in a yield reduction of ca. 42% over the plough-based tillage while in the treatments with herbicide application yield restrictions were small. According to many investigators (Cox et al., 1990; Dubas, Menzel, 1999; Gregorich et al., 1993; Machul, 1995; Menzel, Dubas, 2003) the major cause of decreased yields under the direct seeding management was reduced plant density and hence reduced number of cobs per unit area. By contrast, Machul and Księżak (2007)

Table 3. Weight of 1000 grains [g].

Management system	RZD Grabów			SD Baborówko		
	2007	2008	2009	2007	2008	2009
Monoculture – zero tillage	253	259	242	271	286	304
Monoculture – conventional tillage	262	245	255	279	294	303
Crop rotation	260	260	252	289	292	311
LSD (α = 0.05)	6.95	5.98	4.63	5.23	3.69	6.21

Table 4. Parameters of maize cobs.

Management system	Weight [g]		Number of seeds per cob	Proportion of rachis [%]	Cob measures [cm]	
	cob	grain			length	diameter
			Grabów			
Monoculture – zero tillage	119.2	108.7	434.0	10.9	17.3	3.7
Monoculture – conventional tillage	119.2	108.1	437.3	11.1	17.3	3.7
Crop rotation	121.4	107.9	440.7	11.0	17.3	3.8
LSD (α = 0.05)	ns	ns	ns	ns	ns	ns
			Baborówko			
Monoculture – zero tillage	151.2	132.3	450	12.1	17.9	4.0
Monoculture – conventional tillage	153.0	136.0	460	12.1	17.9	4.0
Crop rotation	165.1	147.2	474	12.8	18.3	4.0
LSD (α = 0.05)	5.24	4.12	12.3	ns	ns	ns

ns – non significant

Table 5. Plant density at harvest [thousand plants ha^{-1}].

Management system	RZD Grabów			SD Baborówko		
	2007	2008	2009	2007	2008	2009
Monoculture – zero tillage	86.3	71.8	88.7	88.4	72.3	89.4
Monoculture – conventional tillage	87.2	73.8	89.1	89.0	74.1	90.8
Crop rotation	87.7	73.2	89.8	89.4	74.9	91.3
Mean	86.8	72.9	89.2	88.9	73.8	90.5

Table 6. Grain moisture at harvest [%].

Management system	RZD Grabów			SD Baborówko		
	2007	2008	2009	2007	2008	2009
Monoculture – zero tillage	34.2	31.8	32.7	33.8	32.2	27.9
Monoculture – conventional tillage	33.0	30.0	30.3	32.9	31.3	27.0
Crop rotation	32.0	29.2	29.6	32.3	30.8	25.9
Mean	33.1	30.3	30.9	33.0	31.4	26.9
LSD (α = 0,05)	1.82	1.64	1.60	ns	ns	1.59

ns – non significant

Table 7. Plant height# of maize at harvest [cm].

Management system	RZD Grabów			SD Baborówko		
	2007	2008	2009	2007	2008	2009
Monoculture – zero tillage	200	139	205	210	148	195
Monoculture – conventional tillage	190	160	218	194	162	210
Crop rotation	192	160	224	190	164	215
Mean	194	153	216	198	158	207

measured from base to tip of the plant

Table 8. Grain yields of cereals [t ha^{-1}] in the treatment involving crop rotation.

Cereal species	RZD Grabów				SD Baborówko			
	2007	2008	2009	average	2007	2008	2009	average
Spring barley	4.46	4.76	3.00	4.07	4.12	3.57	5.1	4.26
Winter wheat	7.16	6.69	5.29	6.38	5.07	7.48	7.3	6.62
LSD (α = 0.05)	0.48	0.51	0.33		0.29	0.98	0.96	

failed to observe any variation in the yields of rotated vs. continuously cropped maize, regardless of the seedbed preparation method.

In both maize-growing regions the weight of 1000 grains from directly seeded maize grown under monoculture was lower than that from maize grown in crop rotation. There was no significant effect of seedbed preparation method on the proportion of rachis, cob length and diameter, and at Grabów no effect was also found on number of seeds per cob, cob weight and weight of seeds per cob. By contrast, when grown in crop rotation at Baborówko the maize had a higher number of seeds per cob, higher cob weight and higher weight of seeds per cob than maize grown under continuous management regardless of seedbed preparation method (Tables 3 and 4).

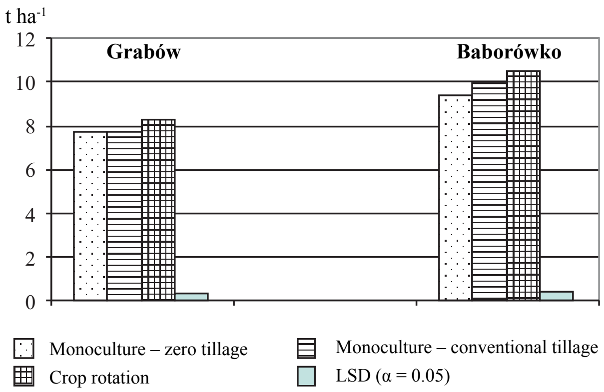

Fig. 1. Maize yields as affected by seedbed preparation (averages of 2007–2009).

The effect of seedbed preparation method on the number of plants per unit area at harvest was also slight (Table 5). It is only in 2008 that a substantially lower than planned plant density was recorded in all studied treatments. Results obtained by Machul (1995) and Menzel and Dubas (2003) indicate that plant density in the treatment with conventional tillage was much higher that that in those non-tillage treatments in which the seeds remained uncovered at seeding. The same effect was observed concerning number of cobs per unit area. Szymankiewicz (1987, 1988) holds the opinion that increased soil compactness and decreased soil capillary capacity may result in a reduced plant density per unit area.

Over the three years, the grain from maize grown under the management that involved monoculture and direct

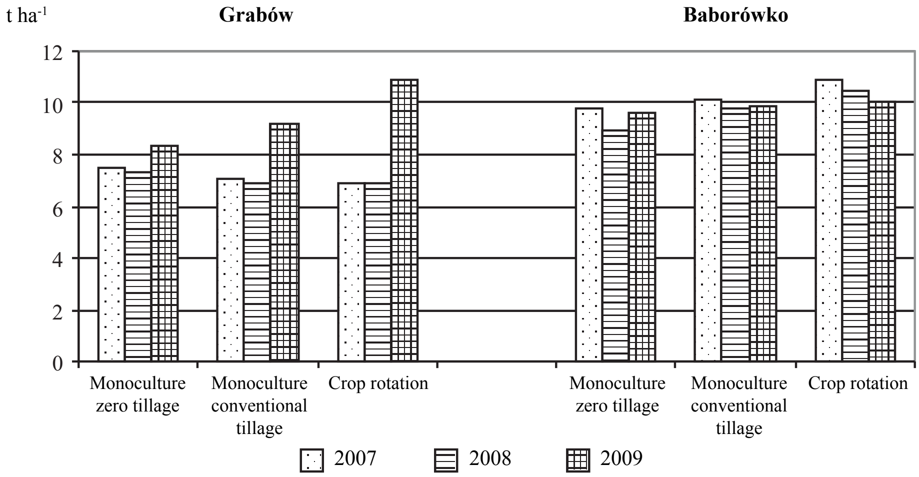

Fig. 2. Maize yields as affected by seedbed preparation method.

seeding was found to contain ca. 2% more moisture that the grain from maize under rotation (Table 6). Similar results were obtained by Griffith et al. (1988), Kaspar et al. (1987), Machul (1995), and also by Menzel and Dubas (2003). By contrast, Dubas and Szulc (2006) found but a slight increase in grain humidity in the directly seeded treatments. As stated by Gregorich et al. (1993) it can be caused by inferior growth conditions of young seedlings caused by lower soil temperatures. Additionally, in the 2009 experiments of this study higher moisture was found in the grain from the middle-eastern region as compared to that from Greater Poland

In the period under evaluation, recorded plant height in maize varied substantially depending on seedbed preparation method (Table 7). In 2007, in the non-tillage treatments the plants were taller than those grown in the treatments where conventional tillage was applied, both under monoculture and under rotation. Probably, it was caused by the impact of improved soil moisture in the non-tilled vs. pre-sowing tilled treatments. Conversely, in 2008 in both regions the plants were taller in the tilled treatments.

Over the duration of the study, the mean yields of spring barley and winter wheat at Grabów were similar to those recorded at Baborówko, even though in the latter region much higher yields of both crop species occurred in 2009 (Table 8). In both regions, winter wheat gave substantially higher yields than did spring barley. A similar yielding pattern of both species regardless of the growing area was earlier observed by Machul and Księżak (2007).

The average humus content of the 0–30 cm soil layer was much higher in the year 2009 than prior to the onset of the study (Table 9). A much higher rise was recorded in the treatments in which maize was grown continuously regardless of seedbed preparation method. However, the increase in the humus content of the soil cropped to maize in 33% was but slight despite the application of organic fertilizers. The highest humus content was recorded in the 0–10 cm layer under corn cropped continuously from direct seeding. Contrastingly, the humus content of that treatment at a layer of 10–30 cm and of the remaining two treatments at a layer of 0–30 cm varied little. An increased buildup of organic matter in the topsoil and its smaller content in the deeper soil layers occurring under reduced tillage was also observed by Alvarez et al. (1995), Blevins (1983), Dzienia and Sosnowski (1991), Machul (1995), Radecki (1986). Alvarez et al. (1995) state that the accumulation of organic matter in the topsoil increases the organic carbon content by 42–50%. Machul (1995) found changes in humus content to be related to the type of soil (a greater increase occurred in brown soil compared to that in alluvial soil). Szymankiewicz (1987) failed to demonstrate changes in humus content in response to annual changes of crop management method. Śmierzchalski (1980), though, is of an opinion that the buildup of organic matter in the topsoil can be looked upon as a non-beneficial alteration to the soil profile.

When compared at the termination of the study (2009) and before its commencement, the soil pH data indicate a rise in pH regardless of the soil management system (Table 9). Before the trial was started, soil pH was higher at the 20–30 cm layer than at the 0–10 cm layer regardless of the system adopted. By contrast, in the autumn of 2009 in the treatment involving continuously grown and directly seeded maize the pH was lower at 20–30 cm than at 0–20 cm. In the treatments which received conventional plough-based tillage, pH was similar in the soil layers assayed for that trait. As a result of direct seeding applied on the year-by-year basis, acidification of the soil and of the topsoil in particular was observed by Dzienia and Sosnowski (1991), Machul (1995), Radecki (1986). According to Radecki (1986) it is most likely caused by surface application of mineral fertilizers and progressively more shal-

Table 9. Effect of different crop and soil management methods on some soil properties (RZD Grabów).

Management methods	Soil layer [cm]	Autumn – 2006					Autumn – 2009					
		pH in KCl	content [mg/100 g of soil]			humus content [%]	pH in KCl	content [mg/100 g of soil]			humus content [%]	total N [%]
			P_2O_5	K_2O	MgO			P_2O_5	K_2O	MgO		
Monoculture – zero tillage	0–10	6.06	16.3	18.1	4.0	1.17	6.5	19.2	15.4	3.7	1.40	0.089
	10–20	6.29	14.8	16.4	3.2	1.14	6.5	19.0	13.5	3.8	1.28	0.083
	20–30	6.32	15.2	11.7	3.8	1.14	5.9	14.4	13.0	2.8	1.26	0.085
	mean	6.22	15.4	15.4	3.7	1.15	6.3	17.5	14.0	3.4	1.31	0.086
Monoculture – conventional tillage	0–10	6.17	18.0	8.9	3.0	1.11	6.8	24.6	7.6	3.5	1.28	0.083
	10–20	6.42	19.0	11.5	2.4	1.14	6.9	24.3	6.6	3.5	1.28	0.084
	20–30	6.65	20.7	15.4	3.5	1.10	7.0	23.5	7.9	3.7	1.29	0.086
	mean	6.41	19.2	11.9	3.0	1.12	6.9	24.1	7.4	3.6	1.28	0.084
Crop rotation	0–10	6.63	21.3	11.5	3.2	1.10	6.7	21.0	7.4	3.3	1.19	0.080
	10–20	6.73	22.5	13.9	3.2	1.22	7.1	21.0	7.9	3.5	1.22	0.079
	20–30	6.81	21.0	16.2	3.4	1.05	7.1	21.8	8.3	3.6	1.17	0.079
	mean	6.72	21.6	13.9	3.3	1.12	7.0	21.3	7.9	3.5	1.19	0.079

low development of the root system with a concommittant depletion of some elements from the top layers.

Total soil N was found to vary little with different seedbed preparation methods although somewhat less total N was found under rotated maize. Likewise, there was little layer-to-layer variation of that nutrient. The content of P_2O_5 was higher after the trial was completed than before it was started. In the treatment which involved directly seeded and continuously grown maize the content of that nutrient was significantly higher at 0–20 cm than at 20–30 cm. No such differences were recorded in the remaining two treatments. When compared before and after the trial, the K_2O content of the 0–30 cm layer of the soil declined in the autumn of 2009. Likewise, the K_2O content of the soil cropped continuously to directly seeded maize was higher than that found in the two remaining treatments. Furthermore, in the former treatment the 0–10 cm soil layer was higher in K_2O than the soil layers of 10–20 and 20–30 cm. No such response was observed in the remaining two treatments which received plough-based cultivation. MgO content of the soil before and after the trial did not show any major changes. In the treatments which received plough-based tillage MgO content was similar across the soil profile, but it was higher by ca. 30% at the 0–10 and 10–20 cm soil depths than at the 20–30 cm depth. Increased levels of available forms of phosphorus, potassium and magnesium in the upper layers of directly seeded vs. conventionally managed soils were also recorded by Dzienia and Sosnowski (1991), Machul (1995), Radecki (1986). Conversely, Szymankiewicz (1987) states that tillage has no effect on the soil contents of potassium and magnesium. Furthermore, that investigator recorded that potassium and phosphorus contents declined with soil depth regardless of how the soil was managed. Increase in topsoil contents of nitrogen, phosphorus, potassium and carbon is, according to Pudełko et al. (1994) caused by reduced leaching and smaller erosion.

CONCLUSIONS

1. The highest grain yields, regardless of growing area, were obtained from maize grown under a three-year rotation scheme with organic fertilization. Under such a management the yields were higher by 10% compared to those from a treatment which combined maize monoculture and direct seeding. The grain yields recorded in Wielkopolska (Greater Poland) were higher by ca. 25% than those in the mid-eastern region.
2. In terms of cereal units, lower yields were obtained from the rotation scheme – spring barley, winter wheat – maize than from maize grown under monoculture, regardless of tillage method and growing area.
3. Direct seeding of continuously cropped maize increased grain moisture at harvest by ca. 2%, did not affect number of grains per cob, but decreased weight of 1000 grains
4. The contents of humus and P_2O_5 in the 0–30 cm soil layer were higher and that of K_2O was lower at the termination of the trial that at its commencement, but the content of MgO remained little changed.
5. The contents of humus and K_2O in the topsoil (0–10 cm) under continuously cropped and directly seeded maize were higher that in the 10–30 cm soil layer. Total N varied but little among seedbed preparation methods and also showed little variation across the soil profile.

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Microbial and enzymatic characteristics of soils under pasture mixtures

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Abstract. In the years 2005–2007, twice a year, soil cores (0–20 cm deep x 2.5 cm diameter) were collected from three replicated plots of each of the selected treatments of a field experiment located at Grabów Experimental Station (51°20'58" N, 21°39'5" E). In this experiment pasture mixtures (white clover-grasses) were established on three fields (sites), about 500–600 m apart, and differing with respect to cropping history (preceding crops). Total numbers (cfu) of bacteria and fungi in soils under pasture mixtures were not significantly influenced by the studied factors (preceding crops and seeding rates). Numbers of bacteria from the genus *Azotobacter* were higher in the soil under pasture mixture grown after ploughed meadow (field III), than after spring barley (field I) and after potato (field II). The soil in field III was richest with respect to SOM and $N\text{-}NO_3$ contents and it had pH most favorable for bacteria. Activities of dehydrogenase and phosphatases (acid and alkaline) were highest in the soil under pasture mixtures grown in field III, lower in the soil of field II and the lowest in the soil of field I.

key words: bacteria, enzymatic activity, fungi, numbers, soil, pasture mixtures

INTRODUCTION

Beneficial environmental effects of growing grass or grass-legume mixtures (for forage or grazing) result mainly from large amounts of organic matter that are accumulated in soils under these mixtures (Ghani et al., 2003; Grzegorczyk, Grabowski, 2005). Ghani et al. (2003) compared chemical, biochemical and biological characteristics of some New Zealand soils of the same type but differing with respect to land uses and found that soils under sheep or dairy pastures contained 40–60% more the total organic C than adjacent cultivated soils. The pastoral soils were also richer in microbial biomass-C, labile fractions of soil organic matter (cold and hot-water extractable C) and mineralisable N contents. Moreover grass roots are known to harbor high populations of specific groups of beneficial microorganisms, e.g. arbuscular micorrhizal fungi or root ectotrophic fungus *Phialophora graminicola,* which play an important role in improving plant nutrition (particularly P uptake), soil structure formation or in controlling some plant pathogens (Deacon, 1976; Martyniuk, 1987, Martyniuk et al., 1991; Błaszkowski, 1991; Wright, Upadhyaya, 1998).

In this work we compared selected microbial and biochemical characteristics of soils under temporal pasture mixtures as influenced by preceding crops and seeding rates.

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MATERIAL AND METHODS

For the purpose of this study soil samples were collected from selected treatments of a field experiment located at Grabów Experimental Station (51°20'58" N, 21°39'5" E) belonging to the Institute of Soil Science and Plant Cultivation in Puławy. In this experiment pasture mixtures (white clover-grasses) were established on three fields (sites), about 500–600 m apart, and differing with respect to cropping history (preceding crops). On field I pasture mixtures were sown after spring barley (grown after fodder grass), on field II after potatoes fertilized with farmyard manure and on field III after ploughed meadow. Fields I and II were located on a grey-brown podsolic soil and field III on a degraded black soil. Basic characteristics of these soils are given in Table 1. In the spring of 2004 mixtures of white clover seeds and grass seeds were sown at the following seeding rates: 10 mln, 20 mln and 30 mln seeds ha^{-1}, each containing either 20% or 40% of white clover seeds. Detailed information on this experiment are given by Harasim (2006, 2008). In the years 2005–2007, twice a year (in the spring – April or May, and in the fallow – October), soil cores (0–20 cm deep x 2.5 cm diameter) were

Table 1. Basic chemical characteristics of soils.

Soils	Soil organic matter [%]	pH (H_2O)	Content [mg/100 g soil DM]			
			$N-NO_3$	P	K	Mg
I[A]	1.39	6.35	2.21	7.5	14.2	2.8
II	1,27	6.60	1.76	7.6	6.6	3.0
III	2.43	7.0	7.17	4.4	3.5	6.7

A – Preceding crops: field I – spring barley, field II – potato, field III – meadow

Table 2. Numbers of colony forming units [cfu g^{-1} soil DM] of *Azotobacter* spp., total bacteria and fungi in soils under white clover-grass pasture mixtures as influenced by preceding crops and some agro-technical factors.

Preceding crops A	Agro-technical factors B	*Azotobacter* spp.	Total bacteria [cfu x 10^7]	Total fungi [cfu x 10^5]
Field I	1	6.0	2.07	1.20
	2	0	1.93	0.79
	3	2.0	1.70	1.30
	4	2.0	1.97	1.40
	mean	2.50	1.92	1.17
Field II	1	13.0	1.97	1.73
	2	7.0	1.63	1.53
	3	1.0	2.10	2.07
	4	9.0	1.77	1.47
	mean	10.30	1.87	1.70
Field III	1	5.0	1.93	1.10
	2	3.0	1.54	1.27
	3	18.0	1.67	1.51
	4	71.0	1.68	1.15
	mean	24.30	1.70	1.26
LSD for:	A	20.5	ns	ns
	B	49.1	ns	ns
	Interaction AxB	ns	ns	ns

A – see Table 1
B – agro-technical factors:
1 – 10 mln seeds ha^{-1}, 20% of white clover seeds in seeding mixture,
2 – 30 mln seeds ha^{-1}, 20% of white clover seeds in seeding mixture,
3 – 10 mln seeds ha^{-1}, 40% of white clover seeds in seeding mixture,
4 – 30 mln seeds ha^{-1}, 40% of white clover seeds in seeding mixture
ns – non significant

collected from three replicated plots of each of the selected treatments (Table 2). The soil samples consisting of 9 cores taken from each treatment were sieved through a 2 mm sieve and stored in a refrigerator (4°C). The soils were analyzed for: total numbers (colony forming units) of bacteria counted on 1/10 PCA medium (Difco) and fungi on Martin's medium (Martyniuk et al., 2001), numbers of bacteria from the genus *Azotobacter* on nitrogen-free agar medium, soil dehydrogenase activity using TTC method (Casida et al., 1964), phosphatases activity by *p*-NPP method (Tabatabai, Brenner, 1969), pH (in H_2O) and water content (gravimetrically, after drying in 105°C). Data were statistically analyzed by two-way analysis of variance (ANOVA).

RESULTS AND DISCUSSION

The soils in fields I and II were similar with respect to almost all, with the exception of K content, chemical properties, as shown in Table 1. In comparison to these soils the soil in field III contained markedly higher amounts of soil organic matter (SOM), $N-NO_3$ and Mg but lower amounts of P and K. This soil had also the highest pH in water (about 7.0). Field III was located on a meadow, which was ploughed down in 2003 and after seedbed preparation it was re-seeded with the pasture mixture in the spring of 2004. Higher contents of SOM and $N-NO_3$ in the soil of this field resulted from grass sod transformation and mineralization processes that took place after meadow cultivation. Data on yields and herbage composition of the pasture mixtures grown in these fields have been analyzed by Harasim (2006, 2008). The average yields of herbage obtained in this experiment were in the order of field I > field II > field III. The share of white clover stalks in swards was also of similar order.

Numbers of soil bacteria and fungi shown in Table 2 represent mean results of six analyses performed twice a year during three growing seasons (2005–2007). Total numbers (cfu) of bacteria and fungi in soils under pasture mixtures were not significantly influenced by the studied factors (preceding crops and seeding rates). The mean number of bacteria from the genus *Azotobacter*, which are able to fix atmospheric nitrogen, was higher in the soil under pasture mixture grown in field III, than in the soils from fields I and II (Table 2). The soils in field III was richest with respect to SOM and $N-NO_3$ contents, which resulted from transformation and mineralization of the plowed-down plant residues of previous meadow. This soil had also the pH most favorable for bacteria from the genus *Azotobacter* (Barnes et al., 2007; Martyniuk, Martyniuk, 2003). Populations of *Azotobacter* spp. in the examined soils were generally low and ranged from 0–1 cell (cfu) g^{-1} in the soil samples taken from field I and II to 71 cfu in the soil from field III. These numbers are comparable with those found in our previous study on the occurrence of *Azotobacter* spp. in Polish soils. In this survey bacteria from the genus *Azotobacter* were not detected in about 50% of Polish soil and in other soils numbers of these bacteria were generally below 100 cfu g^{-1} (Martyniuk, Martyniuk, 2003).

Table 3 shows activities of dehydrogenase and phosphatases (acid and alkaline) in soil samples collected in autumn seasons of the years 2005, 2006 and 2007. For the spring samples similar results were obtained (data not presented). The mean activities of the enzymes shown in Table 3 were the highest in the soil under pasture mixtures grown in field III, lower in the soil of field II and the lowest in the soil of field I. These differences were generally significant at $\alpha < 0.05$. Although the total numbers of bacteria and fungi in the soils under pasture mixtures were not significantly influenced, with the exception of *Azotobacter* spp. (Table 2), by chemical properties of the soils, it seems that

Table 3. Activities of dehydrogenase [µl H_2/100 g soil DM] and phosphatases [µg pNP/g soil DM] in soils under white clover-grass pasture mixtures as influenced by preceding crops and some agro-technical factors.

Preceding crops A	Agro-technical factors B	Dehydrogenase			Acid phosphatase			Alkaline phosphatase		
		2005	2006	2007	2005	2006	2007	2005	2006	2007
I	1	12.5	10.0	12.1	56.9	64.3	63.6	35.9	43.2	34.0
	2	10.6	8.5	7.5	50,3	72.8	56.4	45.3	44.1	34.5
	3	10.4	7.6	8.9	57.1	58.0	53.8	39.8	47.1	39.3
	4	9.2	9.8	10.9	56.0	68.5	57.8	38.7	44.5	40.1
	Mean	10.7	9.0	9.9	55.1	65.9	57.9	39.9	44.7	37.0
II	1	15.2	14.8	19.6	73.1	94.3	79.8	36.6	57.3	49.2
	2	13.3	11.5	17.5	73.1	98.5	76.7	36,1	40.7	39.7
	3	14.5	13.2	15.6	83.0	83.5	73.2	39.7	54.5	39.4
	4	9.2	18.2	14.9	75.9	83.9	76.4	35.7	43.0	43.4
	Mean	12.9	14.4	16.9	76.3	90.0	76.5	37.0	48.9	42.9
III	1	29.0	39.1	38.9	169.7	213.7	196.5	126.9	185.6	145.8
	2	34.9	45.1	40.3	158.8	210.3	184.1	122.9	185.2	136.2
	3	31.9	36.7	36.7	158.5	209.9	186.4	119.6	163.0	140.6
	4	34.6	42.3	40.0	152.7	198.1	181.0	140.2	183.0	152.2
	Mean	32.6	40.8	39.0	159.9	208.0	186.2	127.4	179.2	143.7
LSD for:	A	1.4	1.1	1.3	3.0	5.3	4.4	2.5	4.9	3.8
	B	1.8	1.4	1.6	3.8	6.8	5.7	3.2	6.3	4.9
	interaction AxB	3.1	2.4	2.8	6.6	11.8	ns	5.6	11.0	8.5

A, B – see Tables 1 and 2
ns – non significant

the activity of soil microorganisms might be affected by these properties. The soil under pasture mixture grown in field III containing the highest amounts of organic matter and NO_3-N displayed also the highest activity of soil microorganisms, as manifested by dehydrogenase and phosphatases activities (Table 3). There are numerous studies showing close relationships between various soil fertility indicators (SOM and nutrient contents) and soil microbial and biochemical properties (Barnes et al., 2007. Ghani et al., 2003; Kandele, Murer, 1993; Martyniuk et al., 2001). Activities of the enzymes in soils under pasture mixtures were also influenced by the agro-technical factors (Table 3) but the variability of these effects does not allow for a conclusive statement which of these factors had the most important influence on the biological activity of the soils.

Concluding, the results of this study have shown that microbial and biochemical characteristics of the soils under pasture mixtures were influenced mainly by their physico-chemical properties such as pH, organic matter and nutrient contents, but not by the studied agro-technical factors (preceding crops and seeding rates).

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The estimation of water demands of a determinate and traditional cultivars of faba bean (*Vicia faba* L.)

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Abstract. The experiment was conducted in a greenhouse of the Institute of Soil Science and Plant Cultivation – National Research Institute in Puławy, in the Mitscherlich pots containing a mixture of 5 kg of garden soil and 2 kg of sand. The first-order factors were the two faba bean genotypes: 'Nadwiślański' – the traditional type (indeterminate) and 'Tim' – a determinate type, and the second-order factor was the substrate moisture content: 30, 50 and 70% field capacity (FWC), maintained throughout the growing season.

The potted plants were irrigated using the drip irrigation precision instrument coupled with an automatic controller. During the irrigation, the quantity of given water in the pot was recorded. The shortage of water in the soil adversely affected the dynamics of (mass) accumulation by the vegetative and generative organs of both faba bean cultivars, but the stronger negative impact of drought on the yield was observed on a determinate type than on the traditional type of faba bean 'Tim'.

In the initial stage of growth and development of faba bean both forms exhibited similar water requirements. The differentiation between the water requirements of faba bean cultivars became apparent only during the generative development of field bean and lasted from the flowering to the ripening stage. The determinate faba bean cultivar needed less water during the flowering–maturing period than traditional 'Nadwiślański' but water deficit during this period resulted in greater reduction of seed yield of 'Tim' than of 'Nadwiślański'.

key words: faba beans, a determinate cultivar, a traditional cultivar, water needs, soil moisture levels, % FWC, drought stress, growth and development, yielding

INTRODUCTION

Adverse course of the weather conditions, mainly the lack of rainfall during the periods of flowering and pod setting, which are considered as critical to the growth and development of faba bean is one of the most important factors limiting the yield (Demidowicz, 1990; Jasińska, Kotecki, 1993; Michalska, 1993).

The drought occurring during this period causes falling of flower buds and flowers before fertilization, and later also the pods (Jasińska, Kotecki, 1995). As a result of those phenomena, the broad bean is a species the yields of which vary greatly from year to year (COBORU, 2008).

As faba bean yield is not a reliable and it has a relatively long period of vegetation there is not too much interest by farmers in cultivating faba bean although it is a very valuable protein plant. In this situation, the search for genotypes more resistant to drought stress becomes advisable. This issue is important because climate change during the recent years could result in increasingly long drought in spring and summer months in our country (Łabędzki, Leśny, 2008).

Thanks to considerable breeding progress determinate cultivars of bean were developed with modified morphology and differing in growth and development rate from traditional varieties (Martyniak, 1997). Previous studies showed that the determinate growth cultivars of bean give lower yield (Borowiecki et al., 1992; Kotecki, 1994) and are more sensitive to water shortage in the soil than the traditional cultivars (Podleśny, Kocoń, 2006). At the same time they produce a lower yield of biomass which indicates lower water requirements.

The aim of this study was to determine the water needs of morphological differentiated cultivars of faba bean necessary to obtain optimum seed yield and the estimation of the impact of water deficiency in soil on yield variability.

MATERIALS AND METHODS

The experiments were conducted in 2004–2006, in the greenhouse of the Institute of Soil Science and Plant Cultivation, National Research Institute in Puławy, in Mitscher-

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lich's pots containing a mixture of 5 kg of garden soil and 2 kg of sand. Faba bean cultivars were the first factor of the experiment: 'Nadwiślański' – the traditional type (an indeterminate cultivar) and 'Tim' – a determinate type, and the second factor was a substrate moisture content: 30, 50 and 70% field water capacity (FWC) maintained throughout the growing season.

The experiments were conducted in a completely randomized layout. 10 seeds were sown in each pot and after emergence, some seedlings were thinned, leaving five plants in a pot. The following fertilization (g pot^{-1}) was applied: 0.1 – N, 1,1 – P and 1,4 – K in liquid form injected into the irrigation system, on two dates – at the emergence and at the 1st–2nd leaf stage. The plants were watered using the precision instrument for the irrigation of the soil coupled with an automatic controller.

The amount of water given to the pot was recorded. The results were calculated per 40 plants m^{-2} because it is at that density that faba beans are cultivated most frequently. Detailed observations of plant growth and development were carried out during the growing season. Dynamics of the emergence was defined as the percentage of the number of germinated plants with respect to the seeds sown.

To this end, during the emergence the plants were counted at intervals of 24 hours. The measurements of plant height in the major phases of their growth and development were also performed. The plants were harvested on two dates: at the flowering stage and at full maturity. The plant height was measured and the dry matter yield of individual plant organs was determined during harvest made at the flowering stage.

During the harvest performed at the stage of full maturity plant height as well as yield and its components were determined (the number of pods, number of seeds, seed weight and moisture content). The soil was washed from the pots on a fine-mesh metal sieves in order to determine root weight. The results representing the average of the three pots were analyzed statistically using the Tukey's confidence half-interval at the significance level of $\alpha = 0.05$.

RESULTS AND DISCUSSION

The rate of germination and emergence of faba bean depended on the soil moisture. The earliest emergence and the best developed plants were on the soil with a moisture content of 50 and 70% field water capacity. On the soil with 30% field water capacity plant emergence was postponed about 3 days compared to the plants growing in the soil with higher moisture.

Number of germinating seeds and emerging plants significantly depended on the soil moisture. The poorest germination and later emergence of faba bean were observed on the soil with the lowest degree of humidity (30% FWC), and the best on the soil with 50% of FWC. Both, the decrease and the excessive increase in soil moisture content had a negative impact on the germination of seeds (Fig. 1).

The shortage of water in the soil adversely affected the growth of bean plants of both cultivars (Fig. 2) and reduced dry matter yield of the shoots (Fig. 3). The weight of stems and leaves of 'Nadwiślański' cultivar grown in the soil with a moisture content of 50 and 70% FWC was greater than the weight of faba bean growing in soil with a moisture content 30% of FWC respectively by: 34 and 47.5%; for 'Tim' cultivar these values were respectively: 53.8 and 55.4% .

The opposite was true of root weight. The underground mass of faba bean increased significantly along with decreasing soil moisture. This is explained by plant need to develop a longer root system which will be able to reach deeper into soil under water deficit conditions (Starck, 2002). Faba bean cultivar 'Nadwiślański' at all three levels of soil moisture produced a larger root system than did 'Tim' cultivar.

The mass of vegetative and generative organs of faba bean at the ripeness stage depended significantly on soil moisture. The smallest mass of leaves, stems, hulls, seeds and roots was produced by two cultivars of faba bean grown in the driest soil (Fig. 4). It is worth noting that, apart from the mass decrease of the aerial part, the weight of the root sys-

Table 1. Length of interstages periods of faba bean.

Development stages	Nadwiślański			Tim		
	soil moisture [% FWC]					
	30	50	70	30	50	70
Germination − seedling	9	6	6	9	6	6
Seedling – 2–3 leaf stage	18	18	18	18	18	18
2–3 leaf stage – 5–6 leaf stage	24	24	24	24	24	24
5–6 leaf stage – flowering	12	14	16	12	14	16
Flowering – pod setting	11	14	15	9	11	12
Pod setting – seed filling	12	15	16	8	10	12
Seed filling – full maturity	21	24	27	18	23	24
Total	107	115	122	98	106	112

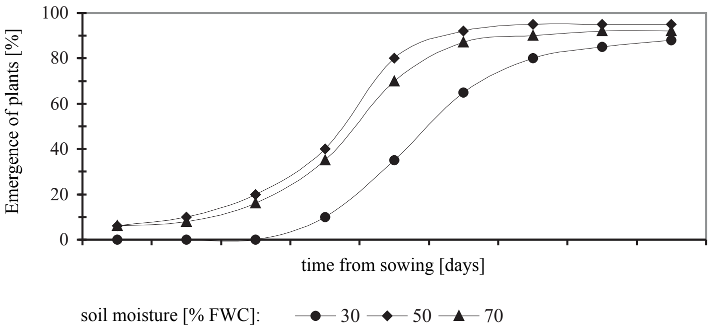

Fig. 1. Emergence of faba bean plants in terms of soil moisture.

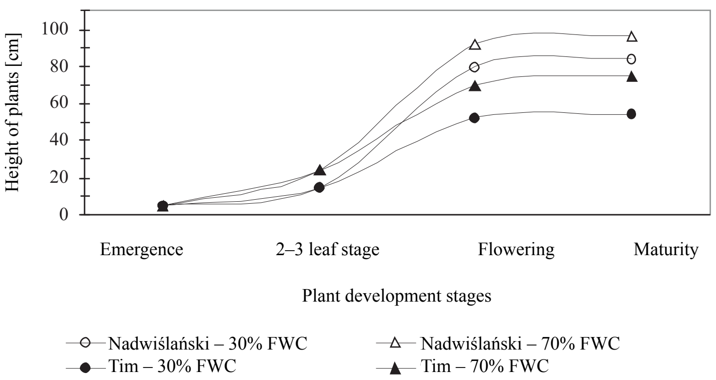

Fig. 2. Height of faba bean plant in dependence of soil moisture.

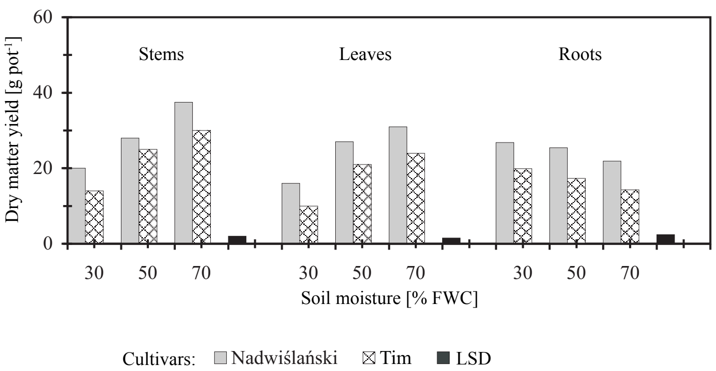

Fig. 3. Yield of dry matter yield of faba bean organs in flowering period.

tem declined as a result of insufficient soil moisture. This should be explained by the earlier maturing of the plants growing under conditions of water deficit and the related early necrosis and decomposition of root system.

The impact of soil moisture on plant growth and development was observed. The duration of some phenological phases of faba bean was shortened along with a reduction of soil moisture. Plants cultivated on soil with lowest degree of moisture (30% FWC) began flowering after 63 days, pod setting after 73 days and reached maturity after 103 days from germination, while for plants grown on soil with moisture 70% FWC these values were: 64, 78 and 117 days respectively (means for both cultivars; Table 1). Earlier flowering of the plants growing under stress caused mutual competition for water, light and nutrients was also observed by Kotecki (1990) and Podleśny (1994) for other crop species.

Water shortage-dependent yield reduction observed at full maturity was, just as that occurring at the flowering stage, more pronounced in cv. ‘Tim’ than in cv. ‘Nadwiślański’.

Higher yields of seeds and pod husks were provided by plants of both cultivars grown under conditions of greater soil moisture.

However, the differences in the yield of generative organs due to reduction of the soil water content were significantly lower in the case of a traditional bean cultivar ‘Nadwiślański’ than a determinate faba bean cultivar ‘Tim’. The reduction of seed yield as a result of a water content decrease from 70 to 50 and 30% FWC of ‘Nadwiślański’ cultivar was 16.3 and 38.8% respectively, and of ‘Tim’ cultivar 20.0 and 53.4%.

The lower ratio of root mass to the aboveground parts also confirms reduced resistance to drought of ‘Tim’ compared to ‘Nadwiślański’ variety (Podleśny, 2001). According to Grzesiak et al. (1997) it is one of the most important indicators largely determining the resistance of plants to drought stress, taken into account in the breeding of cultivars with increased tolerance to drought (Hurd, 1974; Tardieu, Katerji, 1991).

The greater sensitivity of traditional cultivars than that of determinate ones to the shortage of water in the soil is not a rule that applies to all leguminous species. The research of Bieniaszewski et al. (2003) showed that some determinate cultivars of yellow lupine are more resistant to drought than traditional ones.

The reduced yield was a consequence of a significant reduction in the number of pods per plant and number of seeds from one plant (Table 2) while the number of seeds per pod, considered as a varietal characteristic did not change significantly (Xia, 1997). The research of Sammler et al. (1982) and Grzesiak et al. (1989) showed that during drought the bean plants may shed flowers, or even set pods, which, in consequence, results in reduced seed yield.

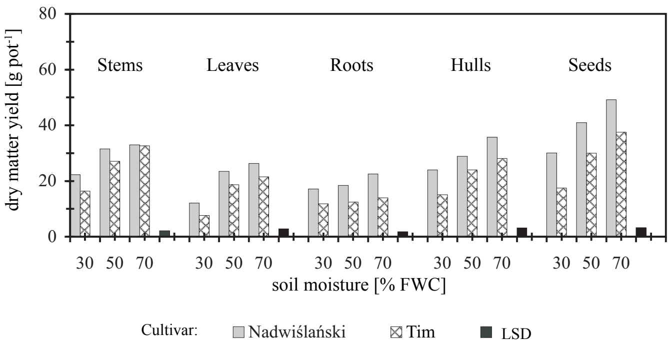

Fig. 4. Yield of dry matter of faba bean plants at maturation period.

Table 2. Components of seed yield of faba bean.

Cultivar	Soils moisture [% FWC]	Number of pods per plant	Number of seeds per pod	Number of seeds per plant	Weight of 1000 seeds [g]
Nadwi-ślański	30	4.6 a	3.1 a	14.4 a	446 a
	50	5.7 b	3.3 a	18.6 b	458 a
	70	6.4 b	3.1 a	19.8 c	459 a
Tim	30	4.0 a	2.4 a	9.6 a	434 a
	50	6.5 b	2.2 a	14.5 b	440 a
	70	6.1 b	2.5 a	15.3 b	440 a

Numbers in columns marked with the same letters do not differ significantly

The authors of other studies suggested that drought may reduce the density of pods per faba bean plant by almost 65% (Mwanamwenge et al., 1999) and decrease seed yield by up to 70% (Lopez et al., 1996). Podleśny (2003) and Bieniaszewski et al. (2003) showed in their previous studies the similar relationships with regard to the yellow and white lupine.

The 'Nadwiślański' cultivar of faba bean needed about 29% more water to produce the optimum yield of vegetative and generative organs than did the cultivar 'Tim' (Table 3). Meanwhile, the research results presented in this study also showed that the 'Nadwiślański' cultivar of faba bean is less sensitive to water deficit in the soil.

It' can therefore be assumed that the 'Tim' cultivar of bean, because of its smaller biomass of organs, consumes less water during the growing season but its weaker root system caused that it requires a more uniform water supply. The long-term water shortage in the soil reduces its yield more severely comparing to the traditional cultivar 'Nadwiślański'.

It is worth noting that the calculated amount of water for the whole period of vegetation is very similar to that obtained in Dzieżyc's studies (1989). It is very interesting because the study of Dzieżyc (1989) was conducted under field conditions, whereas the presented results came from pot experiments.

Table 3. Water requirements of faba bean during the vegetation period.

Month	Nadwiślański		Tim		According to Dzieżyc (1998)
	dm^3 per plant	mm m^{-2}	dm^3 per plant	mm m^{-2}	
April	0.87 a	34.6 a	0.87 a	34.6 a	39.4
May	1.67 b	66.8 b	1.67 b	66.8 b	69.9
June	2.24 c	89.6 c	1.81 b	72.6 b	85.7
July	2.44 c	97.5 c	1.86 b	74.3 b	90.8
August	1.60 b	63.9 b	0.61 a	24.3 a	61.0
Total	8.82	352.4	6.82	272.6	346.8

Numbers in columns marked with the same letters do not differ significantly

Table 4. Water consumption by faba bean plants at different development stages ($dm^3 \cdot pot^{-1}$).

Development stage	Nadwiślański			Tim		
	soil moisture (% FWC)					
	30	50	70	30	50	70
Germination − seedling	0.64 a	1.04 a	2.23 b	0.64 a	1.04 a	2.23 a
Seedling – 2–3 leaf stage	0.82 b	1.54 a	2.87 b	0.82 a	1.54 a	2.87 a
2–3 leaf stage – 5–6 leaf stage	0.65 a	1.11 a	1.87 a	0.65 a	1.11 a	1.87 a
5–6 leaf stage – flowering	0.91 a	1.25 a	2.73 b	0.91 a	1.25 a	2.73 a
Flowering – pod setting	3.40 c	4.54 c	7.43 c	1.54 b	3.24 b	5.44 c
Pod setting – seed filling	1.49 b	2.84 b	6.11 c	1.34 b	4.64 c	9.13 d
Seed filling – full maturity	3.54 d	4.66 c	9.53 d	2.42 c	2.91 b	4.00 b
Total	14.03	23.14	44.12	10.46	17.45	33.96

Numbers in columns marked with the same letters do not differ significantly

The research results obtained by Dzieżyc (1989) probably are related to the traditional bean cultivars which were widely used in agricultural research at that time. Many cultivars of bean with determinate growth are nowadays, on the "National list of cultivars" which increases their importance and justifies that line of research.

There is a lack of studies on determining the water needs of differentiated forms of faba bean at different stages of plant growth and development in literature. Most authors (Demidowicz, 1990; Grzesiak et al., 1989; Sammler et al., 1982) indicate high water needs of plants during flowering and pod setting, but these observations relate to traditional forms of faba bean or faba bean as a species without taking into account its morphologically diverse genotypes.

The data in Table 4 shows that both faba bean cultivars had a similar water demand in the initial period of growth and development. The differentiation between the water requirements of faba bean genotypes studied were revealed practically during the generative development of faba bean and lasted from flowering to ripening stage.

Generally it can be concluded that the determinate cultivar needed less water during flowering stage (considered as a critical) than the traditional cultivar 'Nadwiślański'. On the other hand the deficit of water in that period resulted in a greater reduction of yield of the determinate cultivar 'Tim' than in cv. 'Nadwiślański'.

The strong convergence of obtained results concerning their water needs with the data achieved by Dzieżyc (1989) from the faba bean cultivated in the field conditions shown in the present study suggests that similar water needs depending on the different forms in the particular stages of growth and development of faba bean are present also in the field conditions.

CONCLUSIONS

1. The faba bean plants growing in soil with lowest water content were shorter and some of their phenological stages were altered: they began the flowering, pod setting, and maturity earlier than the plants growing in the highest soil moisture conditions.
2. The reducing of the water content in the soil strongly inhibits the development and yielding of faba bean cultivars. 'Tim' – a form of the determinate growth type gave the lowest yield when grown in the soil with the lowest moisture conditions. The best yield was gained from the indeterminate cultivar 'Nadwiślański' in optimum moisture content of 70% FWC.
3. The reduction of the seed yield obtained from plants growing under conditions of low soil moisture resulted from the significantly lower density of pods per plant and the smaller amount of seeds from a single plant.
4. Both faba bean forms exhibited similar water requirements in the initial period of growth and development. The differentiation between faba bean genotypes studied concerning the water requirements was revealed only during the generative development of faba bean and lasted from the flowering to ripening stage. 'Tim' cultivar needed less water than the 'Nadwiślański' during the flowering-maturing stage, but the water shortage in this period resulted in greater reduction of seed yield of 'Tim' cultivar than it did in that of 'Nadwiślański'.

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Diversity in symbiotic specificity of bacterial strains nodulating lupins in Poland

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Abstract. Bacteria isolated from root nodules of yellow lupin (*Lupinus luteus*), white lupin (*L. albus*), and blue lupin (*L. angustifolius*) were analyzed for their symbiotic specificity. Although these plant species belong to the same cross-inoculation group, they are not always nodulated by the same bacterial strains. There is a certain level of species and cultivar nodulation specificity among analyzed bacterial population. This characteristic is particularly expressed in bacterial populations isolated from white lupin. Serradella (*Ornithopus sativus)* can be nodulated by several analyzed strains of bacteria of both types (fast and slow growing) from root nodules of lupins, but many strains did not induce nodules on serradella roots, or nodules were not effective. Birdsfoot trefoil (*Lotus corniculatus*) is also classified in the same cross-inoculation group with lupins and serradella. Although the analyzed bacterial population has some potential for creating symbiotic systems with *L. corniculatus*, it is not a common phenomenon among lupin microsymbionts and is rather due to a large heterogeneity of the population.

key words: lupin, rhizobia, nodulation, symbiosis, serradella

INTRODUCTION

Lupins are legumes which have been cultivated in Europe for the last 2000 years, used in human and animal feeding, as green manure in agriculture (Rosolem et al., 2002; Jensen et al., 2004) and in soil stabilization. This plant is currently considered a good alternative as an animal foodstuff due to the high quality of its proteins (Erbas et al., 2005; Faligowska et al., 2007). Lupin seeds are a rich source of functional components that are found in modern food. They may be nutritional and non-nutritional compounds and have a positive effect on health, physical development and wellbeing (Lampart-Szczapa, Łoza, 2007). A substantial portion of the world's supply of organic nitrogen is fixed via the symbiosis between root nodulating rhizobial bacteria and leguminous host plants (Postgate, 1998). This association is generally assumed to be mutualistic, but rhizobial strains vary in effectiveness (Burdon et al., 1999) and ineffective bacteria are widespread, indicating that cheating may occur. Therefore there is increasing interest in these plants to be used in sustainable agriculture due to its high potential to provide protein without nitrogen fertilization, estimated at 150–200 kg of nitrogen per ha in symbiosis with rhizobia (Robinson et al., 2000). Using the optimal bacterial strain is critical to obtain the expected results of nitrogen fixation in agricultural practice.

Root-nodule bacteria comprise several distantly related proteobacterial lineages, most notably the genera *Azorhizobium, Bradyrhizobium, Mesorhizobium, Rhizobium*, and *Sinorhizobium* (Sawada et al., 2003), that have acquired the ability to form nodules on legumes and symbiotically fix nitrogen. Lupins, like many other species belonging to the *Leguminosae*, are able to initiate a symbiotic relationship with bacteria of the family *Rhizobiaceae*. In the current taxonomy of this family, there is no separate entity comprising strains nodulating lupins. Different species of lupins (*Lupinus*) and serradella (*Ornithopus*) are effectively nodulated by both slow-growing strains classified within *Bradyrhizobium*, as well as the fast-growing strains of *Rhizobium* and *Mesorhizobium*. Lupin microsymbionts have been characterized to a much lesser extent than, for example, populations of *B. japonicum* or *R. leguminosarum*. Despite the interest of this symbiosis there are few studies about the identity of strains nodulating lupins (Barrera et al., 1997; Stepkowski et al., 2005; Andam, Parker, 2007). The strains isolated to date from effective nodules of lupins in different countries belong rather to the genus *Bradyrhizobium* (Barrera et al., 1997; Rivas et al., 2009). The results showed that the lupin endosymbionts belong to several chromosomal lineages within the genus *Bradyrhizobium* that could represent new species

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of this genus. Among a group of these bacteria *L. albus* bradyrhizobia constitute a lineage that could represent an allelic group present up to date only in within microsymbionts of this species (Velázquez et al*.,* 2010)

The aim of this study was to analyze the nodulation specificity of lupin microsymbionts population isolated from three different species of lupins cultivated in Poland.

MATERIALS AND METHODS

***Lupinus* plants for sampling of rhizobial bacteria and strains isolation.** Field sites from 16 geographical locations in Poland were examined with the goal of isolating and characterizing the indigenous strains of *Rhizobiaceae* able to nodulate lupins plants. A total of 235 strains were isolated and 167 of them were taken for further biochemical characterization. The strains were isolated from 18 varieties of three species of *Lupinus*: 8 varieties of yellow lupin (*Lupinus luteus*), 6 varieties of narrow leaf lupin (*L. angustifolius*) and 4 varieties of white lupin (*L. albus*). The strains originated from places of different lupin cultivation history. At the locations of the plant breeding stations (Nowa Wieś Zbąska, Przebędowo, Wiatrowo) lupins were present often for several years or even yearly (Wiatrowo). At the other locations (Głębocko, Długa Goślina, Lipnica, Gurówko) lupins were not grown for at least 7 years before. On all the places no artificial inoculums were used and all isolated plants contained root nodules. Fifty-eight strains from the entire population were taken to the nodulation tests.

Isolation of rhizobia from nodules. Plant roots were washed with tap water until no soil particles were apparent. Plants were dried lightly with paper towels. Nodules were dissected from roots using scalpels and forceps, and dissected nodules were immediately placed in presterilized centrifuge tubes. Dissected nodules were surface sterilized in 0,1% $HgCl_2$ solution for 2 minutes and then rinsed three times in sterile distilled water. Nodules were then individually crushed with a flame-sterilized glass rod and each resultant slurry was streaked onto two replica plates containing 25 ml of solid AG medium (Somasegaran, Hoben, 1994).

Type of metabolism. One of the distinctive characteristics of the bacteria belonging to the different genera of *Rhizobiaceae* is their influence on culture medium pH (Brenner et al., 2005). Bacterial colonies were grown on Petri dishes on YM agar solid medium (Somasegaran, Hoben, 1994) with 0,5% (ethanol solution) of brome thymol blue (BTB) as pH indicator. Initially the YM medium was adjusted to pH 6.8 (green color). Bacterial growth caused changes of the medium pH as well as color of the pH indicator (yellow when lowering pH and blue when the pH was raised). Each strain of bacteria were inoculated on 3 separate Petri dishes by four-quadrant streaking method and placed in a dark incubator at a temperature of 28^{o}C. After 5 days observations of the growing medium color were carried out. They were considered positive for cases in which a clear bacterial growth was observed, and if at least 2 of 3 repetitions for each strain showed an identical and distinct color change of culture medium.

Relative growth rate of isolates is another important distinctive characteristic of the bacteria belonging to the different genera of *Rhizobiaceae* (Brenner et al., 2005). Relative growth rate of isolates was analyzed in the solid medium. Three groups of isolates with different relative growth rate at a temperature of 28^{o}C were distinguished. Strains classified as slow growing showed single colonies after 3 days, after 5 days colonies were 1–2 mm in diameter. Intermediate strains showed visible growth after 2 days and after 3 days colonies were well formed (ca. 2 mm). Fast growing bacteria showed well formed colonies after 2 days while after 3 days some colonies were joined and difficult to distinguish.

Analysis of nodulation specificity was performed in three independent experiments: on the perlite-vermiculite solid medium on Leonard jars (1st and 2nd experiment) and on an artificial agar slants (3rd experiment) (Somasegaran, Hoben, 1994). Nodulation analyses of bacterial isolates in the greenhouse experiments were carried out using sterilized Leonard jars with a liquid nitrogen free Dilworth medium at pH of 6.8 (setup of Leonard jar is presented on Fig. 1). As the culture medium a mixture of perlite and vermiculite in a 1:3 ratio by volume was used. Seeds were sown in three places in each Leonard jar at a depth of about 1.5 cm. For a single jar the seeds of one plant species analyzed were sown. In each of the three places two seeds of the species were sown. Seeds were infected with bacterial culture in an amount of 1.5 ml of culture in place (two seeds). Single jar was inoculated by the culture of one bacterial isolate. Seeds were covered with a layer of growing medium, and then the entire surface covered with 1.5 cm layer of paraffin coated sand, in order to avoid possible cross-infection during the growing season. At the stage of first leaves just visible one plant of every pair was cut off leaving single plant in every of three places per one Leonard jar. Four repetitions of each combination of plant species x bacterial isolate were used. Leonard jars with biological material were placed in the greenhouse for a period of 35 days. Nodulation specificity analysis of selected bacterial isolates to small-seed legume plants was carried out using the nitrogen free Dilworth medium solidified with agar in the form of slants in glass tubes. Test tubes used were 200 mm of length and 20 mm crosswise. Serradella (*Ornithopus sativus*) and birdsfoot trefoil (*Lotus corniculatus*) were grown on agar slants. Before sowing the seeds were sterilized and scarified as described bellow. Before

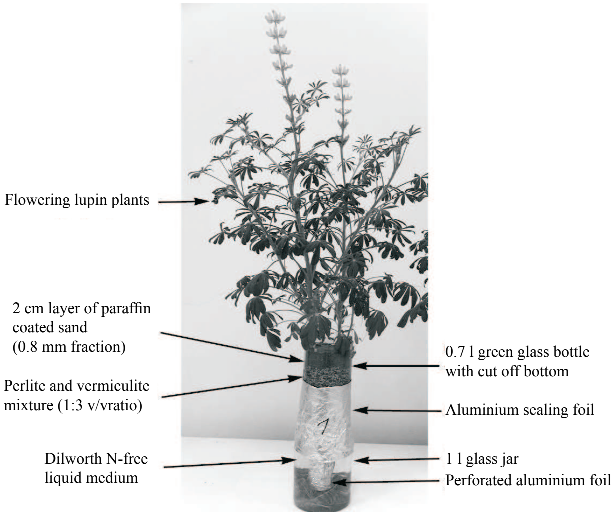


Fig.1. Setup of Leonard jar used for nodulation tests in the greenhouse experiments.

laying on slants the seeds were germinated. Germination was on Petri dishes with agar (in H_2O) in a dark incubator at a temperature of 28°C. Seeds were infected with bacterial culture of 1 ml of culture per tube. Ten repetitions of each combination of plant species x bacterial isolate were used. Photoperiod used: 16 hours artificially lighted and 8 hours darkness.

Seeds sterilization. In the greenhouse experiment three species were grown: yellow lupin (*Lupinus luteus*), serradella (*Ornithopus sativus*) and soybean (*Glycine max* (L.) Merr.). The surfaces of seeds were sterilized before sowing using different procedures, depending on plant species: a) sterilization of lupins and soybeans: Seeds were washed twice with distilled water and placed for 10 min. in a solution of mercuric chloride, and then washed five times with plenty of sterile distilled water; b) seeds of serradella were washed twice with distilled water and placed for 15 min. in concentrated sulfuric acid and then washed at least 10-fold with plenty of sterile distilled water.

Plant seeds used. *L. albus* cv. Wat, *L. luteus* cv. Lidar and cv. Ventus, *L. angustifolius* cv. Sonet and *O. sativus* cv. Igela and cv. Mazurska biała (all above from Poznan Plant Breeding Station in Tulce); *Lotus corniculatus* cv. Skrzeszowicka (from Plant Breeding Station Nieznanice); *Glycine max* (L.) Merr. Var. Nawiko (from Dept. of Genetics, Univ. of Life Sci., Poznan)

RESULTS AND DISCUSSION

There is no major problems with lupins nodulation in the field crops in Poland because many of microsymbiont strains are endogenous and they have the capacity to survive in soils for many years in the absence of their host-plants (Martyniuk et al., 2005). For all field collected plants, root nodules were observed. But their nodulation and nitrogen fixation effectiveness is still unknown. The studied strains were previously analysed and showed large variations in the structure of the population. The high diversity of the lupin microsymbionts were shown by both biochemical as well as molecular analyzes (Pudelko, 2010).

In the group of strains presented herein, two types of metabolism were observed: characteristic of slow-growing bacteria of the *Rhizobiaceae* family (mainly of the *Bradyrhizobium* genus) metabolism, resulting in alkalinization of culture medium, as well as typical for the rapidly growing *Rhizobium* coupled with the acidification of culture medium (Table 1). Interestingly while typical for *Rhizobium* are fast-growing strains, alkalizing *Bradyrhizobia* are usually slow-growing. In the analyzed population all the combinations of growth rate and metabolic type were represented.

The ability of the tested strains to nodulate selected legume species was analyzed. Nodulation of yellow lupin (var. Ventus), soybean and serradella were tested in the first greenhouse experiment in Leonard jars. Serradella has been classified in the same group of nodulation specificity (cross-inoculation group) as lupins, while soybean is nodulated by *Bradyrhizobia* closely phylogenetically related to some of the lupins microsymbionts (Table 2). Nodulation of small-seed leguminous plants – serradella and birdsfoot trefoil were analyzed also on agar slants in the growing chamber.

Table 1. Percentage of isolates representing different metabolism type and different relative growth rate within analyzed population.

Type of metabolism	Relative growth rate of isolates			Total
	slow	interme-diate	fast	
Acidifying strains	17.24%	22.41%	**37.93%**#	77.59%
Alkalizing strains	**10.34%**##	8.62%	3.45%	22.41%

\# typical characteristic for *Rhizobium*
\## typical characteristic for *Bradyrhizobium*

Table 2. Nodulation ability of bacterial strains isolated from three species of lupins with yellow lupin, serradella and soybean as potential plant hosts.

Source host plant for inoculum strains	Yellow lupin (*L. luteus* cv. Ventus)	Serradella (*O. sativus* cv. Mazurska biała)	Soybean (*G. max.* cv. Nawiko)
L. luteus	81.82%	71.43%	0.00%
L. angustifolius	60.00%	21.43%	0.00%
L. albus	42.86%	16.67%	0.00%

Greenhouse experiment showed considerable variation among isolates in their nodulation capability.

Depending on the primary host plant species serving as a source for the bacterial strains we could observe important differences in the nodulation ability within tested population. As many as 81,82% of the bacteria originated from yellow lupin were able to initiate symbiosis with the plants of the same kind while only 42,86% of the strains isolated from white lupin nodulated yellow lupin. That confirms previous observations suggesting higher species specificity among *L. albus* microsymbionts (Rivas et al. 2009). The similar scheme, although at a lower level, we could observe when serradella was used as a host plant. The highest nodulation frequency was for strains originally isolated from yellow lupin, while the lowest from white lupin. We observed strains capable of simultaneous nodulation of yellow lupin and serradella, which confirms the possibility of classifying these two species in the same cross-inoculation group. But there are strains in the analyzed population (mainly isolated from roots of blue lupin) capable to nodulate yellow lupin, which do not induce nodules on the roots of tested serradella plants. Therefore, it seems that not in every experimental case can serradella replace lupin plants in nodulation and nitrogen fixation performance studies. That would suggest, that among all strains capable of lupins nodulation the most versatile are these infecting *L. luteus* plants in the field conditions.

Interestingly, in several cases lack of nodules on the roots of lupins was observed, despite the bacterial strains used as inoculum were isolated from the roots of plants of this kind. However, in the presented nodulation analysis only one variety (Ventus) of one species of lupin (*L. luteus*) was used. That would suggest some level of cultivar specificity among analyzed population. There were no nodulation cases in the analysed population, when soybean was used as the potential host plant. It seems that, despite the high phylogenetic relationship of *Bradyrhizobium japonicum* (soybean microsymbiont), and some bacteria nodulating lupins (Barrera et al. 1997), the latter do not have the capacity to initiate symbiosis with soybeans.

This suggested species specificity for nodulation was confirmed in the second experiment, when total number of nodules, as well as fresh weight of nodules were analyzed. For this experiment we have chosen (from the previous test) six strains capable to effectively nodulate yellow lupin cv. Ventus and representing all six rhizobia metabolic types. The following plants were tested: *L. albus* cv. Wat, *L. luteus* cv. Lidar and cv. Ventus, *L. angustifolius* cv. Sonet.

It is reported that total nodule weight related positively with the quantity of nitrogen fixed, even better than total number of bacteroids of the effective bacterial strain (Wadisirisuk, Weaver, 1985). Data presented in Fig. 2 shows high variability in total nodule weight and hence

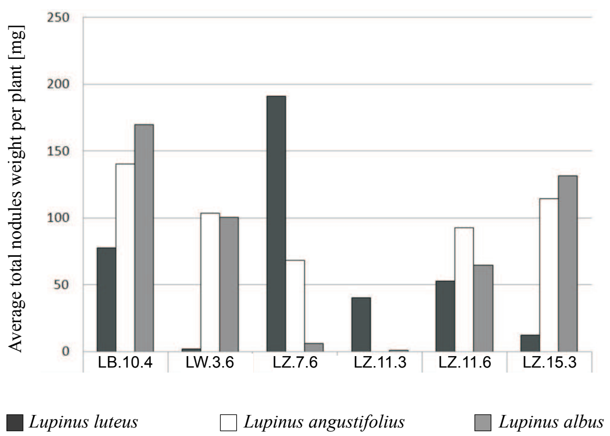
Table 3. Metabolic characteristics of the stains used in 2nd and 3rd experiment.

Type of metabolism	Relative growth rate of isolates		
	slow	intermediate	fast
Acidifying strains	LZ.15.3	LW.3.4	LB.10.5
Alkalizing strains	LZ.7.6	LZ.11.3	LZ.11.6

an estimated nitrogen fixation rate. There is also a certain level of bacterial strain specificity to the plant species.

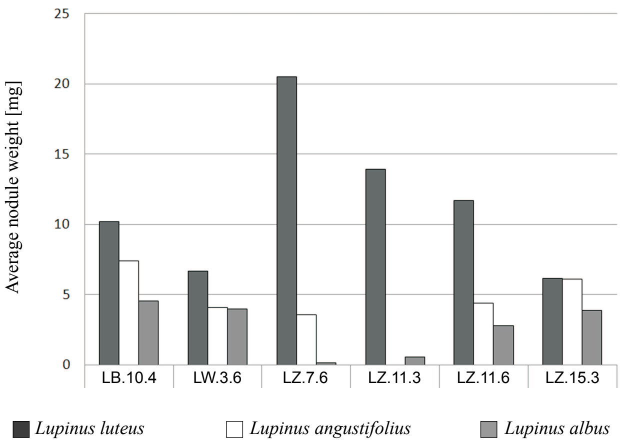
Strain LZ.7.6 (obtained from *L. luteus*) induced nodules weighing 191.22 mg when yellow lupin was inoculated during the experiment. It is almost three times more comparing to blue lupin and 30 times more comparing to white lupin used as a host plants in the nodulation tests with the same strain. The effect was opposite with strain LB.10.4 obtained from roots of white lupin.

After the initial infection, nodules grow and harbor increasing populations of bacteria until the nodules senesce and the rhizobia are released into the soil. However, rhizobial effectiveness in nodules is not always guaranteed. Host species differ in the type of nodules they form, and this can determine the degree to which differentiated bacteroids can repopulate the soil (Denison, 2000; Mergaert et al., 2006). Furthermore, some legumes can hinder the growth of nodules with ineffective rhizobia, thus punishing uncooperative symbionts (Simms, Taylor, 2002). Plants might modulate resource allocation or impose sanctions on individual nodules. Indeed, a recent model by West and colleagues (West et al., 2002) predicts that high rates of N-fixation by rhizobia can be maintained only if plants allocate resources to nodules on the basis of the N-fixation rate of their occupants. While many physiological and biochemical



The strains designation is based on their source host plant (LB – *L. albus*, LW – *L. angustifolius* and LZ – *L. luteus*)

Fig. 2. Average total nodules weight per plant of three lupin species induced by six different bacterial strains.



The strains designation is based on their source host plant (LB – *L. albus*, LW – *L. angustifolius* and LZ – *L. luteus*)

Fig. 3. Average nodule weight on roots of three lupin species induced by six different bacterial strains.

mechanisms might be involved in sanctions or differential allocation, these mechanisms should all produce a consistent phenotype in nodules occupied by ineffective bacteria: small size (Simms, Taylor, 2002).

Nodules smaller than normal are generally an indication of infection by an ineffective or less effective strain of rhizobia. In the analyzed group of strains we could also observe the meaningful differences in the nodule sizes in respect of plant species and strain used as inoculums. The most specialized strains (LZ.7.6, LZ.11.3 and LZ.11.6 in Fig. 3) induced the formation of the biggest nodules on their host plant, while on two other lupin species the nodules (if created) were of marginal size.

Third experiment of nodulation potential carried out on agarose slants generally confirms that in the studied population of bacteria isolated from lupin roots there exists an ability to initiate symbiosis with serradella (Table 4). To some extent, although with a significantly lower frequency, lupin microsymbionts are able to initiate symbiosis also with birdsfoot trefoil.

Attention can be put to the relatively high frequency of ineffective root nodules. The term „ineffective" describes the nodules which do not contain a noticeable amount of leghemoglobine. The emergence of these "white" nodules confirms the thesis of a limited "compatibility" of lupins nodulating bacteria to serradella. A similar situation to that, occurring with greater intensity, can be observed in systems with birdsfoot trefoil used as a potential host of the symbiosis.

It is worth to note that in this experiment we could observe the presence of noneffective nodules on the serradella roots in systems where strains effectively nodulating lupins rather than serradella were, in the pot experiment. We could suppose that regulated and optimal conditions of temperature and lighting can foster the adoption by the plant of even unauthorized bacterial symbiosis partners, on the other hand, continuous contact of a large number of bacterial cells to plant roots without the competitive impact of biotic and abiotic environment may increase the likelihood of this kind of biological artifact in the very artificial environment in the test tube with agar slants.

Understanding and measurement of the field population complexity can lead to obtaining a high proportion of nodule occupancy by applied inoculant strains under these conditions. As both, the strain richness and genetic diversity of rhizobial populations associated with a given host legume, is likely to vary between sites, selected host, strain and management practice combinations aimed at improving nodule occupancy by inoculant strains would need to be screened for effectiveness. For legume species that harbour inherently diverse populations of rhizobia in their nodules, it may be preferable to select crop varieties that nodulate effectively with the resident rhizobia (McInnes, Haq, 2003), rather than attempting to manipulate the competitive ability of introduced inoculant strains. Nodule induction at high frequency by introduced inoculant strains has been readily demonstrated in soils where indigenous rhizobia are deficient. However, most inoculated legume seed is sown into soils containing established rhizobium populations, and in these situations there are reports of inoculant strains inducing the majority of nodules in the first year and of their progressive disappearance and replacement by indigenous rhizobia in succeeding years. In other instances, indigenous rhizobia were a barrier to the successful introduction of inoculants, resulting in low levels of establishment of the applied strains in the year of inoculation. Therefore, an understanding of the nature of indigenous populations of rhizobium, of the factors that affect their distribution and dynamics, and of their role in

Table 4. Nodulation ability of bacterial strains isolated from three species of lupins with serradella and birdsfoot trefoil grown on agar slants. Percentage of nodulated and not nodulated plants.

Primary host plant for inoculum strains	Serradella			Birdsfoot trefoil		
	effective nodules	nonefective nodules	no nodules	effective nodules	nonefective nodules	no nodules
L. luteus	75.00%	16.67%	8.33%	33.33%	50.00%	16.67%
L. angustifolius	33.33%	41.67%	25.00%	8.33%	16.67%	75.00%
L. albus	20.00%	20.00%	60.00%	20.00%	0.00%	80.00%

inoculant strain competition and persistence is of considerable agricultural significance.

CONCLUSIONS

1. Lupins are nodulated by both fast-growing as well as by slow-growing strains. In the analyzed population majority (more than 77%) is constituted by isolates representing growth rate typical for *Rhizobium*.

2. Lupins (*L. luteus, L. angustifolius* and *L. albus*) are cross nodulated by strains of bacteria isolated from root nodules of yellow lupin, white lupin and blue lupin. However, they form a common cross-inoculation group, species nodulation specificity can be observed. This characteristic is particularly expressed in bacterial populations isolated from white lupin.

3. Serradella is nodulated by several analyzed strains of bacteria from root nodules of lupins of both types (fast and slow growing), but as much as 50% of the strains studied did not induce effective nodules on the serradella roots in the glasshouse experiment. In test tubes nodulation analysis showed that 24% of strains do not produce root nodules, and a further 27% of the strains induced inefficient roots nodules on serradella. These observations confirm that lupins and serradella can be classified within the same cross-inoculation group but they also call into question the usefulness of serradella (no doubt more easily grown in the laboratory) as a substitute of lupins in the studies of nodulation and nitrogen fixation efficiency.

4. *Lotus corniculatus* is also classified in the same cross-inoculation group with lupins and serradella. Presented work shows however that classification should be regarded rather as conditional and not absolute. Although the analyzed bacterial population has some potential for creating symbiotic systems with birdsfoot trefoil, it is not a common phenomenon among lupin microsymbionts and is rather due to a large heterogeneity of the population.

5. Soybean, despite reports of high phylogenetic relationship between its microsymbiont (*Bradyrhizobium japonicum*) and bradyrhizobia nodulating lupins, is probably not nodulated by the same strains as lupins and other species belonging to the same group of nodulation specificity. There was not a single case of inoculation, resulting in the formation of symbiotic system involving strains belonging to the analyzed population.

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Yielding of *Festulolium braunii/Trifolium pratense* mixture, depending on the proportion of mixture components

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Abstract. Potential of legume-grass mixtures production exceeds total yield of each component. The yield depends on species/cultivars composition as well as on their participation in sowing mixture. The aim of this study was to assess the effect of red clover cultivar and its proportion of the seeding mixture on the yield of mixtures with *Festulolium*.

A field experiment was conducted in two three-year series in 2005–2007 and 2006–2008 at the Institute of Soil Science and Plant Cultivation – Agricultural Experimental Station Grabów (51°21' N; 21°40' E), in a split-plot design. Two factors were tested: percentage of red clover seeds in mixture (40, 60, 80% compared to pure sowing), and clover variety: Nike (2n), Parada (2n), Bona (4n), Jubilatka (4n). Sulino cultivar of *Festulolium* was used in all mixtures.

The botanical composition of sward was identified during the study. Dry matter yields and crude protein yield from 1 ha were determined. The results were statistically analysed in conformance with the design of experiment. The significance of differences was examined using Tukey's test at $\alpha = 0.05$.

Festulolium is a species suitable for the mixtures with red clover. The yields of dry matter and crude protein of mixtures depended significantly on the share of components. The mixture with the share of 80% of clover seeds and 20% of festulolium proved to be the best in terms of the yield. The least advisible was the mixture containing 40% of red clover and 60% of festulolium. Tetraploid varieties of red clover are more useful for mixtures with festulolium, especially under favourable weather conditions.

key words: legume-grass mixtures, mixture composition, *Festulolium*, diploid cultivars, tetraploid cultivars

INTRODUCTION

The introduction of grasses and legumes for crop rotation aims to improve the forage base for the animals, and simultaneously reduces costs of feed production (Grzegorczyk, 1999; Goliński, 1998). It also takes into account the need for maintaining the biological balance of ecosystems, which is the fundamental principle of sustainable development of agriculture (Duer, 1999; Nowak, Sowiński, 2007; Søegaard et al., 2007). Production potential of legume-grass mixtures is greater than that of a monospecific crop of each component. The formulation of appropriate recommendations concerning the composition of mixtures, tillage treatment and usage systems is difficult, due to the high variability of the effects, induced by the interaction between habitat and plants in the sward (Jelinowska, Staniak, 2007; Zannone et al., 1986). Maintaining of appropriate proportion of grasses and legumes can be obtained, among others, by proper selection of species and varieties, and their participation in the sowing mixture. The optimal performance and quality of green forage is provided by clover-grass mixtures, which, according to different authors, should contain from 30–50% of clover and 50–70% of grass (Ciepiela et al., 1998; Kessler, Lehman, 1998; Kryszak, 2003). In the field conditions red clover gives good yield in the mixture with festulolium (Kryszak, 2003; Søegaard, Weisbjerg, 2007), but due to high competitive ability of that hybrid, its part in sowing mixture should be significantly reduced (Borowiecki, 1997; Ostrowski, 2000; Staniak, 2008). Another important factor is the proper choice of forms and varieties of red clover. Some of the tetraploid varieties exhibit higher yield and better durability, and therefore should be suitable for cultivation in the mixtures (Gaweł, Bawolski, 1995; Borowiecki, Ścibior, 1997).

The aim of this study was to assess the yielding potential of festulolium mixtures with selected di- and tetraploid red clover varieties and pointing the most beneficial seed proportion of the seeded mixture in terms of the yield.

METHODS

A field experiment was conducted in two three-year series in 2005–2007 and 2006–2008 at the experiment sta-

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tion RZD IUNG-PIB Grabów (51°21' N; 21°40' E). Festulolium (Sulino cv.) mixtures with red clover were sown on the lessive soil, very good rye complex. pH of soil was neutral before setting the experiment; phosphorus, potassium and magnesium per 1000 g of soil was: P – 170 mg (high content), K – 122 mg (low content), Mg – 36 mg (low content), C – 0.7%, humus – 1.2%. The experiment, conducted according to the split-plot scheme was arranged in four replications, on plots of 22 m^2 size till the harvest. Two factors were included in the experiment: the contribution of clover seeds in the mixture (40, 60, 80%), compared to the mass of seeds sown in pure sowing: festulolium – 40 kg ha^{-1} (750 plants m^{-2}), diploid varieties of red clover 12 kg ha^{-1} (320 plants m^{-2}), tetraploid varieties of red clover 15 kg ha^{-1} (390 plants m^{-2}) and the variety of clover: Nike (2n), Parada (2n), Bona (4n), Jubilatka (4n). Mixtures were sown on the 14th April 2005 and the 9th May 2006, in rows every 12 cm, without a protection plant. The following doses of mineral fertilization (kg ha^{-1}) were used in the year of sowing: N – 60 (30 +30), P – 26 and K – 66, in the year of full usage: N – 90 (30 +30 +30), P – 22, K – 66 (33 +33). At the first series, in the sowing year, two swaths of green matter were collected, whereas in the first year of utilization – three cuts, and in the second year – four cuts. In the second series, in the sowing year, only one cut was harvested, whereas in the first and second year – four cuts. The botanical composition of sward was identified at the research. The dry matter yields and crude protein yield from 1 ha were determined, on the basis of the dry matter content (by weight at 105°C) and total protein (by Kjeldahl method). The results were statistically elaborated for the randomized split-plot layout. The significance of differences was examined using Tukey's test at significance level $\alpha = 0.05$.

The weather conditions during the experiment varied significantly; they were characterized particularly by high variability of precipitation (Table 1). In 2005, heavy rainfall occurred in May and July, while the significant deficiencies of water were noted in April, June and August. The year 2006 was even less favorable for the growth and development of grasses. Two summer months: June and July were particularly dry which was also accompanied by high air temperature (in July it exceeded the multi-year mean average by 4.1°C). Heavy rainfalls occurred only in August, and were almost three times higher than the average of the region. Between 2007 and 2008, rainfall during the growing period was respectively 30 and 12% higher than long-term average, and its distribution was more balanced. The plant growth proceeded in the first series of experiment (2005–2007) in the less favourable weather conditions. The moisture conditions were better in the second series of studies (2006–2008).

RESULTS AND DISCUSSION

Botanical composition of sward

The emergence of plants was quite good and balanced, but the participation of clover seedling in the plant emergence was significantly lower compared to the quantity of seeds sown (Table 2). In 2005, the participation of red clover in the emergence was from 26 to 56%, while in 2006, from 39 to 61%, which was largely influenced by high competitive ability of festulolium in relation to legumes (Borowiecki, 1997; Ostrowski, 2000; Ścibior and Gaweł, 2004).

The participation of clover in the dry matter yield, in 2005, was similar to the share of clover after emergence, while in 2006, due to adverse moisture conditions, was much smaller. This was also reflected in a smaller share of clover in dry matter yield at the first year of utilization (2007). The major impact of weather conditions on the contribution of individual components in the yield of subsequent regrowths was also reported by Harkot and Trąba (1998) and Sowiński et al. (1997, 1999). The clover in the year of sowing was significantly more sensitive to soil moisture deficits than festulolium, due to the poorly formed root system. The festulolium hybrid was more sensitive to adverse conditions in the first year of utilization. It confirms the results of Borowiecki (2002) and Wilman et al. (1998) for festulolium susceptibility to drought, as well as earlier studies of the author (Staniak, 2004).

Table 1. Meteorological conditions during vegetation periods of mixtures.

Years of research	Rainfall [mm]#						Temperature [°C]##					
	month											
	IV	V	VI	VII	VIII	IX	IV	V	VI	VII	VIII	IX
2005	-28.8	27.0	-24.7	48.8	-38.2	-6.4	0.9	0.1	-0.6	1.7	0.2	1.6
2006	-8.9	-3.6	-32.8	-74.0	144.0	-36.2	1.3	0.2	0.7	4.1	0.6	2.3
2007	-25.7	17.6	28.9	8.5	76.7	27.4	1.0	1.8	2.0	0.9	1.8	-0.4
2008	32.9	30.6	-19.3	1.4	-20.5	20.9	1.3	-0.3	0.9	0.6	1.6	-0.7
Mean from the years 1871–2000	39.0	57.0	71.0	84.0	75.0	50.0	7.7	13.4	16.7	18.3	17.3	13.2

the difference between the total precipitation and long term average in the given month.
the difference between the average daily air temperature and average long term temperature of each month, during the given month.

Table 2. Emergence [%] and mean share of red clover in the dry matter yield of mixtures [%].

Share of red clover (%)	Red clover cultivar	Emergence		Share of red clover in dry matter yield					
				sowing year		1st year of utilization		2nd year of utilization	
		series							
		I (2005)	II (2006)	I (2005)	II (2006)	I (2006)	II (2007)	I (2007)	II (2008)
40	Parada	11.7	11.2	13.9	3.9	46.0	24.5	54.6	71.9
	Nike	7.6	15.2	7.5	5.2	33.8	25.7	50.2	67.5
	Bona	8.3	18.0	7.6	7.9	44.8	34.4	54.5	74.1
	Jubilatka	13.7	18.3	9.6	8.0	48.3	32.6	54.0	75.8
	mean	10.3	15.7	9.6	6.2	43.2	29.3	53.4	72.3
60	Parada	17.8	23.5	19.2	3.9	61.5	27.2	65.8	72.0
	Nike	28.9	27.0	19.0	5.3	53.1	36.2	63.1	70.6
	Bona	18.7	30.7	16.8	13.2	45.9	47.3	58.9	82.8
	Jubilatka	19.8	22.6	20.0	14.2	61.2	39.3	61.5	82.4
	mean	21.3	26.0	18.8	9.2	55.5	37.5	62.3	77.0
80	Parada	35.1	53.1	24.9	21.2	64.6	44.6	68.5	79.4
	Nike	46.1	52.7	21.3	18.2	65.1	42.1	67.0	75.2
	Bona	44.9	49.8	28.6	36.5	61.7	54.4	68.4	83.6
	Jubilatka	53.2	39.9	31.3	28.7	67.6	43.5	69.6	82.2
	mean	44.8	48.9	26.5	26.2	64.8	46.1	68.4	80.1

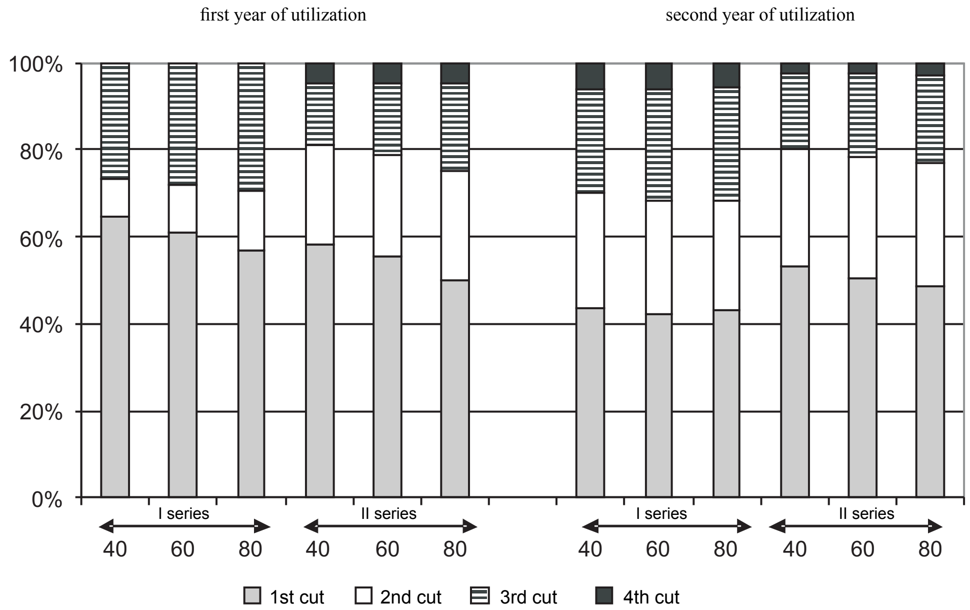

Fig. 1. The percentage participation of cuts in the whole annual dry matter yield of the mixtures.

Yield distribution

The contribution of individual cuts to the annual yield is important in multi-harvested perennials production. The reduction of the share of the first cut in favour of the second and the third one is evaluated positively in the production of feed (Wilczek, 2008).

The proportion of individual cuts in the annual dry matter yields depends on the participation of individual components (Fig. 1). The weather conditions during the study also had strong influence on this trait. A favourable share of the first cut was recorded in the mixtures with the largest participation of clover (80%), although the differences were more pronounced in the better weather conditions (series II), where the first cut was on average 50% of total yield. With 40% share of clover in the mixture, in these weather conditions, the first crop accounted for around 56%. A small amount of rainfall during the second vegetation regrowth in 2006 (series I) significantly reduced its share in the yield, while high rainfall in August increased

Table 3. Dry matter yields of festulolium-red clover mixtures [t·ha-1]

Share of red clover [%]	Cultivar of red clover	Sowing year		1st year of utilization		2nd year of utilization		Total yields	
		series							
		I (2005)	II (2006)	I 2006)	II (2007)	I (2007)	II (2008)	I	II
40	Parada	3.23	2.69	9.3	12.2	14.5	12.1	27.0	29.7
	Nike	2.76	3.04	8.1	13.6	12.9	11.6	23.7	31.0
	Bona	2.99	2.75	9.3	15.1	14.0	14.1	26.3	35.1
	Jubilatka	2.99	2.67	9.3	16.8	14.6	15.1	27.0	38.0
60	Parada	3.41	2.32	10.3	14.5	16.2	13.7	30.0	33.6
	Nike	3.73	2.16	9.9	16.0	16.6	14.0	30.2	35.4
	Bona	3.10	2.59	9.9	18.3	14.9	16.3	27.9	40.9
	Jubilatka	3.87	2.84	11.2	17.2	16.4	15.9	31.4	39.5
80	Parada	4.57	2.42	11.1	17.6	17.9	14.8	33.6	38.3
	Nike	3.85	3.16	11.0	18.5	16.7	16.7	31.5	42.2
	Bona	4.17	3.28	12.0	19.9	16.8	19.1	32.9	46.5
	Jubilatka	4.26	2.93	11.7	18.5	17.6	15.8	33.5	41.0
Mean for share of red clover									
40		2.99	2.79	9.0 a	14.4 a	14.0 a	13.2 a	26.0 a	33.5 a
60		3.53	2.48	10.3 ab	16.5 b	16.0 ab	15.0 b	29.9 ab	37.3 b
80		4.21	2.95	11.5 b	18.6 c	17.2 b	16.6 b	32.9 b	42.0 c
Mean for cultivar of red clover									
	Parada	3.74	2.42	10.2 ab	14.8 a	16.2	13.5 a	30.1 ab	33.8 a
	Nike	3.45	2.79	9.7 a	16.0 a	15.3	14.1 ac	28.4 a	36.2 a
	Bona	3.42	2.87	10.4 ab	17.7 b	15.2	16.5 b	29.0 ab	40.8 b
	Jubilatka	3.71	2.81	10.7 b	17.5 b	16.2	15.6 bc	30.6 b	39.5 b
Mean for cultivar form									
Diploid varieties		3.59	2.63	9.9 a	16.3 a	15.8	13.8	29.3	31.8 a
Tetraploid varieties		3.55	2.84	10.6 b	18.7 b	15.7	16.0	29.8	36.5 b

Numbers in columns followed by the same letters do not differ significantly

the share of the 3rd regrowth. Greatest production capacity of the plant mass of the third cut comparing to the previous one is a rare phenomenon in the literature (Ćwintal, Kościelecka, 2005). The major impact of weather conditions on this trait was also reported by other authors (Wilczek, 2008; Ćwintal, 2008; Kryszak, 2001; Sowiński et al., 1997). The selection of varieties did not affect significantly the contribution of individual cuts in the annual yield, as confirmed by the results of Ćwintal and Wilczek (2004).

The dry matter yield

The weather conditions during the study were the most differentiating factor of the yields. The summer drought in 2006 had an especially great impact, which caused almost total loss of crops in the second cut (series I). Under these conditions, total dry matter yields of mixtures in the first year of utilization were on average 37% lower than the yields of mixtures in the first year of utilization under favourable weather conditions (series II).

There was a significant interaction during the years, which was the result of the varied response of the varieties to the weather conditions, and therefore the treatment average is given for each year separately. The seed proportions of individual components were shown to have a significant effect on mixture yields (Table 3). The most efficient were mixtures containing 80% of red clover at sowing, whereas the least efficient one was that with 40% share of the red clover.

The significant differences were found in all years of utilization, as well as in the total yields, although this trend was also maintained in the sowing year. The rise of the total dry matter yields averaged 26%, compared to a mixture with 40% share of legumes. This is confirmed by Gaweł (2009), who showed that a mixture of red clover, meadow fescue and festulolium, with 60% share of legumes was more efficient than a mixture with 40% of clover. The better yields of the mixtures with higher clover share were also reported by other authors (Sowiński et al., 1997, 1999). In terms of the cultivar factor, the mixtures of festulolium with tetraploid varieties of red clover showed higher productivity. The increase of the total dry matter yields averaged 15%. Under less favourable weather conditions (series I) the mixture that involved cv. Jubilatka gave the best yield whereas yields of mixtures with varieties: Bona, and Parada did not differ significantly. The mixture involving the diploid cultivar Nike was the poorest one. Under the better moisture conditions (series II), a mixture with Bona and Jubilatka gave significantly higher yield. These results were confirmed in the research by Borowiecki (1997), in which the mixture of festulolium with tetraploid red clover cultivar Ulka gave higher yield than the mixture with diploid Nike cultivar. Investigations by Gaweł and Bawolski (1995) gave similar result: the mixtures of fescue with tetraploid red clover (regardless of cultivar) gave generally higher yields than the diploid clover mix. In COBORU (Broniarz, 2006) studies the cultivar Bona gave higher yield than Parada. Wilczek (2008) showed that the weather conditions with periodic shortages of water lead to the greater efficiency of green mass of Bona cultivar (in the monocultivar sowing). The same conditions lead to the greater efficiency of the dry mass of Parada cultivar, which may indicate lower demand for water of that cultivar.

The yields of crude protein

The total protein production from 1 ha was significantly differentiated by the share of clover at sowing mixtures. The greatest yield of protein was obtained from the mixtures with the largest share of legumes (80%) and in both series was 44% greater than that from a mixture with 40% share of legumes (Table 4). A similar relationship was demonstrated by Sowiński et al. (1999), who obtained by an average of 371 kg ha^{-1} more protein from the mixture with 70% share of clover seeds in a mixture with different species of grasses (ryegrass: annual, hybrid, perennial, meadow fescue) than from the sowing mixture in inverse proportion. Staniak (2008) also confirmed in other investigations the beneficial effect of increased participation of red clover in canopy mixtures on the yield of protein. The cultivar of clover was also the factor influencing the obtained yield of protein.

The mixture of tetraploid varieties showed the greater efficiency of protein, although the significant differences were recorded only in the second year of utilization and in the total yields of the 2nd series of the experiments. The total crop yield increase averaged 16%. Under the favourable weather conditions (series II) the most efficient cultivar was Bona while under the shortage of moisture, Jubilatka was the best. The lowest yield of protein was obtained from the mixtures with Nike cultivar. The greater efficiency of true protein of tetraploid cultivar of red clover Karo as compared with diploid cultivar Dajana was reported by Ćwintal and Wilczek (2004). No significant differences in

Table 4. Total protein yields of mixtures [kg ha^{-1}].

Share of red clover [%]	Red clover cultivar	Sowing year		1st year of utilization		2nd year of utilization		Total yields	
		series							
		I	II	I	II	I	II	I	II
40	Parada	443	397	1123	1156	1945	1682	3511	3236
	Nike	430	409	1101	1259	1972	1349	3503	3017
	Bona	468	347	1112	1788	1896	1895	3475	4030
	Jubilatka	459	407	1289	1781	2091	2121	3839	4309
60	Parada	593	352	1460	1670	3021	2181	5074	4203
	Nike	592	350	1276	1536	2585	2002	4453	3888
	Bona	449	408	1080	2194	2482	2286	4011	4889
	Jubilatka	621	462	1796	1858	2553	2443	4970	4762
80	Parada	740	333	1608	2128	2871	2471	5219	4932
	Nike	634	427	1476	2282	2650	2439	4760	5148
	Bona	696	449	1734	2424	2850	3092	5280	5965
	Jubilatka	769	378	1736	2194	2996	2426	5501	4998
Mean for share of red clover									
40		450 a	390	1156 a	1496 a	1976 a	1762 a	3582 a	3648 a
60		564 a	392	1403 ab	1815 a	2660 b	2228 ab	4627 b	4435 b
80		710 b	397	1638 b	2257 b	2842 b	2607 b	5190 b	5261 c
Mean for cultivar of red clover									
	Parada	592	361	1397	1652 a	2612	2112	4601	4124 ab
	Nike	552	395	1284	1692 ab	2402	1930	4238	4017 a
	Bona	538	401	1308	2135 b	2409	2424	4255	4961 b
	Jubilatka	616	416	1607	1944 ab	2547	2330	4770	4690 ab
Mean for cultivar form									
Diploid varieties		572	378	1341	1672	2507	2021 a	4420	4071 a
Tetraploid varieties		577	409	1458	2040	2478	2377 b	4513	4825 b

Numbers in columns followed by the same letters do not differ significantly the efficiency of the protein between two varieties: Bona (4n) and Parada (2n) were noted by Wilczek (2008).

CONCLUSIONS

1. The differentiation of seed participation in the mixture of festulolium with red clover significantly affected the amount of dry matter and protein yields. The most efficient were the mixtures of 80% share of clover in the sowing, while the least-yielding were the mixtures with 40% share of that species. The rise of the total yield of a dry matter averaged 26%, whereas proteins 44%.

2. The weather conditions, particularly humidity and the selection of red clover varieties for the mixtures with festulolium significantly effected on their yield. The most useful proved to be tetraploid varieties of red clover. In conditions of shortage of moisture the highest yields of dry matter and total protein were obtained from the mixture with the cultivar Jubilatka, while the poorest yields were gained from the mixture with diploid cultivar Nike. The mixtures of varieties Bona and Jubilatka were characterized by greater efficiency of dry matter and total protein under the favourable weather conditions.

3. Festulolium is a species suitable for the mixtures with red clover. The mixture with the share of 80% of clover seed and 20% of festulolium proved to be the best in terms of the yield. More useful for mixtures with festulolium are tetraploid varieties of red clover.

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Variation in micronutrients concentration in lupine, pea and maize during the vegetation period on sandy soils

Research communication

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Abstract. In a two-year field experiment, established on sandy soil, tendencies in the changes in Cu, Zn, Mn and B concentrations in aerial parts of lupine, pea and maize were observed, from early vegetation season to the inflorescence stage. In each year, plant material samples were collected four times at 10-day intervals. Soil properties and total precipitation from the onset of the vegetation season to the termination of sample collection affected the level of the four microelements in plants. The effect was particularly strong in the case of copper. Although the concentrations of the microelements were different in the two years, certain tendencies in the modification of their levels could be observed during the vegetative season. The content of Cu in aerial parts of all the test plants tended to decrease distinctively as the plants grew older. Fluctuations in the levels of Zn, Mn and B, which depended on the plant species and environmental conditions, did not produce such clearly defined tendencies.

key words: papilionaceous plants, maize, microelements in plants, changes in concentration of microelements

INTRODUCTION

As plants grow and develop, they experience changes in the chemical content of the tissues. It is commonly believed that the concentration of nutrients in young plants is higher than in more mature ones. Many studies have focused on the dynamics of nitrogen uptake (Plenet, Lemaire, 2000) and other macroelements (Beale, Long, 1997; Fricks et al., 2001), or else on the seasonal changes in the mutual ratios of particular macroelements in plants (Guillard, Allinson, 1989). However, there is scarcity of reports on the dynamics of the uptake of microelements during the vegetation season. Most of such papers deal with the uptake of heavy metals on polluted soils (Joner, Leyval, 2001; Yang et al., 2006).

The purpose of the present study has been to determine the direction of changes in the concentration of microelements in aerial parts of two papilionaceous plants and maize on sandy soils, from the early vegetative growth to the inflorescence stage.

MATERIALS AND METHODS

The material for the research was composed of plant samples collected in 2005–2006 from fields at the IUNG-PIB Experimental Station in Jelcz-Laskowice, cropped with lupine, pea and maize. The forecrop for cv. Taper yellow lupine and cv. Wiato pea was spring barley followed by winter wheat. The maize cultivar LG 2244 was also grown in a crop-rotation system after cereals. In both years, N fertilization was applied as ammonium nitrate and the PKMg nourishment was introduced as a complex fertilizer Polimag 305. The rates of the nutrients introduced under lupine and pea were: N – 40 kg ha^{-1}, P – 25 kg ha^{-1}, K – 70 kg ha^{-1} and Mg – 17 kg ha^{-1}. Under maize, the following doses were applied: N – 150 kg ha^{-1}, P – 28 kg ha^{-1}, K – 80 kg ha^{-1} and Mg – 19 kg ha^{-1}. Pea and lupine were sown on 11 April 2005 and 24 April 2006, whereas maize was sown on 23 May 2005 and 24 May 2006.

Fields under the crops differed for the properties of the arable layer of soil (Table 1). In general, they were sandy soils, containing little organic carbon. The soil reaction was acidic or strongly acidic. Soil analyses were performed according to the methods used in chemical and agricultural stations. The content of P and K plant available forms was determined with Egner-Riehm method and Mg was tested using Schachtschabel method. The micronutrients were extracted from soil using 1 M HCl, after which Cu, Zn and Mn were determined with the AAS method and the level of B was established by colorimetry.

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Table 1. Some physicochemical properties of the top soil layer in the trial fields.

Field	Percentage of fraction in mm			C org.	pH in	Concentration [mg kg^{-1}]						
	1.0–0.1	0.1–0.02	<0.02	[%]	KCl	P	K	Mg	Cu	Mn	Zn	B
Pea 2005	57	26	17	0.65	4.4	49	159	33	3.3	186	7.2	0.9
Pea 2006	44	36	19	0.67	5.3	74	156	32	3.0	181	9.6	0.8
Lupin 2005	55	28	16	0.50	4.3	46	123	31	3.7	158	7.1	0.8
Lupin 2006	36	40	24	0.60	5.0	58	152	43	3.0	166	8.1	0.6
Maize 2005	62	23	15	0.74	4.8	65	90	44	2.8	156	7.0	0.6
Maize 2006	53	26	21	0.69	5.2	71	189	30	2.9	177	8.6	0.7

Table 2. Weather data from the onset of the vegetation season to the last date of sample collection.

Year	Average temperature [°C]					Sum of precipitation [mm]				
	March	April	May	June	March–June	March	April	May	June#	March–June
2005	1.3	9.3	14.2	17.0	10.5	12.3	20.3	86.2	21.6	140.4
2006	0.0	9.4	14.1	18.3	10.8	28.9	50.2	29.9	45.8	154.8

the data for June comprise the information only for the 1st and 2nd decade of this month.

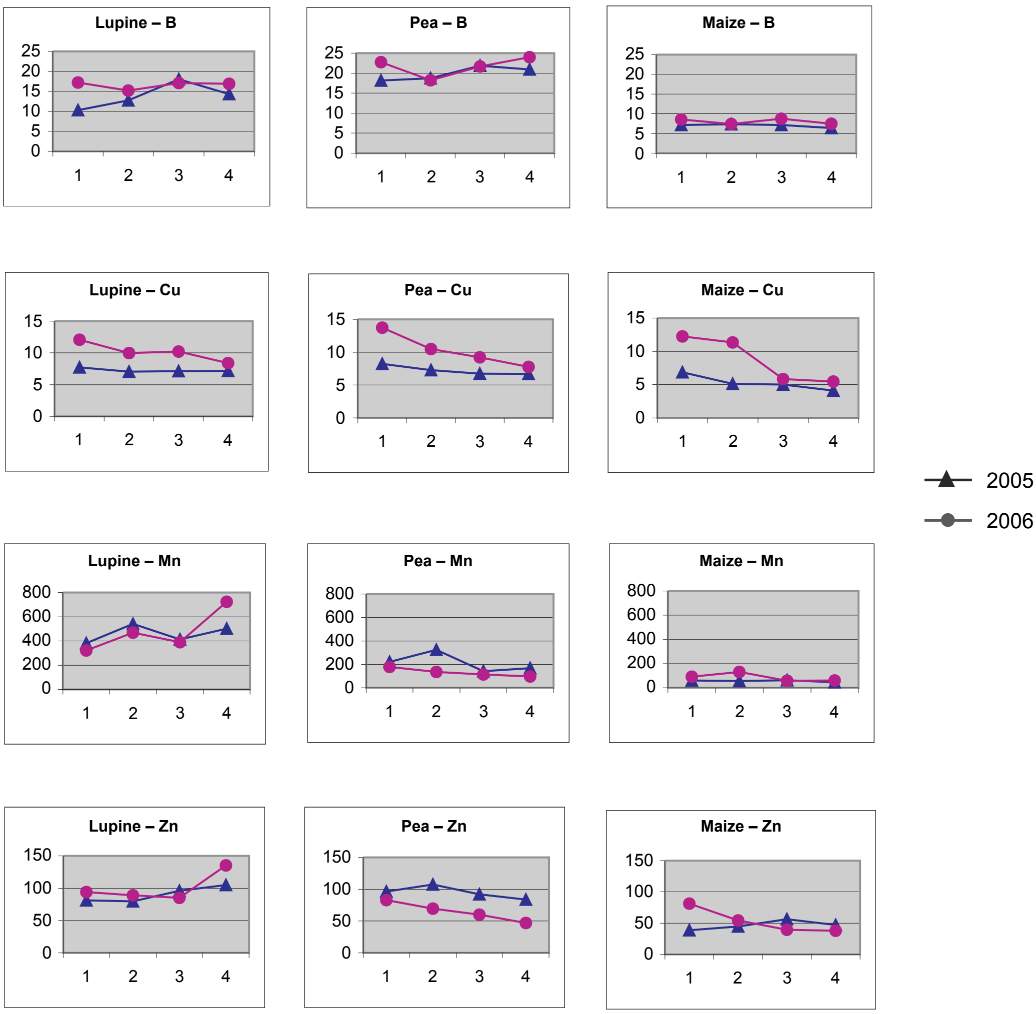

Fig. 1. Content of microelements [mg kg^{-1}] in dry matter of aerial parts of plants on four dates during the vegetation season (1-4) in both years.

The weather conditions between the two years, from the early vegetative growth to the last date of sample collection, varied greatly (Table 2). Particularly large differences occurred in the total rainfall and rainfall distribution.

Plant samples were taken from 12 fixed points, each covering 4 m^2, set up on each plot. In each year, aerial parts of plants were sampled on four dates, at 10-day intervals, starting on the thirtieth day after sowing. The growing stages of plants agreed in both tested years. On each of the four dates, whole aerial plant parts were cut over an area of 1 m^2.

Once the plant samples were dry ashed in a muffle furnace, hydrochloric acid dilutions were prepared to determine the content of B by ICP method, and the content of Cu, Mn and Zn by AAS method. For determinations of B and Mn, certification material NCSZC 76008 was used, while the determinations of Cu and Zn were conducted using the in-house material – IPE plant samples (Cu – IPE 06.2.4, Zn – IPE 06.2.4) obtained from interlaboratory research work conducted in the Netherlands.

RESULTS AND DISCUSSION

The levels of the analysed microelements were varied depending on the plant species and environmental conditions, especially soil properties and weather. The level of Cu for all the three plant species was evidently higher in 2006 than in 2005 (Fig. 1). It can be assumed that the availability of this microelement for plants in the year 2006 was superior. The soil from crop fields in 2006 had larger sorptive complex than the fields in 2005, as indicated by a higher content of <0.02 mm fraction and usually organic carbon (Table 1). The content of total Cu is higher in soils of larger exchange complex, and the mobility as well as availability of this element is conditioned by levels of organic matter in soil (Kabata-Pendias, Pendias, 2001). The mobility of Cu is enhanced by organic compounds, which are released during the decomposition of organic matter. Solubility of copper in soil depends mainly on the presence of dissolved organic carbon (Roömkens et al., 1999; Impellitteri et al., 2002). In 2006, the total rainfall was larger, particularly in the season preceding the sampling. The amount of precipitation in March and April 2006 was twice as large as in the analogous time in 2005 (Table 2). Soil moisture is another important aspect in decomposition of organic matter. Higher soil moisture content in 2006 facilitated the release of copper to soil solution, which meant better availability of this element to plants.

Differences in the concentrations of the other microelements in the plants, caused by the differences in the climatic and soil conditions, were not as big as for copper. Noteworthy is the higher level of Zn and Mn in pea plants in 2005, which may have been a result of the soil pH being lower than in 2006 (Fig. 1, Table 1).

Although the concentrations of the microelements in plants were different between the two years, certain tendencies in their fluctuations could be observed as the plants continued to mature (fig. 1). Modifications in the Cu content were decreasing during the vegetation season, with the decline being larger in maize rather than in the two papilionaceous plants. The other two elements, Mn and Zn, remained stable or tended to decrease only in maize and pea. In lupine plants, the concentration of Mn and Zn immediately before the flowering stage was higher than initially. The content of B in plants gradually increased in the consecutive growing stages or else remained unchanged during the whole period examined.

CONCLUSIONS

1. Among the studied four micronutrients a clear alteration of their concentration in plants over the successive phases of growth (independent of plant species), occurred only for copper. The content of Cu in the aboveground parts of peas, lupins and maize decreased as the plants aged.
2. Changes in the content of Zn, Mn and B over the growing season were not consistent and depended on plant species, soil conditions and weather.

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Agricultural and forest biomass as feedstock in the manufacture of solid biofuels

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Abstract. The study involved 15 types of pellets produced from biomass from agricultural, forestry and production residues. The aim of this study was to determine moisture, ash content, calorific value and net calorific value of biofuels. Pellet made from pure wood sawdust was characterized by the best quality in terms of value of energy (the highest calorific value and the lowest ash content) and met the requirements of DIN 51731. The calorific value of pellets from agricultural residues was lower by about 10% and its ash content almost 900% higher than that of post-production sawdust. Pellets from biomass of perennial crops and production residues, despite their flaws, are likely to be future-attractive, renewable biofuels, in particular for applications in power industry and municipal heating.

key words: biomass, energy crops, post-production residues, pellets, ash content, calorific value

INTRODUCTION

Nowadays, the main source of biomass for energy purposes are forests and timber industry. However, in the light of the law regulation, the structure of the biomass source must be changed to the increase of the share of biomass from agricultural production residues and agro-food industry (Dz.U. nr 156, poz. 969), which is a big challenge for agriculture (Budzyński et al., 2009; Grzybek, 2008; Kuś, Faber, 2009).

One of the major sources of biomass is to be long-term energy crops grown on agricultural land: native species of willow (*Salix* L.), Poland-acclimatized Sida (*Sida hermaphrodita* R.), giant perennial grass – Miscanthus (*Miscanthus x giganteus* J.M.Greef & M.Deuter) and other (Grzybek, 2006; Kuś, 2008; Podlaski et al., 2009; Stolarski, 2004, 2009; Stolarski et al., 2006, 2008, 2009; Szczukowski et al., 2000, 2004).

Solid, fresh biomass as a fuel compared to mineral resources such as coal in terms of physicochemical properties of fuel is less attractive. This is due to (among other reasons) too low density of biomass, making it difficult to transport, storage and dosing for the boilers. In addition, a wide range of moisture from a few percentage points to as much as 50–60% and low volumetric energy content hinder the distribution of biomass in the basal form. Therefore, electric utilities are interested in processed biomass, refined to the form of pellets. Pellets compared to the original form of biomass have lower moisture, higher density which increases their concentration of energy (Grzybek, 2004; Kowalik, 2002; Stolarski et al., 2005; Szczukowski et al., 2004). However, the calorific value of pellets, and the ash content varies largely depending on the origin of the material used for pelletizing.

The paper posed the hypothesis that the biomass from agricultural and agro-food industry can be the raw material for pellet production, and the quality of produced biofuel will probably be standing close to the one obtained from forest biomass residue. The aim of this study was to determine moisture, ash content and heating net calorific value of pellets produced from agricultural and forestry biomass.

MATERIAL AND METHODS

The study was carried out on 15 types of pellets produced from biomass: perennial energy crops (willow, Sida, giant Miscanthus), agricultural residues (straw of cereals – triticale, straw of hemp (for oil), cereal bran, residues of cleaning grass seeds, sunflower marc), forest residues (wood shavings, the bark of coniferous trees), wood (pine and oak sawdust), food industry (apple pomace, coffee marc) and mixed (75% pine sawdust + 25% cereal bran by weight).

The representative samples of pellets of each type of biomass were collected. The assays for each trait were per-

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formed in the laboratory of Department of Plant Breeding and Seed Production (University in Olsztyn) in three replications. Pellet moisture was determined by dryer-weight method (by PN-80/G-04511 standard). Then, individual samples of biofuels were crushed in the analytical mill "IKA FMC 10 basic", using sieves with mesh diameter of 0.25 mm. The ash content was determined in so prepared analytic samples (by PN-80/G-0512 standard). In addition, the heat of combustion of the biofuels was determined in the calorimeter IKA C 2000, based on the dynamic method (by PN-81/G-04513 standard) and the calorific value was calculated accounting for their moisture in the working state.

The results were analyzed using computer package STATISTICA 9.0 PL. Arithmetic means and standard deviation were calculated for the studied traits. The multiple-comparison SNK test which groups together means of similar values was applied to identify statistically homogeneous groups at $\alpha = 0.05$.

RESULTS AND DISCUSSION

The moisture of pellets was low, averaging 9.2% (Fig. 1). Pellets produced from pine sawdust had the lowest moisture content (6.3%). Slightly, but significantly higher humidity was found in pellets from oak sawdust. In the remaining fuels the value of this trait ranged from 7.3% for Sida to 12.9% in the pellets from apple pomace. The requirements of DIN 51731 standard allow the pellets with a moisture content of 12% to be put on the market. Pellets derived from apple pomace and cereal bran did not meet that standard. In other studies, moisture of pellets also varied in a narrow range from 7.04% in the pellets from beech sawdust to 12.04% for pellets made from sunflower marc (Stolarski et al., 2007).

The ash content of pellets produced from different types of biomass averaged 3.5% (Fig. 2). The pellets produced from oak sawdust was characterized by the lowest, positive value of that trait (0.5% DM). Statistically, they were in the same homogeneous group with pine sawdust pellets (0.9% DM). The ash content in the pellets from willow biomass was 1.8% DM. The apple pomace pellets were in the third group of fuels in terms of ash content. Whereas, in the fourth group of fuels in terms of this value were fuel from sunflower marc, bark of conifers, wood shavings, mixture of sawdust and bran, and the biomass from Sida, Miscanthus and hemp (2.3–3.2% DM). The highest ash content (11.1% DM) of the examined biofuels was found in pellets made from the residue from cleaning of grass seeds. In the pellets from straw the ash content was 7% DM, while in bran it was by 0.7 percentage points lower.

The cited standard DIN 51731 permits on the market pellets with ash content below 1.5%. In this study these criteria were met by pellets made from clean, post-production oak and from pine sawdust without bark. Pellets made from other biomass did not meet the criteria of this standard. However, it could be used primarily as industrial pellets to be applied in the power industry and in municipal heating.

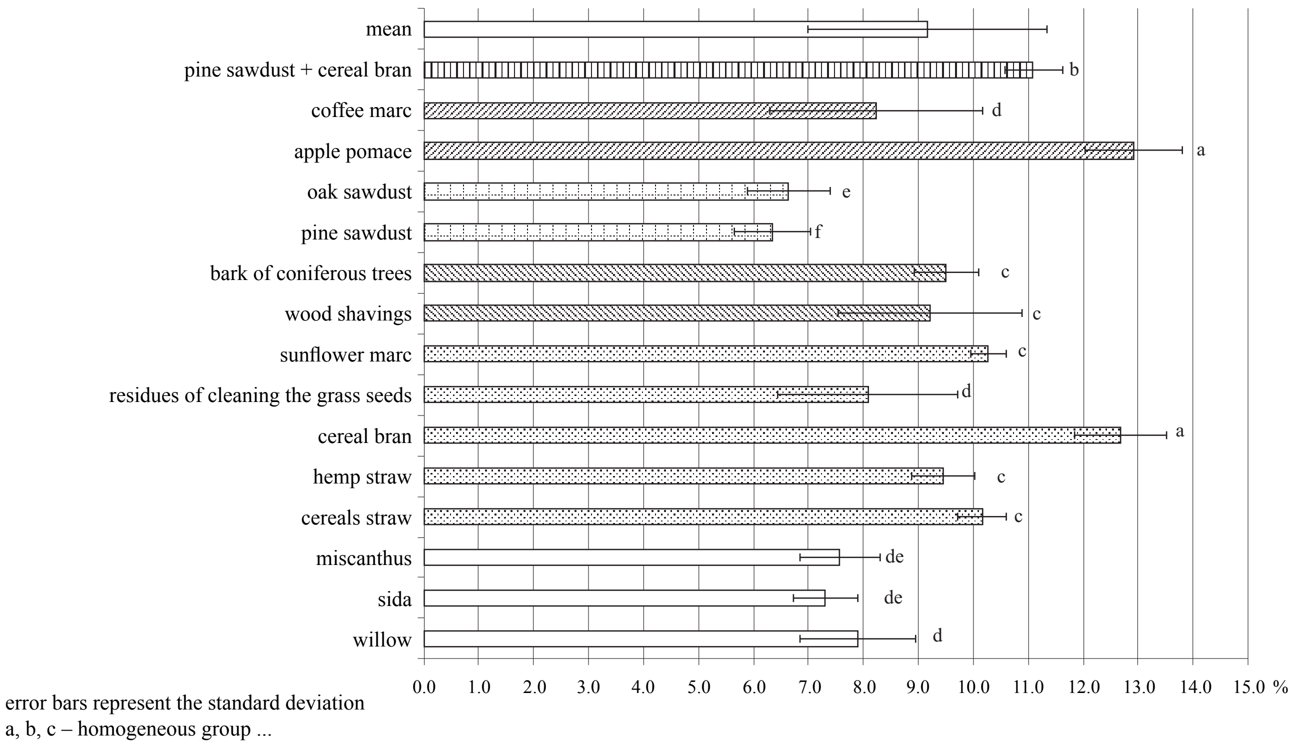
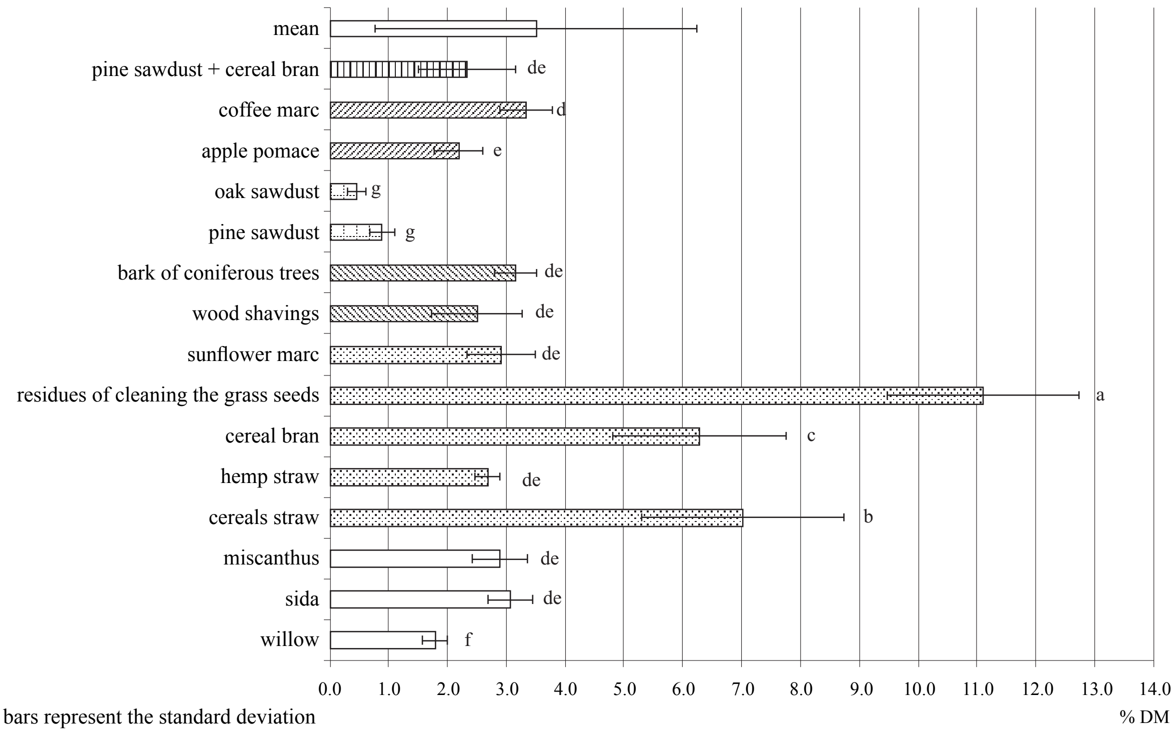
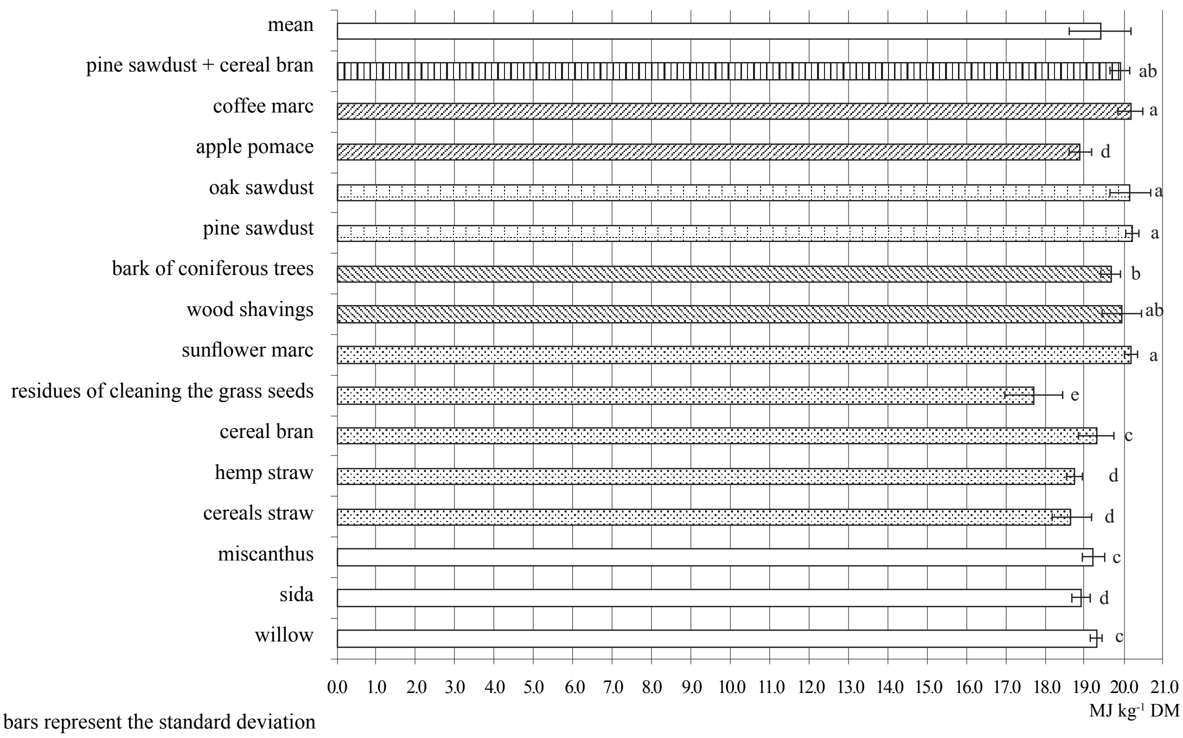


Fig. 1. Pellet humidity from various types of biomass [%] (source: authors' own research)



error bars represent the standard deviation
a, b, c ... – homogeneous group

Fig. 2. The ash content of pellets of different types of biomass [% DM].
(source: authors' own research)



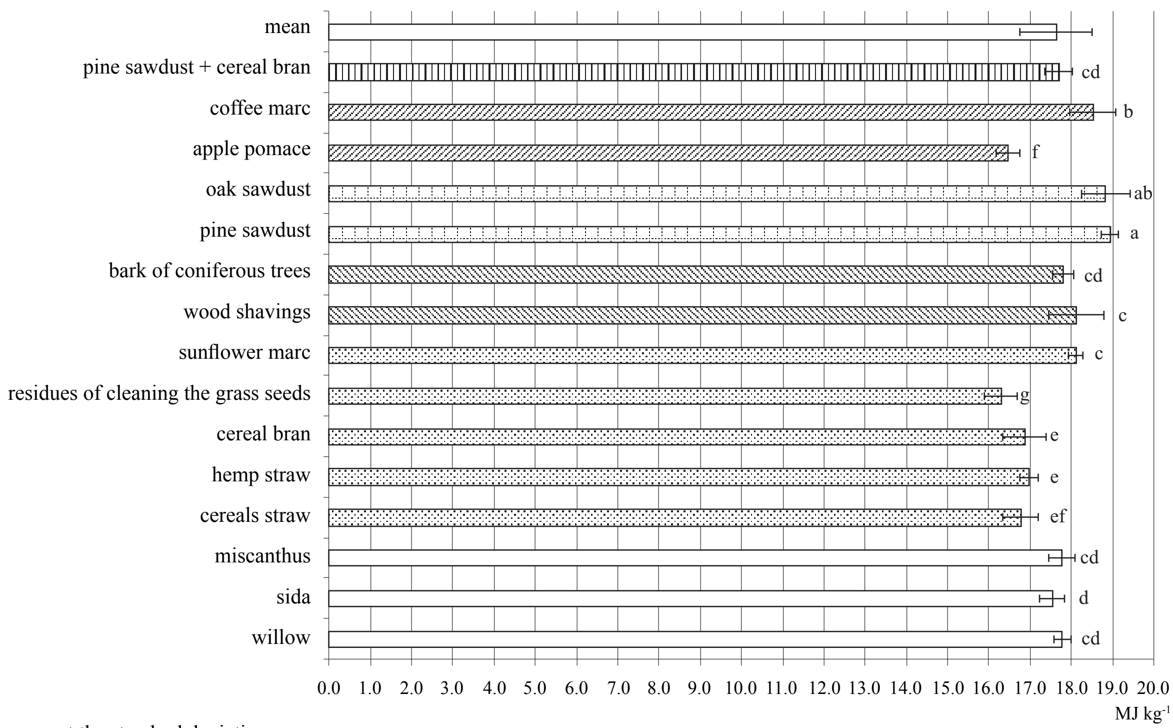
error bars represent the standard deviation
a, b, c ... – homogeneous group

Fig. 3. Combustion heat of pellet of different types of biomass [MJ kg^{-1} DM].
(source: authors' own research)

In other studies, the ash content of willow pellets was lower (1.4% DM), and for Sida pellets was higher (3.4% DM) than limit value (Stolarski et al., 2005). In subsequent studies, though, the ash content in these species was respectively 2.2% and 2.7% DM (Stolarski et al., 2007). Król et al. (2010) indicate that the ash content of willow biomass was 2.6% DM and this feature for Sida and Miscanthus biomass was substantially higher, respectively 4.2% and 4.4% DM. The authors cited determined ash in sunflower straw at 5.5% DM and at half that amount (2.65% DM) in sunflower husk. Stolarski et al. (2007) indicate that the ash content in the pellets from the remains of sunflower was 3.5% DM. Varied content of ash in the biomass from the agro-food industry was determined by Borycka (2009). The value of this trait ranged from 1.24% to 3.40% for apple and carrots, respectively.

The combustion heat of tested biofuels was high and averaged 19.4 MJ kg^{-1} DM (Fig. 3). To the homogeneous group of pellets with the highest calorific value were classified: coffee and sunflower marc, oak and pine sawdust. In the second homogeneous group were biofuels derived from the mixture of branches of forest trees and bran with sawdust. The third group included pellets from the bark of conifers. In the fourth group were placed pellets from willow, Miscanthus and cereal bran. In the fifth homogeneous group were biofuels from apple pomace, wheat straw, hemp straw, and Sida. The last homogeneous group with the lowest combustion heat was composed of the pelleted residue from cleaning of grass seeds, a product that contained large quantities of mineral impurities.

The calorific value of individual biofuels taking into account their humidity is shown in Figure 4. In the case of this trait variation in the number of homogeneous groups was greater than that for combustion heat, since as many as ten groups were identified. The highest calorific value was shown by pine sawdust pellets 18.9 MJ kg^{-1}, slightly lower by oak sawdust pellets. High calorific value (18,1–18,5 MJ kg^{-1}) was characteristic of pellets from the coffee marc, those from sunflower marc and from the mixture of branches of forest trees. Pellets from willow, Miscanthus, conifer bark as well as from the mixture of sawdust and bran were characterized by a calorific value of 17.7–17.8 MJ kg^{-1}. However, by far the lowest average calorific value of pellets was found in those made from the residue left in the cleaning process of grass seeds 16.3 MJ kg^{-1}. In other studies, the calorific value of pellets from the sawdust of forest trees ranged from 17.7 to 18.0 MJ kg^{-1} (Stolarski et al., 2007). The pellets made from the biomass of energy crops had a calorific value of about 17 MJ kg^{-1}. In the biofuel made from post-processing residues the calorific value was lower by about 0.6 MJ kg^{-1}. Król et al. (2010) indicate that willow biomass was characterized by a higher calorific value (17.2 MJ kg^{-1}) than the biomass of Miscanthus and Sida (about 16.5 MJ kg^{-1}). In the above-cited studies high



error bars represent the standard deviation
a, b, c ... – homogeneous group

Fig. 4. The calorific value of pellets of different types of biomass [MJ kg^{-1}].
(source: authors' own research)

Table 1. Characteristics of pellets made from different origins of biomass.

Pellets of biomass	Combustion heat [MJ kg^{-1} DM]	Humidity [%]	Ash content [% DM]	Calorific value [MJ kg^{-1}]
Perennial energy crops	19.15 ±0.28	7.59 ±0.83	2.58 ±0.68	17.70 ±0.30
Agricultural residues	18.92 ±0.93	10.14 ±1.74	6.00 ±3.35	17.00 ±0.71
Forest residues	19.81 ±0.42	9.36 ±1.23	2.83 ±0.68	17.95 ±0.52
Timber industry residues	20.19 ±0.37	6.50 ±0.72	0.67 ±0.28	18.88 ±0.43
Food industry	19.54 ±0.72	10.58 ±2.81	2.77 ±0.72	17.49 ±1.14
Mixed	19.90 ±0.25	11.10 ±0.53	2.33 ±0.83	17.69 ±0.33

± standard deviation
Source: authors' own research

calorific value was found in rapeseed cake (19.9 MJ kg^{-1}), whereas in peanut shells the value was 15.7 MJ kg^{-1}. Borycka (2009) showed large variations of the calorific value of biomass residues from fruit and vegetables. The lowest value of that trait was characterized by apple pomace (15.9 MJ kg^{-1}) and the highest by tomato marc (23.3 MJ kg^{-1}).

Based on the results from this study we can confirm that the quality of the pellets as a biofuel can be varied depending on the type of biomass, from which it was produced. When analyzed for quality using the averaged data from this study sawdust from timber industry was found to have the best suitability as a source of energy. It is characterized by highest calorific value and lowest moisture and ash content (Table 1). Pellets from the biomass from perennial energy crops is characterized by a lower calorific value by an average of 1.2 MJ kg^{-1}. However, the calorific value of biomass pellets from agricultural residues was lower by an average of 1.9 MJ kg^{-1} than pellets from timber industry residues. The ash content of pellets from the biomass of perennial crops, forest residues and food industry residues was 4-fold higher than in the pellets from pure sawdust. By contrast, pellets from agricultural residues biomass contain, on average, almost 9 times more ash, which may limit their use in the manufacture of solid fuel – pellets of high quality for individual customers.

Varied ash contents in the tested fuels are not surprising, because the ash content in different types of biomass is affected by: the characteristics of species, the soil on which the plants grow, fertilization rates, the level of contamination by minerals and biomass-acquisition period (Stolarski et al., 2009). The ash content of dendromass is dependent on parts of the plant from which it was acquired and on the fraction of bark and mineral impurities. Therefore, the pellets from sawdust which did not contain bark or impurities was characterized by the lowest value of that trait. Agricultural biomass contained significantly more ash. In order to produce pellets which would meet the requirements of DIN 51731 the products to be created should include, among other things, post-processing residues of timber industry blended with agricultural biomass.

CONCLUSIONS

1. Pellets made from pure wood sawdust was characterized by the best quality in terms of value of energy (the highest calorific value and lowest ash content) and they met the requirements of DIN 51731 standard.
2. Among the solid biofuels produced from treeless biomass a high calorific value and the lowest content of ash was characterized by a pellet from perennial energy crops.
3. Pellets produced from perennial energy crops, agricultural residues, forestry and food industry did not meet the requirements of DIN 51731 standard because of too high content of ash.
4. The calorific value of pellets from agricultural residues was lower by about 10% and the ash content of almost 900% higher than that of sawmill sawdust.
5. Pellets from biomass of perennial crops and production residues, despite their flaws, are likely to be future-attractive renewable biofuels, in particular for applications in power industry and municipal heating.

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Response of durum wheat (*Triticum durum* Desf.) to protection intensity

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Abstract. The field study was carried out in years 2007–2009. The experimental field was located on soil derived from loess silt, which is categorized as good wheat soil complex. The aim of the experiment was to establish the influence of varied plant protection levels: minimal (seed dressing and herbicide) and comprehensive (seed dressing, 2 types of herbicide, retardant, fungicide and insecticide) on the yield, crop structure components and selected physical quality parameters of the grain of spring wheat *Triticum durum* (Desf.) and *Triticum aestivium* (L.). The yield of minimally protected wheat was reduced by approximately 22.6% compared to intensively protected wheat, which was caused by a significant decrease in the number of ears, thousand grain weight (TGW) as well as grain weight and number per ear. Limited plant protection resulted in overall decrease in grain bulk density and uniformity as well as grain glassiness. Depending on a genotype, the yield of durum wheat made up from 68.3% to 85.1% of the yield of common wheat. The grain of durum wheat was characterised by greater TGW, uniformity and glassiness compared to common wheat.

key words: plant protection, grain yield, grain quality, durum wheat, spring wheat

INTRODUCTION

The consumption of resources such as fertilizers or plant protection products is one of the main indicators of cultivation intensity. Yield growth and stabilization are the most frequent results of increased production intensity. It is also important to mention a beneficial or unfavourable influence of production intensification on the quality of harvested crops (Kołodziejczyk et al., 2007). Growing consumer awareness concerning negative effect of agrochemicals on their health, and activity of environmental organisations force producers to strictly limit the use of plant protection products (Panasiewicz et al., 2008; Pruszyński, 2009). However, weed, diseases and insects are still inseparable elements of wheat cultivation. They have to be controlled which should be performed with the use of all available methods and techniques in accordance with the principles of environment protection (Korbas et al., 2008). In Polish climate, beneficial characteristics of varieties should be supported with adequate crop management practices, and limitations in the use of production resources contribute to a decrease in grain quality parameters. Grain harvested at a plantation cultivated without protection during years characterized by intensification of pathogens is characterised by decreased milling quality. On the other hand, grain from weed-infested fields is characterised by low protein and gluten content, low gluten quality and high susceptibility to pre-harvest sprouting (Podolska, 2007). Making the most out of the full yield potential of cultivated plants is only possible as a result of the provision of optimum growth conditions (Nowak et al., 2005), which involves, inter alia, proper plant protection (Panasiewicz et al., 2008). Therefore, the decision concerning the cultivation of durum wheat in Polish climate conditions will be based on, inter alia, sensitivity to pathogenic fungi infection and necessity to use of plant protection products (Pląskowska, Chrzanowska-Drożdż, 2008).

The aim of this study was to determine an influence of two systems of managing pests and diseases on the yield, crop structure components and selected physical quality parameters of spring grain of durum and common wheat.

MATERIAL AND METHODS

The field study was carried out in years 2007–2009 in Experimental Farm Felin owned by the University of Life Sciences in Lublin. The experimental plot was located on soil derived from loess silt, which is categorized as good wheat soil complex. The experiment was carried out as

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a randomized block design with four replications. Wheat varieties were classified as first-order factors. A total of 10 levels of the first factor were taken into account: durum wheat (*Triticum durum* Desf.) – cultivars Navigator (Canadian), Lloyd (American), Chado, Izolda, Kharkivska 27, Kharkivska 39, Kharkivska 41 (Ukrainian), Puławska Twarda (Polish) and $LGR_{896/23}$ line selected in the Institute of Plant Genetics, Breeding and Biotechnology, University of Life Sciences in Lublin; spring common wheat (*Triticum aestivium* L.) – Torka cultivar. Various levels of chemical protection were classified as a second-order factor: minimal – seed dressing Oxafun T 75 DS/WS (at 200 g per 100 kg of grain) and herbicide Chwastox Trio 540 SL (at 2.0 $dm^3 \cdot ha^{-1}$ used in the BBCH 29 phase) and comprehensive – seed dressing Oxafun T 75 DS/WS, herbicides Chwastox Trio 540 SL and Puma Uniwersal 069 EW (1.2 dm^3 ha^{-1} – BBCH 24–25), growth regulator Stabilan 750 SL (1.8 $dm^3 \cdot ha^{-1}$ – BBCH 30–31), Alert 375 SC fungicide (1.0 $dm^3 \cdot ha^{-1}$ – BBCH 41–49) and insecticide Decis 2.5 EC (250 $cm^3 \cdot ha^{-1}$ – BBCH 61–69). Cultivation was typical of the ploughing system. Before the sowing the following fertilization was applied: P – 26; K – 66 and N – 40 $kg \cdot ha^{-1}$. The second dose of nitrogen (40 $kg \cdot ha^{-1}$) was introduced at the beginning of the shooting phase. The area of each plot at harvest was 10 m^2. The seeds were sown at a rate of 500 grains per 1 m^2 in a field previously cropped to winter rapeseed.

Prior to the harvest the number of ears in the area of 1 m^2 was assessed. Once the grains had reached full maturity the harvest was made. Yield, grain weight and grain number per ear and TGW were assessed. The grain samples were subjected to laboratory investigation in order to establish bulk density (in accordance to PN-73/R-74007), grain uniformity (in accordance to BN-69/9131-02) and glassiness (in accordance to PN-70/R-74008). The results were statistically analysed by two-way ANOVA at the significance level of $\alpha = 0.05$. LSD was also calculated. Since during the study the reaction of wheat to the factors under investigation was similar; the results were given as three-year averages.

Table 1. Rainfalls and air temperatures according to the Meteorological Observatory at Felin.

Year	Month						
	III	IV	V	VI	VII	VIII	III–VIII
	Rainfalls (mm)						Sum
2007	30.2	17.4	81.5	87.8	87.0	37.6	341.5
2008	64.8	55.8	101.6	25.9	77.1	45.0	370.2
2009	69.6	2.9	71.1	125.5	57.1	54.7	380.9
Mean for 1951–2000	25.8	40.6	58.3	65.8	78.0	69.7	338.2
	Temperature (°C)						Mean
2007	6.2	8.7	15.0	18.1	19.2	18.4	14.3
2008	3.4	9.3	12.8	17.7	18.3	19.3	13.5
2009	1.4	11.4	13.6	16.4	19.9	19.0	13.6
Mean for 1951–2000	1.0	7.5	13.0	16.5	17.9	17.3	12.2

Over the three year-long cycle (Table 1), the year 2007 was the most favourable for spring wheat yields (in the period from May to July high temperatures and rainfall exceeding the long-term average were recorded, which contributed to intensive growth and development. The year 2008 was characterised by above-average rainfall in May and rain deficiency in June compared to the long-term average. The lowest yield and the lowest quality of grain was harvested in the last year of the study – 2009. In that period, severe rainfall deficiency in April and at the beginning of May inhibited the emergence, growth, and development of spring wheat, while subsequent frequent rainfalls contributed to weed infestation.

RESULTS

Regardless of varieties and lines, the use of the minimal level of chemical protection significantly reduced the yield of spring wheat compared to comprehensive protection (Table 2). The decreased use of chemical agents resulted in a decrease in wheat yield by from 14.1% (Izolda) to 29.5% (Kharkivska 41 and $LGR_{896/23}$). The yield of common wheat (6.59 t ha^{-1}) was significantly greater than the yield of durum wheat (5.07 t ha^{-1} on average). Depending on a genotype, the yield of durum wheat was lower than

Table 2. Grain yield and number of ears of spring wheat (means for 2007–2009)

Cultivars and line		Yield of grain [$t \cdot ha^{-1}$]			Number of ears per 1 m^2		
		M	C	mean	M	C	mean
Navigator		4.12	4.87	4.50	383	456	420
Lloyd		4.02	5.38	4.70	345	408	376
Chado		4.78	5.92	5.35	343	405	374
Izolda		5.18	6.03	5.60	368	423	396
Kharkivska 27		4.37	5.86	5.11	340	403	372
Kharkivska 39		4.35	5.43	4.89	301	360	330
Kharkivska 41		4.64	6.59	5.61	351	448	399
Puławska Twarda		4.16	5.77	4.96	394	501	447
$LGR_{896/23}$		4.08	5.79	4.93	362	402	382
Torka		5.91	7.28	6.59	485	552	518
Mean		4.56	5.89	–	367	436	–
$LSD_{0.05}$	a		0.702			55.6	
	b		0.189			15.0	
	a×b		ns			ns	

M – minimal plant protection
C – comprehensive plant protection
a – for cultivars and line
b – for protection levels
a×b – for interaction cultivars and line × protection levels
ns – not significant

the yield of common wheat by 14.9–31.7%. Among the compared cultivars and lines of durum wheat, Ukrainian varieties (Kharkivska 41 and Izolda – respectively 5.61 and 5.60 t ha^{-1}) were proven to be characterized by the greatest productivity. Navigator, Lloyd and Kharkivska 39 varieties were significantly less productive (4,50–4,89 t ha^{-1}). Polish Puławska Twarda variety and $LGR_{896/23}$ line yielded at a similar level; however, the yield was significantly lower than that of the Torka variety.

The intensification of chemical protection in relation to the minimal level of the use of chemical agents resulted in an significant increase in the number of ears per m^2, TGW as well as grain weight and number per spring wheat ear (Table 2 and 3). Regardless of the level of plant protection, the common wheat had significantly higher number of ears per m^2 than all varieties and the line of durum wheat. However, the analysed genotypes showed significant variation in the value of this crop structure components. Kharkivska 39 (330 ears), Lloyd, Chado and Kharkivska 27 varieties as well as $LGR_{896/23}$ line (372–382 ears) were characterised by a small number of ears; while Puławska Twarda showed a significantly larger number of ears (447 ears). It was also observed that the TGW of durum wheat amounted to 43.3 g on average and was greater by 22% compared to the TGW of common wheat. The Ukrainian varieties showed high TGW, especially Kharkivska 39, and the latter proved to

Table 3. Yield structure elements of spring wheat.

Cultivars and line		1000 grain weight (TGW) [g]			Number of kernels per ear			Weight of grains per ear [g]	
	M	C	mean	M	C	mean	M	C	mean
Navigator	42.5	42.8	42.6	25.9	26.3	26.1	1.099	1.103	1.101
Lloyd	41.5	43.7	42.6	28.0	30.8	29.4	1.167	1.371	1.269
Chado	44.4	45.8	45.1	32.8	33.7	33.2	1.455	1.521	1.488
Izolda	43.4	43.4	43.4	32.3	33.7	33.0	1.384	1.448	1.416
Kharkivska 27	43.2	45.9	44.5	30.3	32.7	31.5	1.342	1.476	1.409
Kharkivska 39	46.0	47.6	46.8	32.2	33.0	32.6	1.451	1.522	1.486
Kharkivska 41	44.4	46.3	45.3	31.4	33.0	32.2	1.394	1.510	1.452
Puławska Twarda	37.3	39.2	38.3	29.4	30.7	30.1	1.074	1.189	1.132
$LGR_{896/23}$	39.3	42.3	40.8	29.7	34.4	32.1	1.158	1.471	1.314
Torka	35.0	36.1	35.5	35.9	37.7	36.8	1.229	1.337	1.283
Mean	41.7	43.3	–	30.8	32.6	–	1.275	1.395	–
$LSD_{0.05}$ a		3.18			4.15			0.2552	
$LSD_{0.05}$ b		0.86			1.12			0.0684	
$LSD_{0.05}$ a×b		ns			ns			ns	

Explanations in Table 2

Table 4. Quality of spring wheat grain.

Cultivars and line		Test weight [kg m^{-3}]			Grain uniformity [%]			Grain glassiness [%]	
	M	C	mean	M	C	mean	M	C	mean
Navigator	726	747	736	86.1	90.3	88.2	77.6	81.4	79.5
Lloyd	695	721	708	75.6	81.2	78.4	77.1	79.9	78.5
Chado	735	764	750	91.1	92.6	91.8	75.3	79.8	77.5
Izolda	763	768	766	88.8	90.5	89.7	70.6	75.9	73.3
Kharkivska 27	722	742	732	84.7	89.9	87.3	68.7	75.7	72.2
Kharkivska 39	749	756	752	92.8	94.5	93.6	69.9	74.1	72.0
Kharkivska 41	722	738	730	89.5	92.5	91.0	70.2	77.3	73.8
Puławska Twarda	768	786	777	85.4	87.5	86.5	64.0	69.4	66.7
$LGR_{896/23}$	710	735	723	80.5	84.7	82.6	80.0	83.0	81.5
Torka	750	776	763	75.6	79.7	77.6	39.3	44.8	42.0
Mean	734	753	–	85.0	88.3	–	69.3	74.1	–
$LSD_{0.05}$ a		25.6			6.31			7.26	
$LSD_{0.05}$ b		6.9			1.71			1.96	
$LSD_{0.05}$ a×b		ns			ns			ns	

Explanations in Table 2

be significantly more advantageous than the Polish line and variety as well as Navigator, Lloyd and Izolda. The number of grains per ear in common wheat was greater by 18.2% on average compared to durum wheat. The Ukrainian varieties were characterised by a significantly greater number of grain per ear than the Navigator variety. On the other hand, grain weight per ear of Ukrainian cultivars was significantly greater than that of Navigator and Puławska Twarda varieties.

Bulk density, uniformity and glassiness were to a large extent determined by the level of plant protection and genetic characteristics (Table 4). The intensification of chemical protection resulted in an increase in all the analysed grain quality factors. The varieties that showed the lowest bulk density and grain uniformity was $LGR_{896/23}$ and Lloyd while Chado and Kharkivska 39 showed significantly greater values of those indicators. Grain that showed the greatest bulk density was that of Puławska Twarda and Izolda – respectively 777 and 766 kg m^{-3}. A large percentage of glassy grain was found in $LGR_{896/23}$ line (81.5%) and in Navigator, Lloyd and Chado (77.5–79.5%). The grain of the Polish variety Puławska Twarda was significantly less glassy (66.7%). It was found that the grain of durum wheat was slightly more uniform (by 10.1 percentage points on average) and significantly more glassy (by 33.0 pp on average).

DISCUSSION

One of the most important factors of the comprehensive wheat management system critical for high grain yield is plant protection (Rachoń et al., 2002). Spring durum wheat and common wheat reacted to the use of full chemical protection by an increase in yield by 22.6% on average. The results obtained by Rachoń et al. (2002) show that the use of fungicide, insecticide and retardant resulted in an increase in yield by 1.15 t ha^{-1}. Also other studies showed greater wheat yield achieved through the use of comprehensive plant protection (Podolska et al., 2004) or in better crop management conditions (Kołodziejczyk et al., 2007, Kwiatkowski et al., 2006). On the other hand, according to a study carried out by Pląskowska, Chrzanowska-Drożdż (2008) and Woźniak (2006), protection intensity did not influence the yield of durum wheat. The analysis of the influence of applied plant protection products on ear number per m^2, TGW and grain weight and number per ear showed a significant increase in the value of these elements after the application of comprehensive protection. Other authors have found a similar beneficial influence of intensification of the use of chemical agents on the size of wheat grain (Ciołek, Makarska, 2004), number of ears per m^2 (Kołodziejczyk et al., 2007), grain number per ear (Kwiatkowski et al., 2006) and grain weight per ear (Rachoń et al. 2002).

Rachoń (1997), summarizing results of other studies, said that cultivation of durum wheat is profitable, when its yields are from 70–75% of those of common wheat. In the presented studies, the yield of durum wheat made up from 68.3% to 85.1% of the yield of common wheat. The potential yield of durum wheat smaller than that of common wheat has been also shown by other authors (Rachoń, 1999; Rachoń et al., 2002; Rachoń, Szumiło, 2006; Segit, Szwed-Urbaś, 2009; Woźniak, 2006). The higher yield of *Triticum aestivum* compared to *Triticum durum* is primarily attributable to a significantly grater number of ears, as highlighted in other papers (Rachoń, 1997, 1999; Szumiło, Rachoń, 2008; Woźniak, 2006). According to the literature (Ciołek, Makarska, 2004; Rachoń, 1997, 1999; Rachoń, Szumiło, 2002), a basic quality parameter – TGW – is higher in durum wheat than in common wheat. A similar relation has been found in this study. Grain number and weight per ear of the compared varieties and lines of durum wheat varied, which was also observed by Rachoń et al. (2002).

The analysis of this experiment shows a significant increase in the values of physical quality indicators of wheat after application of comprehensive chemical protection. According to Ciołek and Makarska (2004) the application of pesticides has only a slight influence on glassiness of durum wheat grain. Similarly, Nowak et al. (2005) and Kwiatkowski et al. (2006) found that wheat grain tends to improve its filling after the intensification of chemical application. However, experiments carried out by Podolska et al. (2004) show that the intensification of chemical protection had a positive influence on bulk density. Quality parameters were also determined by the genetic factor, which has a strong influence on grain quality. A variety carries information about its potential quality, which can be achieved in typical weather and agricultural conditions (Podolska, 2007). The research carried out by Rachoń (1997, 1999) and Szwed-Urbaś et al. (1995) shows greater bulk density of common wheat than that of durum wheat, which in the case of most of the compared genotypes was also observed in this study. On the other hand, durum wheat showed slightly higher grain uniformity than common wheat. Similar relation can be found in the literature (Rachoń, 1999). Also grain glassiness, among other quality parameters, determines the quality of durum wheat. The greater the value the better the processing characteristics, since milling results in obtaining more groats than flower. In milling industry to produce good quality semolina, hard and glassy grain is required, since it allows coarse semolina to be produced, which is characterised by low water absorption – desirable quality in pasta production (Segit, Szwed-Urbaś, 2009). In this study, durum wheat grain has been proven to be significantly more glassy compared to common wheat grain, which has been confirmed by other studies (Ciołek, Makarska, 2004; Rachoń, Szumiło, 2002).

CONCLUSIONS

1. The application of comprehensive plant protection, compared to limited protection, increased the spring durum wheat yield by 22.6% on average. An increase in yield was caused by an increased ear number per m^2, TGW, grain number and weight per ear.

2. The limitation of chemical protection resulted in a significant reduction of bulk density, glassiness, and uniformity of spring wheat grain.

3. Depending on a genotype the yield of spring durum wheat made up from 68.3% to 85.1% of that of common wheat.

4. Durum grain was characterised by greater TGW and glassiness and slightly higher uniformity compared to common wheat grain.

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The evaluation of grain and flour quality of spring durum wheat (*Triticum durum* Desf.)

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Abstract. The quality of grain and baking properties of the flour from spring durum wheat (*Triticum durum* Desf.) were evaluated (one breeding line and three cultivars of the durum wheat along with a common wheat (*Triticum aestivum* L.) used as a benchmark). The research material for the investigation came from the field experiments conducted in soil and climate conditions of Lublin voivodship. The scope of the investigation included the evaluation of the physical and chemical indicators of the grain and flour quality: 1000 grain weight, test weight (kg hl^{-1}), the percentage of grain fractions, falling number, content and weakening of the gluten and Zeleny test for the sedimentation rate. The baking properties of the flour on the basis of the analysis of the selected rheological characteristics were also identified. The flour water absorption, resistance and softening of the dough and the valorimetric value of the flour were estimated. Durum spring wheat, exceeded common spring wheat in terms of 1000 grain weight, grain accuracy, the quantity of gluten and water consumption of the flour. By contrast, the falling number and the sedimentation test were higher in the common wheat. The rheological properties (water absorption of the flour, resistance and softening of the dough, the valorimetric value) indicate a good or at least sufficient technological quality of the flour obtained from milling the grain of spring durum wheat. Within the comparison of the durum wheat genotypes, Chado cultivar was distinguished by favourable physical parameters of grain, low weakening of gluten and good results of the farinographic assessment.

key words: spring durum wheat, grain quality, flour quality, rheological properties of dough

INTRODUCTION

All over the world there is a tendency to increase the consumption of products from durum wheat, which are considered as healthy, wholesome and nutritious. The most popular articles with a grain of this species are pasta and couscous (Elias, 1995). Some parts of this grain are milled to wheat flour destined for the bread production. Hence, of interest is not only the pasta value but also the baking value of the grain (Gąsiorowski, Obuchowski, 1978). Bread from durum wheat is produced in the Middle East and North Africa on a grand scale, where almost half of the grain of durum wheat is used to produce different types of bread. Moreover, in Europe, especially in Italy, regional bakery wares are made from that wheat (Boggini et al., 1995; Elias, 1995). The demand for this type of bread is increasing due to its specific characteristics (Palumbo et al., 2000). The bread produced from durum wheat compared to common wheat bread is usually characterized by a slower staling, and thus a longer shelf life, taste and pleasant aroma and a more aesthetic peel and yellow crumb (Golik, 2000; Raffo et al., 2003). However, that cereal also shows undesirable technological characteristics in the raw material destined for the bakery, which can be largely attributed to the specificities of the grain gluten proteins (Elias, 1995; Pasqualone et al., 2004). The differences in the share of each protein fraction contributes to the fact that the dough of durum wheat has different rheological properties from the dough of common wheat (Gąsiorowski, Obuchowski, 1978), which is closely connected with the smaller volume of durum wheat bread (Hareland, Puhr, 1998; Szumiło et al., 2009). However, with certain genotypes of wheat with strong gluten, showing a good balance between flexibility and elongation of dough you can still get the correct volume and appearance of the bread, so in this respect they are similar to high-quality bread wheat (Edwards et al., 2007). Thus, it is advisable to increase knowledge of the baking quality of durum wheat, which may increase the commercial value of this species and open alternative markets (Boggini et al., 1995).

The aim of this study was the evaluation of the grain quality and flour baking properties of selected genotypes of spring durum wheat in comparison with spring common wheat.

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MATERIAL AND METHODS

The experimental material consisted of spring wheat grains derived from one of the field experiments conducted from 2007 to 2008 by the Department of Plant Cultivation in the Felin Experimental Farm (51°22' N, 22°64' E), belonging to the University of Life Sciences in Lublin. The field experiment was conducted on a soil classified into good wheat complex, on the stand after the winter rape. The soil tillage was typical for the plough system. Nitrogen, phosphorus and potassium fertilization amounted to: P – 26, K – 66 and N – 40 kg ha^{-1} and was applied before sowing. The second dose of nitrogen was applied as a top- dressing at the rate of 40 kg ha^{-1}. Weed control was achieved by the use of herbicides Puma Uniwersal 069 EW and Chwastox Trio 540 SL. Stabilan 750 SL was used to prevent lodging in cereals. Fungal diseases and pests were combated by using the formulas: Alert 375 SC and Decis 2.5 EC respectively.

The breeding line $LGR_{896/23}$ of the hard wheat (*Triticum durum* Desf.) was used for analysis (selected at the Institute of Genetics, Plant Breeding and Biotechnology, UP Lublin, Poland) and Lloyd cultivar (American one), and Chado and Kharkivska 27 (Ukrainian cultivars). The grains of common wheat (*Triticum aestivum* L.) cultivar Torka were used as a benchmark. The scope of investigation included the evaluation of the physical and chemical indicators of the grain and the flour quality and baking properties of the flour on the basis of analysis of selected rheological characteristics of the dough. The evaluation of the physical characteristics of the grain was made according to the standard methods (Jakubczyk, Haber, 1983). After prior purification of the raw material and bringing the material up to the optimum moisture a few traits were determined: 1000 grain weight (TGW), test weight (kg hl^{-1}), and share of grain thickness fractions. Test weight was measured by a densitometer, using the measuring container with a volume of 1000 cm^3. The grain was separated by Vogel sieves into four thickness fractions: >2.8 mm, 2.5–2.8 mm, 2.2–2.5 mm and <2.2 mm. The flour were obtained by milling the grain in the laboratory mill (type QC-109). The following traits were determined: the falling number – by Hagberg-Perten method (apparatus type SWD-83), the gluten content – by method of the leaching of the gluten from the dough by sodium chloride (by the mechanical device for measuring gluten concentration type SŻ), then the following traits were evaluated: weakening of the gluten and Zeleny test for the sedimentation rate. Furthermore, analyses of flour and dough were led by farinograph, on the basis of the generally accepted methods (Jakubczyk, Haber, 1983): flour water absorption, the dough resistance and the valorimeric value of the flour – with a template (valorimeter).

The course of weather conditions in years of study (2007 and 2008) was much diversified. In 2007, April was distinguished by the deficiency of rainfall, which adversely affected the germination of wheat. However, in the period from May to July a high temperature and rainfall in excess of the long-term average were recorded (1951–2000). These conditions favoured the intense growth and development of the plants. April in 2008 was warm, with rainfall surpassing long-term norm, thus promoting germination of cereals. May was characterized by moderate temperatures and a large excess of rainfall, whereas in June shortages of rainfall occurred. However, the temperature and the precipitation in July were similar to long-term normal.

The significance of differences between mean values of quality indicators were evaluated by analysis of variance at the significance level $\alpha = 0.05$ and the least significant difference was determined using Tukey's test.

RESULTS

The grain of all durum wheat entries (the cultivars and the line) stood out significantly with the greater weight of 1000 grain (an average of 31.3%) than that of cultivar Torka (Table 1). Similarly, test weight of grain varied significantly with the genetic factor. It was observed that the values of this index of the grain quality were at a high level, both in the case of the durum wheat (802–815 kg m^{-3}), and the common wheat, which indirectly allows to forecast a good technological quality of the grain. In cereals harvested in 2008, a higher test weight and bigger TGW were found in comparison to 2007. By comparing the percentages of the individual fractions in the grain of the durum wheat and the common wheat it was found that the common wheat was characterized by a greater participation of small grains (the fractions less than 2.5 mm) and medium-sized grain (2,5–2,8 mm). Among the durum wheat genotypes, a large share of the average grain fractions was recorded in line $LGR_{896/23}$ and Lloyd cultivar, and the most plump grain (fraction above 2.8 mm) was found in Ukrainian cultivars, especially in Chado cultivar.

The falling number of flour from grain of durum wheat cultivars and lines was low (Table 2), indicating a high activity of amylolytic enzymes. The flour obtained from common wheat was characterized by nearly twice as high value of the falling number (low activity of alpha-amylase), and smaller quantities of gluten (on average less by 8.0 percentage points) than the durum wheat flour. The significant variation of gluten amount in flour among the cultivars and lines of durum wheat was found. Line $LGR_{896/23}$ and Lloyd cultivar were characterized by a greater gluten yield than the Chado and Kharkivska 27 cultivars. However, the gluten weakening ranged from 0.8 to 2.9 mm depending on the genotype of durum wheat and was significantly higher in the Kharkivska 27 and Lloyd cultivars than $LGR_{896/23}$ line and Chado cultivar. The sedimentation index determined for durum wheat genotypes was on average 14.0 cm^3, thus indicating insufficient baking quality of the flour. A significantly higher value of the sedimentation test

Table 1. Physical traits of spring wheat grain.

Line and cultivars		1000 grain weight (TGW) [g]			Test weight [kg m^{-3}]			Fractions of grains [%]			
		2007	2008	mean	2007	2008	mean	<2.2 mm	2.2–2.5 mm	2.5–2.8 mm	>2.8 mm
LGR $_{896/23}$		37.5	46.2	41.9	779	825	802	3.8	10.7	23.5	62.0
Lloyd		44.5	46.7	45.6	809	813	811	3.6	10.7	22.3	63.4
Chado		42.9	51.4	47.1	791	839	815	1.0	3.9	12.3	82.8
Kharkivska 27		44.2	48.4	46.3	796	821	809	2.2	7.4	20.1	70.3
Torka		31.4	37.5	34.5	765	818	792	5.6	19.3	40.5	34.6
Mean		40.1	46.0	–	788	823	–				
$LSD_{0.05}$	a		1.41			8.4		–			
	b		0.62			3.7					
	a×b		2.00			11.9					

a – for line and cultivars
b – for years
a×b – for interaction: line and cultivars × years
ns – not significant

Table 2. Flour quality parameters of spring wheat.

Line and cultivars		Falling number [s]			Gluten content [%]			Gluten weakening [mm]			Zeleny test [cm^3]		
		2007	2008	mean	2007	2008	mean	2007	2008	mean	2007	2008	mean
LGR $_{896/23}$		102	201	152	31.5	34.1	32.8	1.1	1.7	1.4	14.5	11.4	12.9
Lloyd		101	226	164	31.9	35.7	33.8	1.8	4.0	2.9	14.8	12.1	13.4
Chado		111	181	146	25.8	36.6	31.2	0.4	1.2	0.8	13.5	15.5	14.5
Kharkivska 27		116	172	144	26.3	36.0	31.2	1.6	3,0	2.3	15.2	14.9	15.1
Torka		336	254	295	26.2	22.3	24.3	0.3	0.5	0.4	45.4	31.1	38.3
Mean		153	207	–	28.4	33.0	–	1.0	2.1	–	20.7	17.0	–
$LSD_{0.05}$	a		20.1			1.45			0.36			2.34	
	b		8.8			0.64			0.16			1.03	
	a×b		28.5			2.05			0.51			3.31	

Explanations in Table 1

Table 3. Physical properties of the flour and the dough from spring wheat.

Line and cultivars		Water absorption [%]			Dough resistance [min]			Dough weakening [B.U.]			Valorimeter value [j.u.]		
		2007	2008	mean	2007	2008	mean	2007	2008	mean	2007	2008	mean
LGR $_{896/23}$		70.0	68.8	69.4	6.94	7.67	7.31	23	17	20	66	69	68
Lloyd		72.7	70.8	71.7	5.28	5.14	5.21	50	50	50	56	56	56
Chado		67.9	68.7	68.3	3.35	12.22	7.79	23	3	13	56	83	70
Kharkivska 27		67.2	69.4	68.3	5.25	4.58	4.92	58	73	66	56	52	54
Torka		65.1	61.7	63.4	9.47	2.45	5.96	14	43	29	75	49	62
Mean		68.6	67.9	–	6.06	6.41	–	34	37	–	62	62	–
$LSD_{0.05}$	a		2.44			0.629			8.3			2.4	
	b		ns			0.276			ns			ns	
	a×b		ns			0.890			11.7			3.5	

Explanations in Table 1

was found in the common wheat which is closely connected with the good quality of gluten. The flour with a greater falling number along with the quantities and weakening of the gluten was obtained from the grain of the cultivars and lines of the durum wheat harvested in 2008.

The quality of flour obtained by milling of the durum wheat grain was determined by the farinograph, based on the examination of the physical characteristics of the dough at the time of its creation – Table 3. Evaluated genotypes significantly affected the rheological properties such as water absorption of the flour, resistance and softening of the dough. The common wheat flour showed the lowest water absorption, whereas the flour of Lloyd cultivar was characterized by increased water absorption compared to the Ukrainian cultivars. The dough of Chado cultivar and $LGR_{896/23}$ line stood out with a good resistance and small weakening among the compared genotypes of durum wheat. A resistance and softening of the dough from common wheat flour in comparison with durum wheat genotypes had intermediate values. The average valorimeter value of the flour from durum wheat was 62 u. The flour from $LGR_{896/23}$ line, Chado durum wheat cultivar and Torka common wheat cultivar were characterized by a good valorimeter value, whereas the valorimeter value of the flour from grain of Kharkivska 27 and Lloyd cultivars was assessed as satisfactory. In 2008, Chado cultivar showed a greater resistance of the dough and a valorimeter value than in 2007, but smaller weakening of the dough value.

DISCUSSION

The results of investigation show a significantly greater weight of 1000 grains of durum wheat than TGW of common wheat. The smaller plumpness of common wheat grain as compared to hard wheat was also observed in other studies (Ciołek, Makarska, 2004; Rachoń, Szumiło, 2002). The better filling of the durum wheat grain causes its dimensional weight, to be about 10 kg m^{-3} higher than other wheat (Gąsiorowski, Obuchowski, 1978). A similar regularity is indicated by the results of this study. According to Cacak-Pietrzak et al. (2005b) in the spring wheat (*Triticum aestivum*) yield, percentage fraction of grains with a thickness greater than 2.8 mm is lower than the fraction 2,5–2,8 mm. It was confirmed by the researchers' investigations. However, in the case of *Triticum durum* grains the situation is reversed.

In determining the grain quality attention is paid to the enzymatic properties, especially to the amylolytic activity, which is characterized by the falling number. It defines the usefulness of the tested grains for further usage (Knapowski, Ralcewicz, 2004). This ratio depends on the genetic properties of the cultivar and the weather conditions during ripening and harvesting of the grain (Cacak-Pietrzak et al., 2005a). Relatively low values of the falling number were obtained for the flour from the cultivars and lines of durum wheat, which indicates the high activity of amylolytic enzymes and reduces the usefulness of the tested grain for bread production. The higher amount of gluten and its enhanced quality demonstrates the suitability of the flour for baking the bread (Podleśna, Cacak-Pietrzak, 2006). Under the experimental conditions durum wheat was characterized by higher amount of wet gluten than common wheat. This is confirmed in other studies (Ciołek, Makarska, 2004; Rachoń, Kulpa, 2004; Rachoń, Szumiło, 2002; Woźniak, 2006). One of the features which indicates the quality of gluten is the weakening. Gluten characterized by good quality should have a low weakening value – less than 10 mm (Podolska, 2007). The results show that in terms of quantity of wet gluten and its weakening, all genotypes of durum wheat meet the requirements for raw materials for baking. By contrast, the low sedimentation rate of the wheat (Zeleny test) noted in this research tends to the opposite conclusion. The higher values of this parameter in durum wheat were noted by other authors (Woźniak, 2006; Woźniak, Staniszewski, 2007).

According to Gąsiorowski and Obuchowski (1978) stresses occurring during the milling of grains of *T. durum* often affect the starch granules which are very sensitive to the mechanical action, so that they are substantially damaged. In turn, the degree of starch damage in wheat flour has a significant impact on its water absorption, which implies the damaged starch granules absorb much more water (Laskowski, Różyło, 2004; Sapirstein et al., 2007). As it was expected, the flour made from the grain of the compared genotypes of the durum wheat absorbed more water than the common wheat flour. The physical properties of the dough from *Triticum durum* are similar to properties of the dough of "average" to "very weak" common wheat. The dough of durum wheat compared to the dough of the common wheat is not so strong. The reason is that the gluten from durum wheat flour is weaker than the common wheat gluten (Gąsiorowski, Obuchowski, 1978). In the authors' research, the dough made of flour from durum wheat cultivars (Chado) and line ($LGR_{896/23}$) was characterized by good resistance and low softening, which is due to the weakening of the structure of dough, mainly gluten (Rachoń, Kulpa, 2004). However, the flour from grain of these genotypes was distinguished by high valorimeter value, which demonstrates the usefulness of the studied raw materials for bread baking. Nevertheless, to fully assess the baking usefulness of durum wheat grown under soil and climatic conditions of Poland, further studies are needed.

CONCLUSIONS

1. Spring durum wheat, exceeded spring common wheat in terms of thousand grain weight, grain accuracy, the quantity of gluten and water absorption of the flour. However, the common wheat was characterized by far higher values of falling number and rate of sedimentation.

2. The rheological properties (water absorption of the flour, resistance and softening of the dough) indicate a good technological quality of flour from the milling of the grain of spring durum wheat.

3. Within the compared genotypes of durum wheat cultivars, Chado cultivar was distinguished by favourable physical parameters of the grain, low gluten weakening and good results of the farinographic assessment.

4. Preliminary results indicate the usefulness of durum wheat grain for bread making, but further research should be done related to baking process.

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