ISSN 2081–2787

Institute of Soil Science and Plant Cultivation
National Research Institute

No. 3
December 2010

Polish Journal of Agronomy

formerly

PAMIĘTNIK PUŁAWSKI

Puławy, POLAND

Polish Journal of Agronomy
(formerly Pamiętnik Puławski)

Polish Journal of Agronomy is a new nationwide English-language scientific journal. It is launched by the Institute of Soil Science and Plant Cultivation – National Research Institute. The new journal is a continuation of the former Pamiętnik Puławski. Its scope will cover topics connected to broad-sense crop production and agriculture-related environmental issues. The peer-reviewed journal will publish original research papers and invited review articles.

Published by Institute of Soil Science and Plant Cultivation – National Research Institute in Puławy, Poland
Director: *Wiesław Oleszek*

No. 3 **December 2010**

CONTENTS

Printed in Department of Extension and Information
Institute of Soil Science and Plant Cultivation – National Research Institute
Puławy, Poland

Polish Journal of Agronomy
2010, 3, 3–9

Visible and near-infrared spectrophotometer for soil analysis: preliminary results

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Abstract. Precision farming, a fast growing technique, is based on within-field variability at the field or farm scale and demands some information about soil physical and chemical properties. In this paper, we present the use of visible and near infrared reflection spectroscopy (VIS-NIRS) in the 400–2200 nm spectral range to predict soil acidity, available Mg, P, and K content, soil organic carbon (SOC) content, and soil clay fraction (<0.002 mm) content, and then present an on-the-go spectrophotometer for in situ measurements of reflectance spectra. Some optimistic preliminary results have been obtained for the prediction of SOC, clay, available Mg, and K content with r^2 (predicted vs. measured values) varying from 0.64 to 0.69. Results also emphasise the importance of the calibration scheme.

key words: precision agriculture, visible and near infrared spectroscopy (VIS-NIRS), soil organic carbon (SOC), partial least square regression (PLS), on-the-go spectrophotometer

INTRODUCTION

Management of agricultural systems is dependent on demands to control the cost of production and to increase productivity. To obtain a better response from inputs in agriculture, numerous analyses are needed so that those inputs can be applied where they best fill their purpose (van Vuuren et al., 2006). VIS-NIRS technology has the potential to detect fine-scale spatial variability of soil. Furthermore, results can be very accurate as Viscarra Rossel and McBratney (2008) have shown in their review. Unfortunately, transition from standard soil testing approach to the adoption of VIS-NIRS method for precision agriculture need a shift of mentality as underlined by van Vuuren et al. (2006).

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Received 3 August 2010

VIS-NIRS has been used in agriculture for assessing grain, fertilisers and soil qualities (Ben-Dor and Banin, 1995; Faraji et al., 2004; Mohan et al., 2005) and has proven to be a rapid, convenient means of analysing many soil constituents at the same time. Soil properties that have been calibrated with VIS-NIRS include the determination of soil moisture, SOC content, electrical conductivity (EC), cation exchange capacity (CEC), soil acidity, some macro- and microelements (Dunn et al., 2002; Velasquez et al., 2005).

Absorption in the near-infrared spectral region (780–2500 nm) is dominated by molecules that contain strong bonds between light atoms. Specifically, these are molecules that contain C-H, N-H or O-H bonds. This makes the near infrared region particularly useful for measuring forms of carbon, nitrogen and water. VIS-NIRS is a rapid and non-destructive analytical technique that correlates diffusely reflected near-infrared radiation with the chemical and physical properties of materials (Chang and Laird, 2002). One interesting advantage of VIS-NIRS is that the size of spectrometers is rather small so that they can be field-portable (Christy, 2008).

The objective of this work was to investigate the usefulness of VIS-NIRS in determining various soil chemical property (SOC content, soil acidity, content of available Mg, K, and P) and a single physical properties (clay content) in topsoil (0–25 cm) from a soil sampling grid field. With that aim in mind, two calibration schemes have been elaborated. Results discrepancies between the two calibration schemes are discussed in relation to predicted sample localisation and soil texture variability.

MATERIAL AND METHODS

Sample collection and preparation

One hundred and twenty soil samples were collected from a soil sampling grid (Figure 1) in the experimental station of the Institute of Soil Science and Plant Cultivation

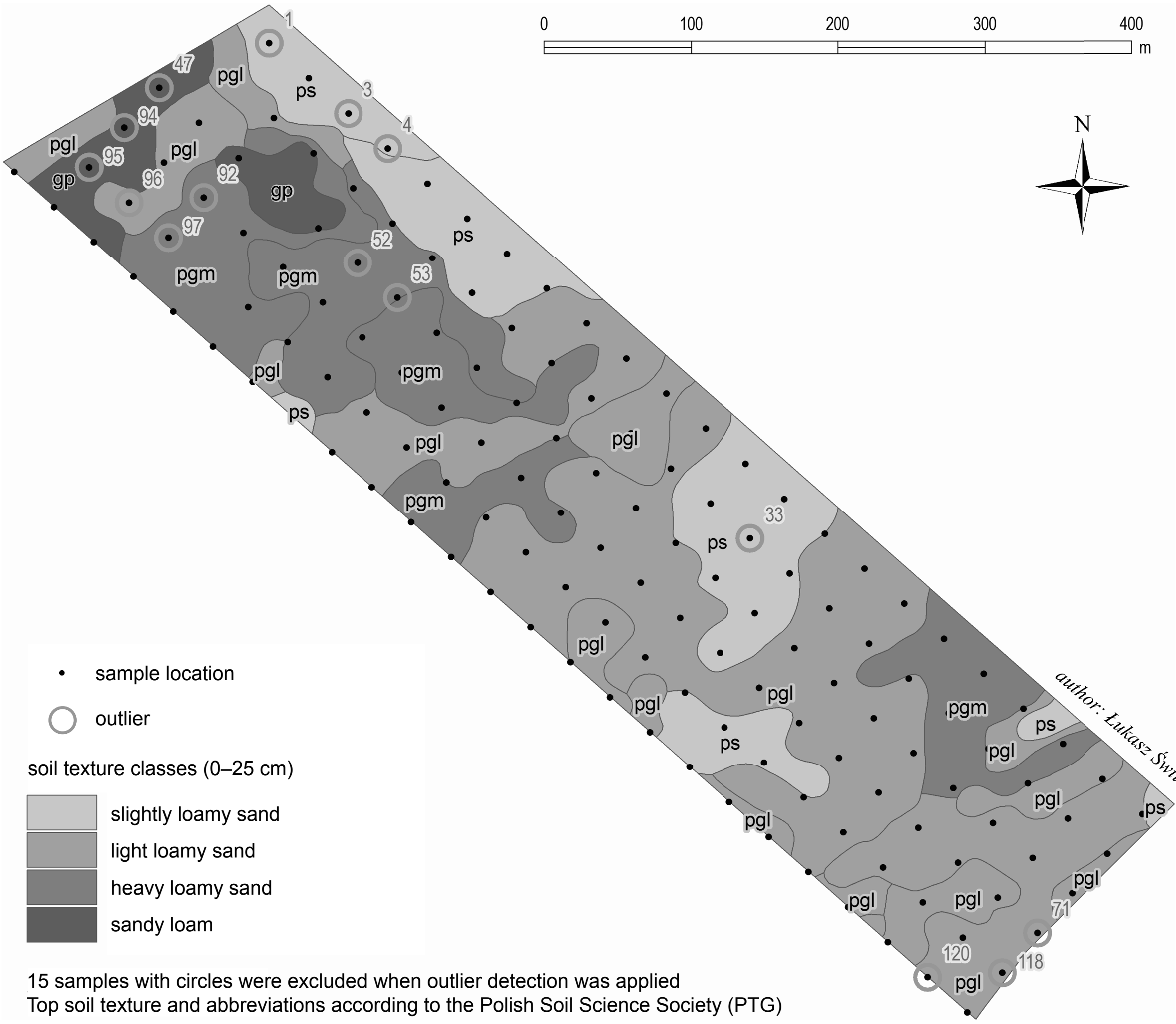


Fig. 1. Map illustrating the Baborówko field and sample locations

(IUNG) in Baborówko near Poznań (Wielkopolska, Poland). The grid belongs to field nº1 the area of which is 9 ha. The station conducts its field trials on an area of 53,6 ha (3 fields) in the following crop rotation: winter wheat, spring barley and winter rape according to the rules of precision agriculture. IUNG uniform agrotechnics recommendations for specific crop technologies and integrated agriculture rules are applied. The soil type at this station is a podzoluvisol (World Reference Base for Soil Resources – WRB). A considerable topsoil texture mosaic is observed on the whole research area but also on the field nº1 in question. The soil grid is georeferenced by means of a global positioning system. Coordinates of the grid centre are N 52º35'1.6", E 16º38'50.47". Samples were collected in the topsoil (0–25 cm), air dried and no further treatments were applied in order to be able to compare results with on-the-go measures in the future.

Chemical analyses

Chemical analyses of the samples were performed by the IUNG Main Chemical Laboratory in Puławy. The soil acidity values were measured potentiometrically in 1M KCl solution. Soil organic carbon content was determined by the Tiurin method (K dichromate digestion). The contents of available K, P, and Mg (mg 100 g^{-1} of soil) were determined in calcium lactate-extractable K, calcium lactate-extractable P, and calcium chloride-extractable Mg. The Mastersizer 2000 apparatus with Hydro MU attachment from the Malvern Company was used for the laser diffraction method for particle size distribution. The Mastersizer 2000 is designed for standard determination of grain size distribution of particles within the size range of 0.002–2 mm. It makes use of laser light scattered on measured particles and converts it into particle size distribution. Measurements were made in three replications (a new portion of air-dry soil poured into the measuring system being treated as a replication). Calculations of particle size distribution were carried out using the Fraunhofer and Mie theories (in the case of Mie theory the refractive index of 1.57 and absorption index of zero were used). The laser light wavelength in the apparatus was 466 nm for blue and 633 nm for red light. Measurements (understood as averaging of 30 000 images of laser light diffraction recorded by the detectors) lasted 60 s (30 s for blue and 30 s for red light) and were carried out directly one by one.

System overview

The samples were scanned from 400 to 2220 nm with the Veris VIS-NIR spectrophotometer in bench top mode (Veris Technologies, Salina, KS, USA) by means of the Veris Spectrophotometer Software V1.69. The sample holder is placed against the face of the sapphire window of the shank module that contains a tungsten halogen lamp and fibre optics for transmission to the spectrometer. Samples were scanned 20 times and averaged by the software and data were collected every 5.5 nm of the electromagnetic spectrum. The absorbance of the scanned sample is given by the relation log (1/R) where R is the reflectance.

On-the-go machine

The Veris spectrophotometer acquires absorbance measurements of soil while being pulled through the field. It is built in a shank mounted on a toolbar, and pulled behind a tractor. The shank is lowered into the ground to approximately 7 cm and pulled through the soil at 6 to 10 km h^{-1}. The device makes measurements through a sapphire window mounted on the bottom of the shank. The texture of the sapphire keeps it clean through its journey into the soil. The device is described at length in Christy (2008). The main feature of this spectrophotometer is the possibility to perform real-time measurements via NIR spectroscopy. Another interesting feature is that all spectra pretreatments are realised on the field i.e. (1) data extraction, (2) filtering, (3) clustering. Outliers are removed during the filtering process using Mahalanobis distance. The aim of these 3 steps is to determine the best soil sampling location. According to spectral properties and principal component analysis, with the help of a fuzzy c-means algorithm, clusters of observations are realised. There are as many samples locations as clusters. Each cluster is representative of the overall spectral variation (Naes, 1987). The number of clusters decided by the operator. The sample location is computed to be close to the centre of the cluster. These samples are needed to create VIS-NIRS calibrations for quantitative predictions. The last treatment is the interpolation using Gaussian weighting to average spectra near a sampled location. Spectra near the location are weighted higher than spectra far away. Then the NIR calibration and validation methods are to be chosen by the operator and laboratory computed.

VIS-NIRS calibration

The spectrum from each sample was matched with laboratory analysis data to create a database for calibrations. A multivariate calibration model is required to obtain some practical information from the VIS-NIRS spectra. The Partial Least Square Regression (PLS), a popular multivariate calibration technique for quantitative analysis of NIR spectral data, was used to determine the best correlation between the chemical data and spectra data. The PLS is a dimension reduction technique that seeks a set of latent variables by maximizing the covariance of two variable blocks (i.e., spectra X and concentration Y). Data were calibrated and tested using the R software – Version 2.11.1 (R Development Core Team, Vienna) and the R *pls* package from Mevik and Wehrens (2007). The *pls* package implements a leave-one-out cross-validation on the calibration set. Ten components are taken into account for the PLS regression of the calibration models. In leave-one-out cross-validation each sample is omitted and predicted using a calibration made from the remaining samples. The number of components to use for validation is given by the root mean squared error of prediction (RMSEP) of the calibration set. The calibration models were then used to predict the chemical parameters of the sample prediction sets.

Two sets of calibration were prepared without outlier treatment and two sets with outlier detection based on a new method from Filzmoser et al. (2005). The method is automated to identify outliers in multivariate space and to distinguish between extreme values of a normal distribution and values originating from a different distribution.

RESULTS

Three samples were not analysed for all chemical properties and therefore were removed from the sample database leaving 117 samples. Sample names have not been changed so that they were easier to position on the map. Mean, standard deviation and ranges for each chemical and physical parameters are shown in Table 1. The SOC values displayed a narrow range 0.71–1.45% conforming with typical content levels of Polish soils. The mean clay content is generally lower than the usual content of Polish soils.

Ten randomly selected soil sample spectra are presented in Figure 2. As can be seen, the spectral bands largely

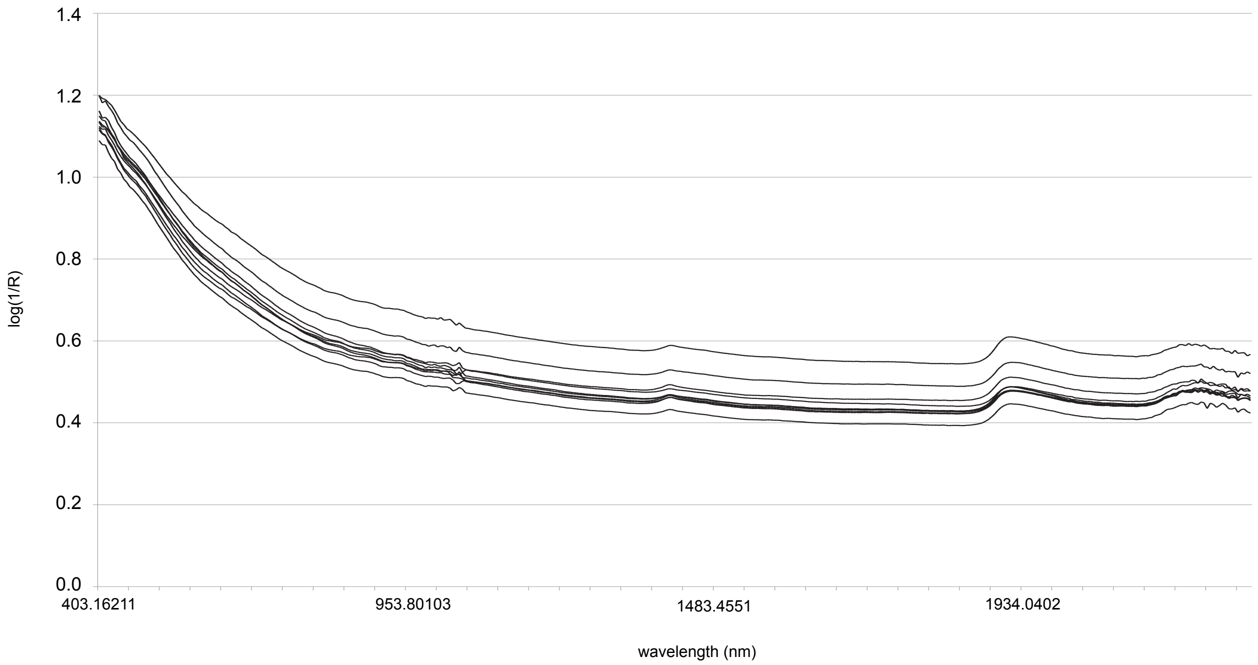
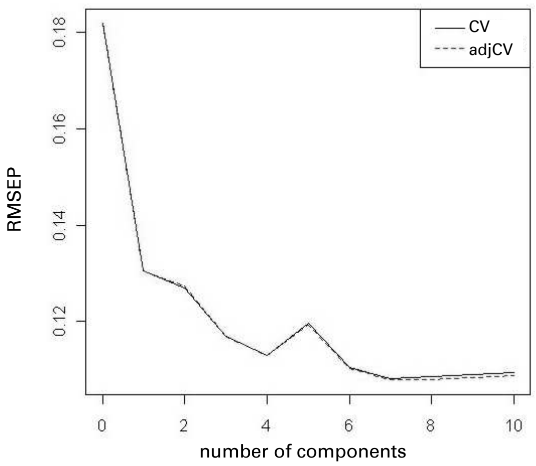


Fig. 2. Near-infrared spectra of 10 randomly selected soil samples.

Table 1. Chemical and physical characteristics of soil samples used in this study.

Variable	Mean ± SD	Range	Mean ± SD	Range
	Series 1–2		Series 3–4	
SOC	1.11 ± 0.20	0.71–1.80	1.08 ± 0.16	0.71–1.45
pH	6.22 ± 0.52	4.99–7.57	6.17 ± 0.47	5.00–7.48
Mg	6.63 ± 2.50	3.10–15.9	6.29 ± 1.97	3.10–12.4
K	13.91 ± 4.95	5.10–44.5	13.07 ± 3.68	5.10–23.4
P	14.40 ± 5.74	5.70–33.6	13.64 ± 4.92	5.70–26.2
clay	1.99 ± 0.82	0.10–3.67	1.95–0.80	0.10–3.38

117 samples were used without outlier detection: series 1 and 2
102 samples left after outlier detection: series 3 and 4
SD – standard deviation
pH – soil acidity
SOC – soil organic carbon content (% soil)
Mg, K, P – content of available Mg, K, P (mg 100 g^{-1} of soil)
clay – clay content (% soil < 0.002 mm)



CV – cross-validation estimate
adjCV – bias-corrected cross-validation estimate
RMSEP – root mean squared error of prediction

Fig. 3. Cross-validated RMSEP curves for the soil organic carbon content: series 1.

overlap. The raw (log 1/R) spectra of all soils have similar shapes with the strongest absorption in the visible range and small peaks (e.g. 1400 and 1900 nm) in the near infrared region. One can deduce from this that spectral exploitation is only possible with the help of advanced multivariate statistics. Three to five components for the validation of the different calibration sets were generally sufficient. As can be seen in Figure 3 which presents as an example the estimated RMSEP as functions of the number of components for the SOC content of the first series, four components seem to be enough.

Without outlier treatment

The first calibration was undertaken by taking the samples from the series as they come in sequence from the grid. The samples were divided into two sets. The first 90 samples were used for calibration and the last 27 samples of the series were used for the validation of the calibration. The second calibration scheme was designed as in Dunn et al. (2002). Starting from sample n°1, data of every fourth sample were moved to a separate file for use as the validation set (30 samples). In that case, samples to be predicted are distributed evenly on the whole area of the field. Coefficient of determination r^2 and root mean squared error of prediction (RMSEP) of the two calibration procedures without outlier detection are given in Table 2 and 3. RMSEP is an indicator of how close validation standards – standards that are not in the calibration – are to the calibration line. There are two results of prediction, the leave-one-out (r_1^2, $RMSEP_1$) for the cross-validation of the calibration set and the validation for the prediction set (r_2^2, $RMSEP_2$). It is to notice that sometimes for a small dataset, only the cross-validation r_1^2 and $RMSEP_1$ are used to test the prediction ability of a model.

The best predictions (r_2^2) are achieved for soil organic carbon content (SOC) and for the content of available Mg for both the two calibration schemes. The first scheme giving somewhat better predicted values. The calibration scheme of series 2 shown better predictive ability for available K and clay content than series 1.

It can be noticed that the prediction obtained for the second series with the sample validation set evenly scattered in the field (calibration scheme 2) are overall better than for the first series where the sample validation set focused on the western border of the field (calibration scheme 1). With the first scheme, four of six properties were very poorly predicted or not at all for the content of available P, but presented reasonable cross-validated prediction r_1^2 (Table 2).

With outlier treatment

Because in large databases outliers can be easily unnoticed, we decided to check for outliers using the Filzmoser et al. (2005) method. All outliers detected were removed (circles in Figure 1) on the base of their chemical properties. Calibration schemes are the same as above. It gives for scheme 1 a calibration set of 85 samples with a validation set containing 17 samples (series 3) and for scheme 2 a calibration set of 78 samples with a validation set of 24 samples. Table 4 and 5 present the r^2 and RMSEP for the two calibration with outliers detection. The prediction of series 3 without outliers are worse than the prediction with all samples, especially the regression coefficient of the validation sets (r_2^2). All chemical properties but SOC were not or very poorly predicted. It is to notice that the r_1^2 are much better than the r_2^2 and sometimes even satisfactory (Mg or SOC content). The calibration scheme 2 of the fourth series of samples predicted the SOC and available K content. All other cross-validated and predicted properties were unsatisfactory (r^2 0.30–0.48).

Overall, the calibration scheme 2 is better working with or without outliers.

Table 2. Calibration and prediction results for soil properties: series 1.

Variable	r_1^2	$RMSEP_1$	r_2^2	$RMSEP_2$
SOC	0.61	0.11	0.65	0.14
pH	0.55	0.36	0.24	0.49
Mg	0.71	1.14	0.69	2.16
K	0.51	3.63	0.29	4.15
P	0.41	4.61	0.01	5.42
clay	0.61	0.50	0.30	0.58

pH – soil acidity
SOC – soil organic carbon content (% soil)
Mg, K, P – content of available Mg, K, P (mg 100 g^{-1} of soil)
clay – clay content (% soil < 0.002 mm)
r_1^2 – calibration set regression, r_2^2 – validation set regression,
RMSEP – root mean squared error of prediction

Table 3. Calibration and prediction results for soil properties: series 2.

Variable	r_1^2	$RMSEP_1$	r_2^2	$RMSEP_2$
SOC	0.64	0.12	0.50	0.14
pH	0.38	0.39	0.40	0.46
Mg	0.69	1.39	0.51	1.50
K	0.42	3.96	0.61	3.05
P	0.28	4.75	0.40	5.10
clay	0.60	0.53	0.64	0.48

Explanations – see Table 2

Table 4. Calibration and prediction results for soil properties: without outlier, series 3.

Variable	r_1^2	$RMSEP_1$	r_2^2	$RMSEP_2$
SOC	0.60	0.10	0.56	0.13
pH	0.47	0.35	0.02	0.46
Mg	0.69	1.02	0.38	2.39
K	0.57	2.43	0.31	2.98
P	0.41	3.76	0.07	5.24
clay	0.51	0.55	0.02	0.96

Explanations – see Table 2

Table 5. Calibration and prediction results for soil properties: without outlier, series 4.

Variable	r_1^2	$RMSEP_1$	r_2^2	$RMSEP_2$
SOC	0.48	0.11	0.68	0.11
pH	0.39	0.36	0.30	0.40
Mg	0.48	1.34	0.48	1.63
K	0.45	2.49	0.68	2.64
P	0.34	3.96	0.32	4.36
clay	0.36	0.41	0.48	0.60

Explanations – see Table 2

DISCUSSION

Results have pointed out that the type of calibration and the use of outlier detection have an important impact on the validity of prediction of the chemical and physical properties of soil samples with NIRS technology. Differences between results of the two calibration can be explained as follows. The validation set of the first scheme was composed of samples situated on the edge of the studied area, and not included in the calibrated area. On the other hand, the validation samples of the second scheme were spread on the whole area and therefore included in the calibration area. In the light of our results, and even if regression coefficients are not absolutely satisfying, it appears that evenly scattered sample datasets are better predicted than sample grouped on the edge of the studied area. This seems logical because of the better representation of chemical and physical properties in both calibration and validation sets for scattered samples.

The first scheme with all samples has failed to predict most of the soil properties but SOC and Mg content. In addition to the fact that predicted samples are outside the calibration area, as said above, samples 94 to 98, from a region of high topsoil texture variability present higher content of clay, available P and K but also Mg. Those five samples have a great impact on the regression result. Removing them improves the prediction. In the future more importance should be given to this region of complicated mosaic topsoil texture. More samples are needed to be sampled in this part of the field to better represent the overall variability of topsoil characteristics and then to improve the prediction ability of the calibration model.

The third and fourth series of calibration were obtained with an outlier treatment. Fifteen samples were removed from the dataset. As can be seen in Figure 1, most of outliers are situated on the border and in the northern part of the field. This can be explained by the fact that other field trials are conducted on neighbour fields and some contamination may occur. Moreover, some of these outliers are located in depleted region (tractor tracks) where sometimes water accumulates and in an area with sandy loam and slightly loamy sand. The effect of water and clay on the other soil properties are that some characteristics are unusually high and are detected as outlier by the algorithm probably without actually being so. As explained above, samples 94 to 98 are somewhat outside the range of values of the soil characteristics. This is another clue as to sample more this part of the field and it shows that choosing sampling location is an essential task in VIS-NIRS.

It seems, in the light of the prediction results that scheme 1 without outliers poorly predicts soil characteristics but for SOC (Table 4). Nevertheless, some properties seems to be predicted with the leave-one-out cross-validation. However, as stated by Dardenne et al., (2000) there is always an over estimation of cross-validation when no validation sets are taken into account. Doing a cross-validation with the calibration and validation set together even improves the cross-validation prediction. The reason for the poorly predicted results can be the fact that there are not enough samples in the calibration set and that removing the outliers is narrowing too much the range of the chemical and physical characteristics, as regression is generally improved when data are widespread.

Scheme 2 greatly improved the prediction of all properties with outlier detection.

Overall, the results of the present study are preliminary and can be improved. One hundred and twenty samples seemed to be enough for that work since most studies reviewed by Viscarra Rossel and McBratney (2008) included 100 to 200 samples. Some of the samples which seem to be far of the regression line will soon be re-analysed, scanned once more and then reinserted in the calibration model. The authors have tried to add several more samples to the models (but unfortunately from another neighbouring field) with a great improvement of the prediction results except for available P. The lack of prediction of available P can be related to the fact that the variable is poorly distributed in comparison with other variables presented in this work. The same reason was assumed by Dunn et al. (2002) for exchangeable Na. In some cases, removing few samples could greatly improve the accuracy of the prediction. Other outlier removing strategies need also to be checked. A data pre-treatment as the one from Chang et al. (2001) increase prediction regression for some properties (e.g. $r^2 = 0.82$ for SOC). Nevertheless, our results for prediction of SOC, clay, available Mg and K content are similar to that of Chang et al. (2001) and of Dunn et al. (2002) even if their studies were based on samples from a much wider area. This work is also a first step towards the analysis *in situ* with the on-the-go VIS-NIRS spectrophotometer. Some maps of chemical properties and especially of the carbon content will be realised and compared with on-the-go maps.

Results presented above are preliminary in nature, 300 more samples from the soil grid (3 fields) will be analysed later, added to the calibration, and compared with the results that will be measured in situ with the on-the-go spectrophotometer. With this aim in mind, the on-the-go spectrophotometer for in situ measurements of reflectance spectra is also described in this paper.

CONCLUSIONS

In the view of our results, near-infrared reflectance spectrometry is a simple to implement, non-destructive method that could be used to predict some soil properties. Results of this study have pointed out that:

1. VIS-NIRS is a suitable method for estimating SOC, clay, available Mg and K content;

2. The calibration scheme is very important and calibration samples should encompass the whole spectrum of chemical and physical characteristics of the soil;

3. More attention has to be paid to areas with important soil texture variability;

4. Outlier detection is not always the best way to obtain a good prediction.

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Polish Journal of Agronomy
2010, 3, 10–14

The effect of foliar fertilisation with Ekolist S on yield and quality of motherwort herb (*Leonurus cardiaca* L.)

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Abstract. In 3-year field experiment on loamy soil effect of foliar fertilization with Ekolist S (containing macro- and microelements) on growth and yielding of motherwort was studied. Ekolist S was applied in form of 1% solution in the first year of vegetation twice: at the end of May and in middle of June, while in the second year – three times: at rosette stage (April/May), 3 week later, and 2 weeks after first harvest. Ekolist S, irrespective of plant age (in first and second year of vegetation) had beneficial influence on plant growth and brought about higher yields of herb (on average by 85% in the first year and by 42% in the second one).

The effect of Ekolist S on quality of herb was not univocal. In the first year of vegetation it resulted in advantageous decrease of stalk contribution in herb but acted conversely on the second year during the first harvest. Effect of Ekolist S on flavonoids content was not clear: noticeable increasing tendency in the first year of vegetation and decreasing in the second (regards first harvest) indicates an interrelation with contribution of stalk in herb (the higher contribution the lower flavonoids content). Theoretical yields of flavonoids (resultant of herb yield and flavonoids content) every year were markedly higher in object, where Ekolist S was applied.

Key words: Ekolist S, motherwort, foliar fertilization

INTRODUCTION

Foliar application of nutrients is effective in culture of leafy species. According to Szewczuk and Michałojć (2003) it is advisable to divide whole dose of fertilizer in 2–3 portions and apply them in form of low concentration liquid in intervals of 10–14 days. Ekolist S is an universal fertilizer assigned for foliar nutrition large group of plants (both – field and horticultural). It contains macro- and microelements (N – 10%, K_2O – 6%, MgO – 2,7% as well as vestigial amounts of boron, copper, iron, manganese, molybdenum and zinc). In the paper there is described the effect of Ekolist S on plants growth as well as on yield and quality of herb one- and two-year-old plants (in first and second year of vegetation).

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Received 5 July 2010

MATERIAL AND METHODS

The experiment was carried out on a loamy soil in years 2001–2004 (plantations established in 2001, 2002 and 2003 were maintained two consecutive years). The soil contained moderate quantity of magnesium, high of potassium and very high of phosphorus (Table 1).

Table 1. Content of macro- and microelements in soil [mg kg^{-1}]

Macroelements:	P		K		Mg
	144		151		51
Microelements:	B	Cu	Mn	Zn	Fe
	0,36	2,7	276,0	11,8	915,0

The most important weather elements (rainfall and air temperature) during motherwort vegetation are presented in Table 2.

Soil fertilization was performed according to recommendation for this species (Mordalski et al., 1994; Załęcki et al., 1994). Fertilizers were applied in the spring of every year in following amounts (in kg of pure nutrient calculated per hectare): N – 80, P – 21.8, K – 66.4. Motherwort seedlings were produced in greenhouse and planted on the field at the beginning of May (at phase 6–8 leaves) in a distance of 50 x 30 cm. The experimental design was randomized blocks (in four replications), plot area 16 m^2.

In the first year of vegetation Ekolist S was applied twice: at the end of May and in middle of June, while in

Table 2. Sum of rainfall and mean daily air temperature during growth period of motherwort

Month	Decade	Rainffal [mm]				Temperature [ºC]			
		2001	2002	2003	2004	2001	2002	2003	2004
April	1	15	6	10	25	9.6	3.5	0.7	5.6
	2	3	2	27	10	5.4	10.3	7.5	9.0
	3	47	10	3	3	10.5	12.0	11.2	9.6
	total/mean	65	18	40	38	8,5	8.6	6.5	8.0
May	1	0	0	9	10	15.4	17.9	16.5	13.8
	2	3	3	41	11	14.2	15.9	14.3	10.9
	3	17	26	22	17	13.5	19.9	19.6	15.4
	total/mean	20	29	72	38	14,4	17.9	16.8	13.4
June	1	28	65	3	4	13.7	15.7	19.1	15.2
	2	13	33	15	26	14.9	18.9	17.0	15.8
	3	7	19	22	20	17.2	18.7	16.1	16.4
	total/mean	48	117	40	50	15,3	17.8	17.4	15.8
July	1	34	69	25	5	20.1	21.5	17.5	16.9
	2	20	53	44	28	22.9	22.9	19.4	17.2
	3	208	4	30	58	23.8	22.5	24.6	20.0
	total/mean	262	126	99	91	22,7	22.3	20.5	18.0
August	1	55	9	1	15	20.4	21.5	20.1	18.8
	2	0	9	7	9	21.6	19.8	19.2	19.6
	3	13	0	20	25	19.2	22.2	17.5	17.3
	total/mean	68	18	28	49	20.4	21.2	18.9	18.6
Total/mean for growth period		463	308	279	266	16.2	17.5	16.0	14.8

the second year – three times: at rosette stage (April/May), 3 week later, and 2 weeks after first harvest (in the last decade of July). Ekolist S was applied in form of 1% solution in amount of 400 l per hectare.

Plants were harvested at flowering stage (in the first year of vegetation – once and in the second year – twice). Before harvest, on every plot 10 plants were measured (height, length of inflorescence, thickness of stem at base and number of branches). Cut herb was dried in drying house with forced air circulation (temperature 40ºC) and weighted after 2 days. Next, in air dry samples (0.5 kg) stalks were separated (the least valuable part of raw material) and their percentage in herb was calculated. In the other samples content of flavonoids (as a hyperoside) was determined by colorimetric method (European Pharmacopoeia, 2003).

Numerical data were statistically analysed with Tukey's test and the least significant differences (at 5% of error) were calculated.

RESULTS AND DISCUSSION

Characteristic of plant growth

In the first year of vegetation motherwort produced one branched stem, the height of it varied from 63 to 98 cm (Table 3). The analysis of weather conditions in vegetative periods shows that very low rainfall in April and May, accompanied by relatively high temperature in 2002 (Table 2) resulted in the smallest plant height. Ekolist S, on average brought about a substantial increase in the height of plants (the exception however was the year 2001 characterized by abundant rainfall).

Number of branches was the most steady feature of the stem (19.6–20.9 in control plants and 18.0–19.3 when Ekolist S was applied). It is characteristic that restraining effect of Ekolist S on ramification in the same time positively affected the thickness of stems (Table 3). Every year spraying plants with Ekolist S resulted in significant increase length of inflorescence (on average by 21%). Positive effect of Ekolist S on height of herbal plants (golden rod, thyme and blue weed) was recorded by Kołodziej (2008) and Król (2008, 2009). In the case of golden rod the marked increase in the length of inflorescences was registered, too (Kołodziej, 2008).

In the second year of vegetation, growth and development of plants were more advanced which enabled two harvests. Plants developed several stems (9.8–10.2 up to first harvest) which were taller than in first year of vegetation, but had much shorter inflorescences (Table 4). Height of plants varied in particular years, Ekolist S however resulted in significant increase of height every year. Number of stems and branches (per stem) did not depend from ap-

Table 3. Effect of Ekolist S on morphological features of motherwort in the first year of growth.

Treat-ment	Year	Height of plants [cm]	Number of branches	Thickness of stem [mm]	Length of inflorescence [cm]
Control	2001	98.1	19.6	6.1	21.1
	2002	63.0	20.0	6.4	21.8
	2003	86.0	20.9	7.0	25.9
	mean	82.4	20.2	6.5	22.9
Ekolist S	2001	97.8	19.3	6.7	26.5
	2002	74.1	19.2	6.9	28.2
	2003	93.2	18.0	7.5	28.4
	mean	88.4	18.8	7.1	27.7
LSD (0.05)		5.3	0.7	0.1	1.7

Table 4. Effect of Ekolist S on morphological features of motherwort in the second year of growth (first harvest).

Treat-ment	Year	Height of plants [cm]	Number of stems per plant	Number of branches per stem	Thickness of stem [mm]	Length of inflo-rescence [cm]
Control	2002	111.0	9.6	11.2	6.3	12.1
	2003	128.1	11.1	11.6	6.1	12.2
	2004	138.5	8.6	13.5	7.3	11.7
	mean	125.9	9.8	12.1	6.6	12.1
Ekolist S	2002	125.8	10.1	11.5	6.9	14.5
	2003	135.5	10.3	11.9	6.8	14.1
	2004	152.1	10.2	12.5	7.7	13.9
	mean	137.8	10.2	12.0	7.1	14.2
LSD (0.05)		4.3	0.9	0.6	0.1	1.5

Table 5. Effect of Ekolist S on morphological features of motherwort in second year of growth (second harvest).

Treat-ment	Year	Height of plants [cm]	Number of stems per plant	Number of branches per stem	Thickness of stem [mm]	Length of inflo-rescence [cm]
Control	2002	67.2	19.6	7.6	3.1	7.8
	2003	61.1	12.8	4.9	3.1	10.4
	2004	40.9	23.9	6.3	2.5	7.9
	mean	56.4	18.8	6.2	2.9	8.7
Ekolist S	2002	73.0	28.8	7.0	3.0	9.1
	2003	72.1	21.8	6.6	3.3	11.1
	2004	48.3	33.3	6.9	2.6	11.1
	mean	64.5	28.0	6.8	2.9	10.4
LSD (0.05)		4.1	2.2	0.5	ns	2.0

ns – not significant

plication of Ekolist S (the only exception was number of stems in 2004). Every year Ekolist S resulted in significant increase thickness of stem and length of inflorescence (on average, respectively by 7% and 17%).

During the second harvest plants had much more stems (their number doubled in the control treatment and almost tripled in case of plants treated with Ekolist S). Their height however did not reach those during the first harvest. Ekolist S caused marked increase height of plants, irrespectively of weather conditions in particular years (Table 5). There is characteristic, that in case of re-growth, Ekolist S had no effect on thickness of stems and length of inflorescences (the only exception was inflorescence in 2004).

Yields and quality of herb

Due to new technology of seedling production (in greenhouse with use of multicell trays), yield of air-dry herb in the first year of motherwort vegetation was very high in comparison with those from productive (farmer's) plantations (Poradnik plantatora..., 1991). Every year application of Ekolist S stimulated growth of plants, especially their height and number of stems, resulting in significant increase of yields (on average by 85%) – Table 6. What is more, Ekolist S caused decrease of stems share in herb (they are an inferior component of raw material). In an experiment led by Kołodziej (2008) with golden rod, Ekolist S resulted in increase of herb yield by 8% while in another experiment with thyme (Król, 2009) foliar fertilization (Ekolist S and Tytanit) generated not only increase of herb yields (13.5–23%) but also beneficial decrease of stem contribution in yields.

Flavonoids content in herb varied in particular years with visible tendency to decrease when higher yields were obtained (Table 6). The effect of Ekolist S on flavonoid content was not univocal and seems to be an unimportant factor in this range. Theoretical yield of flavonoids (being the result of herb yield and content of flavonoids) every year was markedly higher in the treatment where Ekolist S was applied (on average twofold higher).

Yield of two-year-old plants consisted of two crops (first harvest and aftermath). The first crop was over twofold higher than the second one (Table 7). Generally, in the sec-

Table 6. Yield and quality of air-dry herb in the first year motherwort vegetation.

Treat-ment	Year	Yield [t ha^{-1}]	Stems contribu-tion [%]	Flavo-noids content [%]	Theoretical yield of flavonoids [kg ha^{-1}]
Control	2001	2.10	40.5	0.45	9.5
	2002	2.29	40.6	0.36	8.2
	2003	1.99	44.4	0.49	9.8
	mean	2.13	41.9	0.43	9.2
Ekolist S	2001	2.90	39.2	0.49	14.2
	2002	3.34	38.7	0.51	17.0
	2003	5.61	41.7	0.37	20.8
	mean	3.95	39.9	0.46	18.2
LSD (0.05)		0.22	1.6	-	-

Table 7. Yields of air-dry herb during first and second harvest and total yield of flavonoids in second year of plants vegetation.

Treat-ment	Year	First harvest [t ha^{-1}]	Second harvest [t ha^{-1}]	Total yield [t ha^{-1}]	Total theoretical yield of flavonoids [kg ha^{-1}]
Control	2002	3.60	2.18	5.78	24.6
	2003	4.99	2.02	7.01	33.5
	2004	5.65	1.04	6.69	16.8
	mean	4.75	1.75	6.50	25.0
Ekolist S	2002	5.88	3.03	8.91	31.5
	2003	6.36	3.81	10.17	43.7
	2004	6.89	1.68	8.57	24.3
	mean	6.38	2.84	9.22	33.2
LSD (0.05)		0.59	0.76	-	-

Table 8. Quality characteristics of crops in second year of motherwort vegetation.

Treat-ment	Year	Stems contribution [%]		Flavonoids content [%]	
		first harvest	second harvest	first harvest	second harvest
Control	2002	63.0	55.8	0.36	0.53
	2003	60.0	34.3	0.46	0.52
	2004	65.7	42.3	0.26	0.20
	mean	62.9	44.1	0.36	0.42
Ekolist S	2002	63.5	54.4	0.30	0.46
	2003	62.0	31.7	0.40	0.48
	2004	70.2	42.1	0.25	0.42
	mean	65.2	42.7	0.32	0.45
LSD (0.05)		1.8	1.5	-	-

ond year of growth, total yield of herb was by 158% higher than in the first one. Positive effect of Ekolist S was significant every year and at each harvest. On average, Ekolist S resulted in 34% increase of yield during the first harvest and 62% during the second one. In an experiment led by Sugier and Gawlik-Dziki (2009) foliar nutrition of arnica with Ekolist S resulted in an increased yield of inflorescences in the second year of vegetation (by 16.9%) but had no univocal influence on the quercetine content.

Content of flavonoids varied through the years, the lowest being in 2004, when both: yields and contribution of stalks in herb were the highest (Table 7 and 8). In general, flavonoids content was slightly higher in herb of second harvest (aftermath). This phenomenon apparently is related with lesser contribution of stalks in herb (Table 8). Theoretical total yield of flavonoids in two crops varied greatly in years, every year however was markedly higher in the treatment where Ekolist S was applied (on average by 32.8%).

Stem contribution in herb during first harvest of two years old plants was much higher in comparison with one year old plants. During the second harvest, the contribution of stems markedly decreased (Table 8). Ekolist S caused significant increase of stem share during the first harvest, while during the second one did not influence markedly this feature.

CONCLUSIONS

1. Field experiment on foliar feeding of motherwort with Ekolist S (fertilizer containing macro- and microelements) showed positive effects on growth and development of plants in the first and second year of vegetation.
2. Independently of plant age Ekolist S resulted in taller plants, thicker stems and longer inflorescences. In the second year of vegetation foliar feeding additionally brought about increased number of stems (per plant) and branches per stem.
3. Beneficial changes in plant growth and structure resulted in significant increase of herb yields (by 85% in the first year of vegetation and by 42% in the second one).
4. Positive effects of Ekolist S on quality of herb was expressed by the lower contribution of stalks (especially in the first year of vegetation). As regards flavonoids content, it was slightly higher, probably due to decreased contribution of stalks in herb.

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Polish Journal of Agronomy
2010, 3, 15–19

The effect of foliar or soil top-dressing of urea on some physiological processes and seed yield of faba bean

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Abstract. This research was conducted to determine the influence of foliar or soil applied urea fertilizer on symbiotic N_2 fixation, intensity of the net photosynthesis, nitrogen accumulation and seed yield of the faba bean cultivar Nadwiślański. A pot experiment was conducted over two years, in an experimental greenhouse at IUNG-Puławy. Feeding faba beans with urea caused a partial restriction of symbiotic nitrogen fixation, reduced root nodule mass and nitrogenase activity. This inhibitory effect had a temporary character and was lower when urea was supplied to the leaves than to the soil. Feeding faba bean with urea increased the intensity of the net photosynthesis in leaves, which in turn gave greater plant nitrogen accumulation and increased faba bean seed yield (an average increase of 14–15% for foliar feeding and 2–4% for soil top dressing in comparison with no top dressing). Foliar feeding with urea was found to be more beneficial than soil top-dressing, irrespective of the basic dose of N.

key words: faba bean, top-dressing of urea, symbiotic N_2 fixation, photosynthesis, yield

INTRODUCTION

The faba bean (*Vicia faba* L. *ssp. minor* Harz) is a plant which can survive in soils which are poor, medium or rich in nitrogen (N) thanks its ability to symbiotically fix N in association with bacteria of the family *Rhizobiaceae*. High soil N concentrations for faba bean and other legumes restrict symbiotic N_2 fixation (Eaglesham, 1989; Gan et al., 2003; Hardarson, Atkins, 2003; Kage, 1995; Salvagiotti et al., 2008; Wojcieska et al., 1994). The fertilization of legumes with nitrogen is still being investigated (Prusiński, Kotecki, 2006; Zeidan, 2003). Kocoń et al., (1995) and Wojcieska, Kocoń (1997) reported that symbiotic N_2 fixation in the reproductive phase of faba bean development is reduced naturally as a result of decreased nitrogenase activity. This seems to be a result of inadequate N levels to cover the need of the faba bean during pod and seed development. Nitrogen is an essential element for the plant to fulfil its genetic potential yield.

Research into finding an effective method of feeding N to faba bean plants during this essential development period should be focused on gaining knowledge of physiological processes. This includes symbiotic N_2 fixation, intensity of growth and development, intensity of gas exchange, accumulation of N and dry matter (DM) including analyses of the faba bean seed yield. Currently, there is limited knowledge on foliar N feeding of faba bean, and particularly on its influence on physiological processes. Research of Wojcieska and Kocoń (1997) and by this author using marked ^{15}N; identified that faba bean uses N from foliar feeding more efficiently than from soil top-dressing (Kocoń, 2003).

The aim of this study was to evaluate the effectiveness of foliar and soil top-dressed urea on faba bean with respect to symbiotic N_2 fixation, intensity of net photosynthesis, N accumulation and seed yield of faba bean.

MATERIAL AND METHODS

Studies with the faba bean (*Vicia faba* L.) cultivar Nadwiślański were conducted as part of a pot trial over two years in an experimental greenhouse in IUNG-Puławy. A completely randomized design was used in a factorial experiment, the first factor was basal N fertilizer, and the second factor was top-dressing with urea.

Mitscherlich pots were filled with 7.8 kg of quartz sand rinsed with demineralised water. Liming was made by mixing sand with 5 g of $CaCO_3$ pot^{-1}, and adding the following mineral nutrients per pot: 1250 mg P (in $NaH_2PO_4 \cdot H_2O$), 1800 mg K (in K_2SO_4), 390 mg Mg (in $MgSO_4 \cdot 7H_2O$), 50 mg Fe ($(C_6H_5O_7) \cdot 3H_2O$, 10 mg H_3BO_3,

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Received 8 October 2010

Table 1. Urea top dressing scheme.

Basic fertilizer (N mg pot^{-1})	Treatment	Urea top dressing (mg N pot^{-1}) Plant development stage (according to the BBA scale – Ostrowska, Kucińska, 2000)		
		57	62	64
10, 300, 900	Control (C)	0	0	0
	Soil top dressing (S)	150	+150 (300)	+150 (450)
	Foliar application to leaves (L)	150#	+150# (300)	+150# (450)

after addition of 25% extra sprayed N

10 mg $MnSO_4 \cdot 4H_2O$, 1 mg $CuSO_4 \cdot 5H_2O$, 1 mg $ZnSO_4 \cdot 7H_2O$, 0.5 mg $(NH_4)6Mo_7O_{24} \cdot 4H_2O$ and 0.5 mg $CoCl_2$. K, P, and Mg were applied in two doses: half before sowing and half at the 4–5 leaf phase. Microelements were applied before sowing.

Basic nitrogen fertilizer application to the faba bean was in 3 doses: 10 (starter dose), 300, and 900 mg N pot^{-1}. Nitrogen was added to the soil before sowing in NH_4NO_3.

During flowering, some plants were used for urea foliar feeding whilst others for soil top-dressing. As a result of this treatment plants received an additionally 450 mg N pot^{-1} (Table 1), as three equal doses of 150 mg – plants were sprayed by small portions of urea at 7.00–8.00 a.m. for five consecutive days. No surfactant was used while spraying. Prior to this the author (Alexander, 1986; Kocoń, 2003; Skiba et al., 1995) had demonstrated that with spraying, about 25% of the N applied is lost due to unmanageable targeting of the spray solution around the plant. Thus in foliar feeding, the dose of N was increased by 25% with respect to the recommended dose applied to the soil. The soil surface in-between foliar fed plants was shielded with absorbent during treatment to stop the N solution from coming in contact with the soil. Control plants were given basic N fertilizer (before sowing) and were sprayed with water (no top dressing with urea).

In mid April, in both years, 10 faba bean seeds were sown into each pot, inoculated with active *Rhizobium leguminosarum viceae* biotype, (inoculum AS from the collection of the Department of Agricultural Microbiology – IUNG). After germination, plants were thinned to 5 healthy plants pot^{-1}. Soil moistness was kept at a level of 60% of field capacity, and was applied as demineralised H_2O. The reaction of so prepared undersoil in pots measured as pH_{KCl} was about 7.

During the whole period of growth the following measurements were taken at plant development stages (in BBA scale Ostrowska and Kucińska, 2000) described in the Tables 2-4:

a) Measurements of nitrogenase activity using the acetylene reduction method (Hardy et al., 1968; 1973) a gas chromatograph PYE - UNICAM 204 was used. This was done by measuring the entire root system with nodules from a single pot (5 plants). Directly after removal from the soil they were rinsed in water at 18–20°C. Plants were then incubated with 10% C_2H_2, at 25°C for 1 hour. The results (average of three parallel estimates) were in µmol of ethylene pot^{-1}. During harvests, observations were made on root nodules to measure nitrogenase activity. Nodules were then separated from roots to determine their dry weight.

b) Measurements of net photosynthesis and leaves transpiration at the individual plant levels of the main stem (every third leaf, starting with the leaf third from the bottom). This was recorded by measuring infrared absorption, using a CO_2 LCA-4 analyser, working in tandem with a leaf PLC camera. The results are given as arithmetic means for the entire foliage from five replicates. Each repetition on average was measured 40 times. Water use efficiency (WUE) was calculated from the relationship between net photosynthesis to transpiration.

c) Nitrogen content was evaluated from mature plants, with two averaged parallel readings using a spectrophotometer. Nitrogen accumulation was estimated based on the percentage N contents in individual plant parts in their DM. The relative methodological error of determination of N fluctuated between 5 to 8%.

Faba bean plants were harvested at full maturity. Plants were divided into component parts and dried to determine DM yield. The results are the arithmetical mean of 5 replicates after two years research.

Results were statistically analysed using analysis of variance. The least significant difference at P = 0.05 was calculated using Tukey's test.

RESULTS AND DISCUSSION

Pre-sowing soil fertilization with N of faba beans from ammonium nitrate always led to a significant reduction in root nodule mass and limited nitrogenase activity (Table 2). This enzyme is responsible for symbiotic N_2 fixation. The restricting influence of N decreases as soil N is used; therefore, with consecutive sampling, the root nodule mass rose and reached a maximum during pod development. The increase in root nodule mass was not in line with the increase in nitrogenase activity. Nitrogenase activity only increased until flowering and then fell during pod development. The problem of decreased nitrogenase activity in faba bean, and a reduction in symbiotic N_2 fixation during pod development of pods, was indicated by Hardarson and

Table 2. The effect of a basal application of ammonium nitrate and top dressing with urea on the dry mass of faba bean nodules and their nitrogenase activity.

Basal fertilizer [N mg pot^{-1}]	Urea top dressing	Dry root nodule mass [g pot^{-1}]			Nitrogenase activity [μmol C_2H_4 pot^{-1} h^{-1}]		
		Plant development stage#					
		62	64	72	62	64	72
10	C	0.77	0.87	1.18	82.4	139.0	89.8
	S	0.59	0.62	0.82	59.5	109.2	80.8
	L	0.63	0.71	1.02	63.5	118.1	92.6
300	C	0.69	0.70	0.97	51.5	85.6	64.6
	S	0.56	0.58	0.88	39.2	79.5	68.8
	L	0.58	0.60	0.92	42.7	86.3	78.8
900	C	0.34	0.61	1.05	16.6	69.3	63.5
	S	0.14	0.34	0.82	9.6	51.6	60.4
	L	0.22	0.48	1.08	11.3	60.9	69.1
Mean	C	0.60	0.72	1.06	50.2	98.0	72.6
	S	0.43	0.51	0.84	36.1	81.1	70.0
	L	0.48	0.59	1.01	39.2	88.4	80.2
LSD (0.05) for:							
basic fertilization (I)		0.108	0.074	ns	5.74	19.18	7.92
top dressing (II)		0.084	0.058	0.087	4.45	14.69	2.97
interaction							
I x II			0.011			8.84	
II x I			0.128			9.96	

C – control (no top dressing); S – top dressing on soil; L – top dressing on leaves

\# development stages (Ostrowska, Kucińska, 2000): 62 – flowering of first flower bunches; 64 – flowering on three flower bunches; 72 – first visible pods

ns – non significant

Table 3. The effect of a basal application of ammonium nitrate and top dressing with urea on net photosynthesis rate, transpiration rate and WUE of leaves of flowering field bean plants (stage 64 – flowers on three flower bunches).

Basic fertilizer [mg N pot^{-1}]	Urea top dressing	Net rate of photosynthesis [μmol CO_2 m^{-2} s^{-1}]	Transpiration rate [mmol H_2O m^{-2} s^{-1}]	WUE [μmol CO_2 per mmol H_2O]
10	C	10.69	5.75	1.86
	S	11.44	7.25	1.58
	L	12.95	7.75	1.67
300	C	10.72	6.34	1.69
	S	10.98	7.28	1.51
	L	12.23	7.51	1.63
900	C	10.03	7.29	1.37
	S	10.33	7.27	1.42
	L	11.27	7.24	1.56
LSD (0.05) for:				
basic fertilization (I)		ns	0.214	
top dressing (II)		1.140	0.317	
interaction				
I x II			0.448	
II x I			0.371	

For explanation see Table 1, 2.

Atkins (2003), Wojcieska and Kocoń (1997) and in different legumes by Gan et al., (2003) and Ruszkowska et al., (1991). The reduction in nitrogenase activity, which reduces fixation N_2, can negatively affect the yield of faba bean seed, because during the period of high plant demand for N (flowering and pod development), a consequence of insufficient N for the developing pods can be a reduced yield.

Top-dressing of faba beans with urea at the start of flowering negatively influenced root nodule mass and nitrogenase activity. Generally, continual feeding of plants with urea increased nodule mass and nitrogenase activity (Table 2), often exceeding the activity of this enzyme in test pots – not fed N. Foliar feeding of faba bean with urea, during this period, was a more favourable way of supplementing N, compared with side-dressing of N, because side-dressing restricted an important process of symbiotic N fixation. There was a more beneficial influence of foliar feeding of faba bean with urea on symbiotic N_2 fixation, as reported earlier (Kocoń et al., 1995), with work on peas Wojcieska et al., (1994), with soybean Gan et al., (2003), lucerne Ruszkowska et al., (1991). It was associated with a lower soil N concentration in the soil environment of the plants.

The influence of pre-sowing fertilization of soil with ammonium nitrate on the intensity of photosynthesis of faba bean leaves was minimal (Table 3). However, transpiration intensity increased with increased N fertilizer. Plants were more inefficient in managing water during photosynthesis. However, feeding faba beans with urea, (particularly their foliage), always increased the intensity of the net photosynthesis in their leaves. Consequently, it often also increased transpiration intensity; thus, it gave similar indications of the photosynthetic effectiveness of water use – similar to the value of WUE, independent of method of urea feeding (Table 3).

Table 4. The effect of basic application of ammonium nitrate and top dressing of urea on nitrogen accumulation and yield of dry mass in ripened plants (5 plants).

Basal fertilizer [mg N pot^{-1}]	Urea top dressing	Nitrogen accumulation [mg N pot^{-1}]			Dry matter yield [g pot^{-1}]		
		seed	other organs	whole plant	seed	other organs	whole plant
10	C	2431	999	3430	59.1	91.6	150.7
	S	2663	1058	3721	60.2	96.3	156.5
	L	2820	987	3807	66.3	88.2	154.5
300	C	2887	968	3855	61.3	92.1	153.4
	S	2937	929	3866	62.4	87.3	149.7
	L	3002	1025	4027	65.6	87.9	153.5
900	C	2379	1037	3416	51.8	88.2	140.0
	S	2561	994	3555	53.3	86.9	140.2
	L	2960	1032	3992	65.0	89.2	154.2
LSD (α=0.05) for							
basic fertilization (I)					3.64	ns	8.95
top dressing (II)					3.47	ns	7.02
interaction:							
I x II					4.17		
II x I					4.62		

For explanation see Table 1, 2.

Nitrogen belongs to the group of nutrients, which given at an appropriate dose generally positively influence the process of the gas exchange in plants with regard to the intensity of net photosynthesis. Jla and Gray (2004) reported on the increase of net photosynthesis intensity under the influence of N feeding in faba bean and Borowski and Michałek (2000) in broad bean. Starck (2002) and Kościelniak et al., (1990), consider an increase in intensity of this process can be related with increased RuBisCo enzyme activity as well as a rise in assimilate export from leaves to roots. Moreover, a high level of assimilates in plant roots beneficially influenced symbiotic N_2 fixation (Ayaz et al., 2004; Muraoka et al., 1991; Starck, 2002).

Pre-sowing soil fertilization with ammonium nitrate, as a basic N fertilizer, in doses up to 300 mg N increased the amount of N in seed and in the entire plant (Table 4). However, high doses of N applied at 900 mg N dose^{-1} to a pot had a detrimental effect on N accumulations and biomass yield, particularly of seed. Post flowering fertilization with urea of plants in the reproductive phase always leads to a higher amount of N in plants. Consequently, it also lead to increases in yield, especially of seed, independent of basic N fertilisation. Therefore, the most beneficial treatment was foliar feeding with urea (Table 4).

Behairy et al., (1988), Kocoń et al., (1995), Muraoka et al., (1991), Podsiadło (2001), Wojcieska and Kocoń (1997) reported an increase in seed yield of faba bean in plants fertilized with small doses of N. Higher doses of N were less effective (Behairy et al., 1988;, Hardarson, Atkins, 2003; Hardarson et al., 1991; Kulig, Ziółek, 1997; Muraoka et al., 1991; Zeidan, 2003). Similar relations have been reported in other legumes (Borowski, Michałek, 2000; Prusiński, Kotecki, 2006; Salvagiotti et al., 2008).

The reason for the reduced seed yield at the highest dose of N (900 mg) was a reduction in nodules and nitrogenase activity, which reduced symbiotic bonding of N_2 during initial plant growth. After the mineral N was used up the amount of available symbiotically fixed N_2 was not sufficient for the plants needs. Only top-dressing of faba bean with urea (during flowering and pod development) had an effect on the nutritional needs of plants that had been supplemented with nitrogen and consequently gave higher seed yields. In pots, when fed with urea, particularly foliage fed plants had a higher intensity of net photosynthesis. This confirms that urea in doses, as applied in this experiment, favourably interacted with the process of gas exchange of plant leaves and did not reduce plant photosynthesis (Borowski, Michałek, 2000; Jla, Gray, 2004). This work and earlier work using ^{15}N (Kocoń, 2003), and Gan et al., (2003) with soybean, confirms that foliage feeding of faba bean with N during flowering and podding supplements N deficiencies and is more effective than side dressed N fertilizer.

CONCLUSIONS

1. Top-dressing faba bean with urea, as well as basic fertilization with ammonium nitrate reduces root nodule mass and nitrogenase activity. A decrease in nitrogenase activity as a result of N fertilizer is a temporary character after which growth recovers.
2. The restricting influence of fertilizing with urea on symbiotic N_2 fixation was less with foliar feeding N than with soil applied N.
3. Feeding faba bean with urea during reproductive development increased net photosynthesis of leaves, accumulation of N in plants and seed yield. Foliar feeding with urea was much more effective than a side-dressing.
4. This research confirmed that there is a benefit in applying small doses of nitrogen pre-sowing. The yield of faba bean seed increased, when a dose of 300 mg N pot^{-1} was applied, and decreased at a dose of 900 mg N pot^{-1}. It was shown that there was a benefit of foliar feeding with N in the reproductive phase of faba bean development.

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Polish Journal of Agronomy
2010, 3, 20–23

Effect of sowing rate on yields and grain quality of new cultivars of spring barley

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ABSTRACT: Microplot experiments with spring barley were carried out on an experimental field IUNG-PIB in Puławy. They involved 3 sowing rates: 250, 350 and 450 seed m^{-2}. In 2004–2005 the cultivars Nadek, Sebastian, Widawa and Kirsty were investigated, and in 2006–2007: Toucan, Mauritia, Nagradowicki and Tocada. The experiments were conducted on good wheat complex soils (heavy loamy sand on light loam). All cultivars of spring barley responded with yield increase to the increase of sowing rate from 250 to 450 seed m^{-2}. Widawa, Mauritia, Nagradowicki and Tocada showed a higher yield increase in response to high sowing rate compared to medium rate of 350 grains m^{-2}. The increase of grain yield showed by all cultivars at high sowing rate was the result of an increase in the number of ears per unit area (to the highest degree in the cultivar Mauritia). Grain weight per ear (averaged across cultivars) was significantly higher at low sowing rate. The increase in the protein content in grain at high sowing rate was recorded for the cultivars Widawa, Kirsty, Nagradowicki and Tocada. Significantly positive effect of high sowing rate on grain plumpness was found in: Nadek, Sebastian, Toucan and Mauritia cultivars.

key words: spring barley, sowing rate, grain yield, protein content, yield components

INTRODUCTION

The synthesis of research results on the effect of different crop management-related, environmental and biological factors on spring barley yield showed that sowing rate is the strongest factor interacting with other factors on grain yield and yield components (Noworolnik, 2003). Different cultivars of spring barley react differently to an increase in sowing rate (Farack, Hansel, 1987; Kozłowska-Ptaszyńska, 1993; Jedel, Helm, 1995). It is related to their different tillering ability and different light requirements. A great number of recently introduced new malting and fodder cultivars of spring barley gives the reason for a systematic research on their requirements as to the optimal sowing rate in relation to grain yield and its quality. A good malting quality of the cultivars is related to a low protein content of the grain, while the reverse is true of fodder quality. Malt parameters are significantly dependent on barley grain plumpness.

The aim of the research was to examine the reactions of new cultivars of spring barley (yield, yield components, and protein content) to the increase in sowing rate. Moreover, it is important to compare the cultivars for their productive tillering and for the number of grains per ear, because those features are not determined by COBORU. The research hypothesis assumed different impact of sowing rate on yields and protein content in barley cultivars. The ones with weaker tillering are supposed to react more positively to higher sowing rate

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Received 28 November 2010

MATERIAL AND METHODS

Microplot experiments with spring barley were conducted on an experimental field of IUNG-PIB in Puławy. They included 3 sowing rates: 250 (low), 350 (medium) and 450 (high) seed m^{-2}. In 2004–2005 the cultivars Nadek, Sebastian, Widawa and Kirsty were investigated, and in 2006–2007: Toucan, Mauritia, Nagradowicki and Tocada. The experiments were set up on good wheat complex soil (heavy loamy sand on light loam), on a field previously cropped to potatoes and laid out as a split-plot design with four replications between April 2 and 12. The soil was high in phosphorus, potassium and magnesium. Fertilization of 60 kg N, 22 kg P and 58 kg K per ha was used. Barley was manually sown at an amount higher than its sowing norm, and after the emergence, the stand was thinned down to the right plant density (according to the layout). During growth, the plants were mechanically protected against lodging. Weeds were manually removed. Diseases and pests were controlled by using chemical plant protection agents.

Once harvested, the barley crop was evaluated for grain yield, grain yield components, protein content (Kjeldahl method; N × 6.25) and seed plumpness (Vogel sieves). Protein yield was also calculated. The results were statistically evaluated by the analysis of variance, and the significance of differences was evaluated using Tukey's test.

RESULTS AND DISCUSSION

The research showed a significant impact of sowing rate on grain yield of spring barley cultivars and main yield components (Tables 1 and 2). Grain yield increased together with an increase of sowing rate to 450 seed m^{-2}, but in 2004–2005 yield increase (averaged across cultivars) at that sowing rate compared to the sowing rate of 350 seed m^{-2} was more like a tendency. Higher yield increase at high sowing rate compared to medium sowing rate were found for: Widawa, Mauritia, Nagradowicki and Tocada. The increase of grain yield with sowing rate was the result of the increase of ear number per area unit of all cultivars (to the highest degree in the cultivar Mauritia). Grain weight per ear (averaged across cultivars) was significantly higher at low sowing rate. The interaction between sowing rates and particular cultivars occurred (in 2006–2007) for grain weight per ear. A negative impact of high sowing rate on this value was found for Toucan and Mauritia, whereas its changes at Nagradowicki and Tocada were insignificant. The increase of sowing rate caused the decrease in number of grains per ear of the cultivars investigated in 2004–2005 (Table 1), whereas for those investigated in 2006–2007 the interaction between a sowing rate and a cultivar occurred. Number of grains in Toucan, Mauritia decreased at high sowing rate, while it increased for Nagradowicki and Tocada cultivars (Table 2).

In scientific literature we cannot find much information on the comparison of the reaction of new spring barley cultivars to sowing rate. It is mainly concerned with the results of microplot experiments, continually performed in IUNG-PIB in Puławy (Kozłowska-Ptaszyńska, 1993; No-

Table 1. Effect of sowing rate on yielding and yield components of spring barley cultivars (2004–2005).

Cultivar	Sowing rate [seed number per m^2]	Grain yield [g m^{-2}]	Ear number per m^2	Grain yield per ear [g]	Grain number per ear
Nadek	250	791	922	0.86	20.5
	350	866	1090	0.79	18.2
	450	915	1216	0.75	17.8
	mean	857	1076	0.80	18.8
Sebastian	250	860	941	0.92	19.3
	350	946	1132	0.84	17.2
	450	991	1235	0.80	17.0
	mean	934	1103	0.85	17.8
Widawa	250	856	953	0.90	21.1
	350	914	1122	0.82	20.0
	450	975	1264	0.77	19.2
	mean	915	1113	0.83	20.1
Kirsty	250	803	947	0.85	21.1
	350	886	1121	0.79	20.3
	450	927	1238	0.75	20.0
	mean	872	1102	0.79	19.1
Mean	250	828	941	0.88	20.6
	350	906	1116	0.81	18.9
	450	952	1238	0.77	18.3
LSD(0.05) for:					
sowing rate		54	86	0.06	1.4
cultivar		51	ns	0.05	1.3
interaction		59	ns	ns	1.8

ns – non significant

Table 2. Effect of sowing rate on yielding and yield components of spring barley cultivars (2006–2007).

Cultivar	Sowing rate [seed number per m^2]	Grain yield [g m^{-2}]	Ear number per m^2	Grain yield per ear [g]	Grain number per ear
Toucan	250	622	792	0.79	19.8
	350	710	975	0.73	18.7
	450	771	1107	0.70	18.1
	mean	701	958	0.74	18.9
Mauritia	250	632	698	0.90	19.3
	350	770	963	0.80	17.4
	450	863	1125	0.76	17.6
	mean	755	929	0.82	18.1
Nagrado-wicki	250	695	820	0.85	19.0
	350	880	1042	0.84	20.5
	450	972	1170	0.83	20.8
	mean	850	1011	0.84	20.1
Tocada	250	683	687	0.99	22.0
	350	847	939	0.94	21.6
	450	970	1004	0.96	23.1
	mean	833	876	0.96	22.2
Mean	250	657	749	0.88	20.1
	350	798	980	0.83	19.6
	450	898	1102	0.81	19.9
LSD(0.05) for:					
sowing rate		62	76	0.05	ns
cultivar		58	72	0.07	1.4
interaction		71	ns	0.08	1.7

ns – non significant

worolnik, 2007b; Noworolnik, Leszczyńska, 1998, 2000, 2004b) but which involved cultivars older than the ones investigated in this work. It was recorded that the cultivars with weaker tillering, mostly malting ones, show higher increase in grain yield at high sowing rate. The cultivars with stronger tillering show higher plant death rate at high sowing rate. New cultivars of spring barley show less varied reactions to sowing rate (concerning grain yield and its structure) in comparison with older cultivars from the previous research. A high number of ears of some cultivars per area unit under higher sowing rate generally cause a higher decrease in grain yield and grain number per ear. Different reactions of different barley cultivars to sowing rate due to different properties of those cultivars were found also in field experiments in Poland (Noworolnik, 2007b; Noworolnik, 2004a) and abroad (Farack, Hansel, 1987; Jedel, Helm, 1995, Zhao et al., 1988).

Sowing rate-dependent changes in protein content varied from cultivar to cultivar (Tables 3, 4). Non significant differences in protein content were recorded for the cultivars Nadek, Sebastian, Toucan and Mauritia, whereas an increase of this value at high sowing rate was found for Widawa, Kirsty, Nagradowicki and Tocada. Sowing rate did not significantly affect the content of proteins in grains averaged across cultivars. The increase in sowing rate resulted in an increase of protein yield in all the cultivars. A positive significant impact of sowing rate on grain plumpness was found for Nadek, Sebastian, Toucan, Mauritia and for the average of all cultivars (Tables 3, 4). The weight of 1000 of grains did not change significantly under the influence of sowing rate in the cultivars investigated in 2004–2005. A significant negative impact of high sowing rate on the mass of 1000 of grains was found for Nagradowicki, Tocada and for the average of the cultivars investigated in 2006–2007. Slight but varied differences of protein level in the grains of the investigated cultivars under the influence of sowing rate were found also in other works (Bertholdsson, 1999; Eagles et al., 1995; Noworolnik, 2003, 2007a, 2007b, 2008; Pecio, 2002; Zhao et al., 1988). There was also an increase in grain plumpness of spring barley (Bertholdsson, 1999; Eagles et al., 1995; Pecio, 2002) and slight decrease of TGW (Jedel, Helm,

Table 3. Effect of sowing rate on grain quality features of spring barley cultivars (2004–2005).

Cultivar	Sowing rate [seed number per m^2]	Protein content [d.m.%]	Protein yield [kg m^{-2}]	Grain fraction >2.5 mm [%]	1000 grain weight [g]
Nadek	250	11.9	94	79	41.9
	350	11.4	99	86	43.7
	450	11.6	106	89	42.2
	mean	11.6	100	85	42.6
Sebastian	250	10.7	92	82	47.5
	350	10.5	99	87	48.8
	450	10.8	107	89	47.3
	mean	10.7	99	86	47.9
Widawa	250	10.2	87	78	42.6
	350	10.8	99	79	40.8
	450	11.1	108	82	40.2
	mean	10.7	98	80	41.2
Kirsty	250	10.5	84	80	40.2
	350	11.0	97	82	39.0
	450	11.2	104	83	39.2
	mean	10.9	95	82	39.5
Mean	250	10.8	89	80	43.1
	350	10.9	99	84	43.1
	450	11.2	106	86	42.2
LSD(0.05) for:					
sowing rate		ns	7	5	ns
cultivar		0.6	ns	5	2.7
interaction		0.7	ns	7	ns

ns – non significant

Table 4. Effect of sowing rate on grain quality features of spring barley cultivars (2006–2007).

Cultivar	Sowing rate [seed number per m^2]	Protein content [d.m.%]	Protein yield [kg m^{-2}]	Grain fraction >2.5 mm [%]	1000 grain weight [g]
Toucan	250	10.8	67	77	39.7
	350	11.0	78	84	39.0
	450	11.0	85	87	38.4
	mean	10.9	77	83	39.0
Mauritia	250	10.8	68	81	46.7
	350	10.7	82	85	46.2
	450	11.1	96	90	43.5
	mean	10.9	82	85	45.5
Nagradowicki	250	11.5	80	80	44.6
	350	12.2	107	83	41.2
	450	12.6	122	86	39.8
	mean	12.1	103	83	41.9
Tocada	250	11.0	75	78	45.1
	350	11.3	96	81	43.5
	450	11.8	114	82	41.7
	mean	11.4	95	80	43.4
Mean	250	11.0	73	79	44.0
	350	11.3	91	83	42.4
	450	11.6	105	86	40.8
LSD(0.05) for:					
sowing rate		ns	8	6	2.9
cultivar		0.6	9	ns	2.7
interaction		0.8	ns	8	3.3

ns – non significant

1995; Noworolnik, 2003, 2007a, 2007b, 2008; Noworolnik, Leszczyńska, 1998, 2000, 2004a, 2004b; Pecio, 2002) along with the higher sowing rate.

Grain yield and the values of its components varied from cultivar to cultivar. A higher grain yield (averaged across sowing rates) was recorded for the cultivars Sebastian, Nagradowicki and Tocada (Table 1, 2). In the case of Nagradowicki, it was the result of the highest number of ears per area unit; Sebastian and Tocada showed a high grain weight per ear; Sebastian, due to a very high 1000 grain weight (close to Mauritia); and Tocada due to a very high grain number per ear. The lowest weight per 1000 grains was found in Toucan and Kirsty cultivars.

The highest protein level of the grain was found for Nagradowicki, followed by Nadek and Tocada (Table 3, 4). A high protein yield was given by Nagradowicki, Nadek, Sebastian and Widawa. A high grain plumpness (an important feature of malting quality) was found in Nadek, Sebastian and Mauritia, the lowest in Tocada and Widawa. The comparison of yield, yield component and quality traits of different barley cultivars by COBORU cultivar experiments (Lista..., 2008; Wyniki..., 2009) are mostly consistent with the results of this work.

CONCLUSIONS

1. All the cultivars of spring barley reacted positively to an increase in sowing rate from 250 to 450 seed m^{-2}. Widawa, Mauritia, Nagradowicki and Tocada cultivars showed a higher yield increase at high sowing rate in proportion to the average sowing rate of 350 seed m^{-2}.
2. The increase of grain yield at high sowing rate was the result of the increase in the number of ears per area unit of all cultivars (to the highest degree in cv. Mauritia). The weight of grain per ear (averaged across cultivars) was significantly higher at low sowing rate.
3. The protein content increase in grain at high sowing rate was recorded in the cultivars Widawa, Kirsty, Nagradowicki and Tocada. A significant positive effect of high sowing rate on grain plumpness was recorded for Nadek, Sebastian, Toucan and Mauritia.

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Polish Journal of Agronomy
2010, 3, 24–27

An inexpensive process for 350–1100 nm wavelength aerial photography for agro-environmental studies

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Abstract. The aim of this work was to present the possibilities of obtaining four-channels of aerial photographs (blue, green, red, infrared) and their use in agriculture research. Two synchronised and gyro-stabilised digital cameras FujiFilm IS-1 sets installed on a Cessna airplane were used. The digital cameras used in the study were factory set and adapted to perform photos in the 350–1100 nm wavelength. To separate the photographs content into spectral channels, selective filters were used and installed on the lens as well as photographs from two cameras were combined in a GIS computer program. High-resolution multispectral photographs were the source material for the spatial analyses. For example, the analysis can be used in field monitoring – e.g. vegetation index (NDVI, VARI) map drawing. In this work, an evaluation of the cost involved in obtaining this type of data was presented.

Keywords: remote sensing, image processing, computer vision, NDVI, VARI

INTRODUCTION

Low altitude digital photography allows for a quick and cheap acquisition of spatial data. This data, processed by the methods available in geographic information systems (GIS) take on cartometric characteristics. Additionally, their processing allows vegetation index maps to be drawn, one of the main sources of information used for agro-environmental studies (Nieróbca et al., 2008; Pudełko et al., 2008a, 2008b, 2009; Borzęcka-Walker, 2007).

The advantages of using low altitude digital photography are in its low cost of obtaining photographs (the price of a flight) and in timing the date of the flight according to the state of the plant vegetation in the researched area. These opportunities are not always given by either high-resolution satellite images (clouds and flights periodicity) or high altitude aerial photography (clouds at low and middle altitudes). In many cases, there is a need to evaluate the implementation of a spatial variability for a smaller area. Therefore, bearing in mind the financial factor, the use of a cheaper solution rather than professional remote sensing methods is necessary. For this reason, alternative ways of obtaining low altitude digital photos are increasingly used in scientific research and practice. In the literature, there are many examples of methods used such as light aircraft (Bauer at al., 1997; Nieróbca at al., 2007; Pudełko and Igras, 2008), unmanned aerial vehicles (Igras and Pudełko, 2008; Hunt et al., 2005; Rydberg at al., 2007; Sugiura et al., 2005), balloons (Inoue et al., 2000) and kites (Jensen et al., 2007). Another advantage of digital photography is the possibility of taking infrared photographs. Commonly available cameras have built-in filters that eliminate registrations in the channel over 750 nm (near infrared). However, some companies offer cameras to record the full range (350–1100 nm) registered by the CCD (charged couple device) matrix. Such cameras include IS-1 produced by the Fujifilm Inc.

The aim of this work was to present possibilities of obtaining four-channels of aerial photographs (blue, green, red, infrared) based on a Cessna plane and self-made gyro-stabilised platform for Fujifilm IS-1 cameras.

MATERIALS AND METHODS

This project deployed a self-constructed multispectral sensor system for low-altitude remote sensing (MSS-LA-RS) that consisted of two synchronised and gyro-stabilised digital cameras FujiFilm 9MP IS-1 set, which was installed on a Cessna airplane (Figure 1). The images done in Red (600–700 nm), Green (500–600) and Blue (400–500 nm) and IR (700–1100 nm) were captured at the same time by using a radio controlled trigger. The system allows an operator

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Received: 15 March 2010

Fig. 1. Multispectral sensor system for low-altitude remote sensing (MSS-LA-RS) mounted on a Cessna plane.

to preview the area to be photographed in real time. For RGB photography the B+W No. 58 filter was used, and for NIR the B+W 52 UV-IR CUT CHROM. An image processing package ERDAS IMAGINE® 8.4 (Erdas, Inc.) was used for drawing up maps of vegetation indices. NDVI (Normalised Difference Vegetation Index) maps were obtained based on jointly taken Red, Green, Blue and NIR photos. VARI (Visible Atmospherically Resistant Index) map can be used as an equivalent of NDVI maps in the case of only RGB photography (Golzarian et al., 2007; Stark et al., 2000).

The possibilities of obtaining four channels of high-resolution aerial photographs were presented based on the analysis of a chosen RGB-NIR combined photos. The photos were taken on 30.08.2008 from an altitude of 500 m, when the system was tested on IUNG's experimental fields (51° 28'N, 22° 04'E, western Poland).

RESULTS AND DISCUSSION

The MSS-LA-RS test has demonstrated its functionality. Thanks to the possibility of a preview in real time of the image seen by the cameras, the operator has an opportunity to choose the appropriate moment for taking the photographs. Figure 2 shows aerial photos, obtained with the stabilisation mode of cameras, which provides sufficient precision in the vertical direction of the photograph axis and parallel axis according to the direction of the two cameras. This allows the geometrization of the photographs into orthophotomaps and connection of RGB and NIR channels into the 4-channel photography, which was the basis to draw up the NDVI and VARI index maps (Figure 3).

Table 1. A statistical description of the NDVI and VARI maps.

Index	Mean	Median	Std. Dev.	Max	Min
NDVI	-0.02	-0.01	0.37	1.00	-1.00
VARI	0.02	0.02	0.17	0.96	-0.37

The pictures below represent the following forms of land use: ploughed fields, fields with developed vegetation, abandoned area, fields with no developed vegetation, fields with medium developed vegetation, water, artificial area (buildings).

A comparison between the NDVI map and the VARI map shows a high correlation between selected indices on both maps. This suggests a possibility of applying the VARI index as an equivalent of the NDVI index, where the images were made solely in the RGB channels. VARI mostly correlates with NDVI in areas used for agriculture: ploughed fields, fields with developed vegetation, fields with not developed vegetation, fields with medium development of vegetation (Figure 3 and 4 – zones: A, B, D, E). Compared with VARI index, NDVI much better illustrates the areas occupied by buildings or by water (Figure 3 and 4 – zones: G and F). A comparison between the descriptive statistics of both images shows that the NDVI index is more spectrally sensitive. The NDVI image is characterised by a greater value of standard deviation along with more extreme values (Table 1). Therefore, the image classification process is more efficient.

The CCD matrix used in popular digital photography is characterised by a lower sensitivity of infrared registration (Pudełko et al., 2008b). Therefore, the ratio of registered values in the NIR and R channels will differ from the real ratio. It caused the NDVI index figures to be undervalued, which is shown on the presented map (Fig. 3). In contrast, the CCD matrix is especially sensitised to the green and red with blue. Consequently, both analysed indexes presented a large correlation in the registration of the vegetation intensity. An even better contrast can be observed between the vegetation and soil on the VARI maps than on the NDVI maps (B vs. zones A and D on the Figure 3). Only the NDVI index showed a capability of detecting water. In the case of detecting buildings, it seems most appropriate to compare the information with maps of both indices.

Low altitude aerial photography compared with other methods of remote data acquisition seems to be the most economical. The cost of a flight in a Cessna aircraft, which was used to obtain data presented in the work amounted to 130 Euro. In 2008, Fujifilm IS-1 cameras in the United States cost $900.

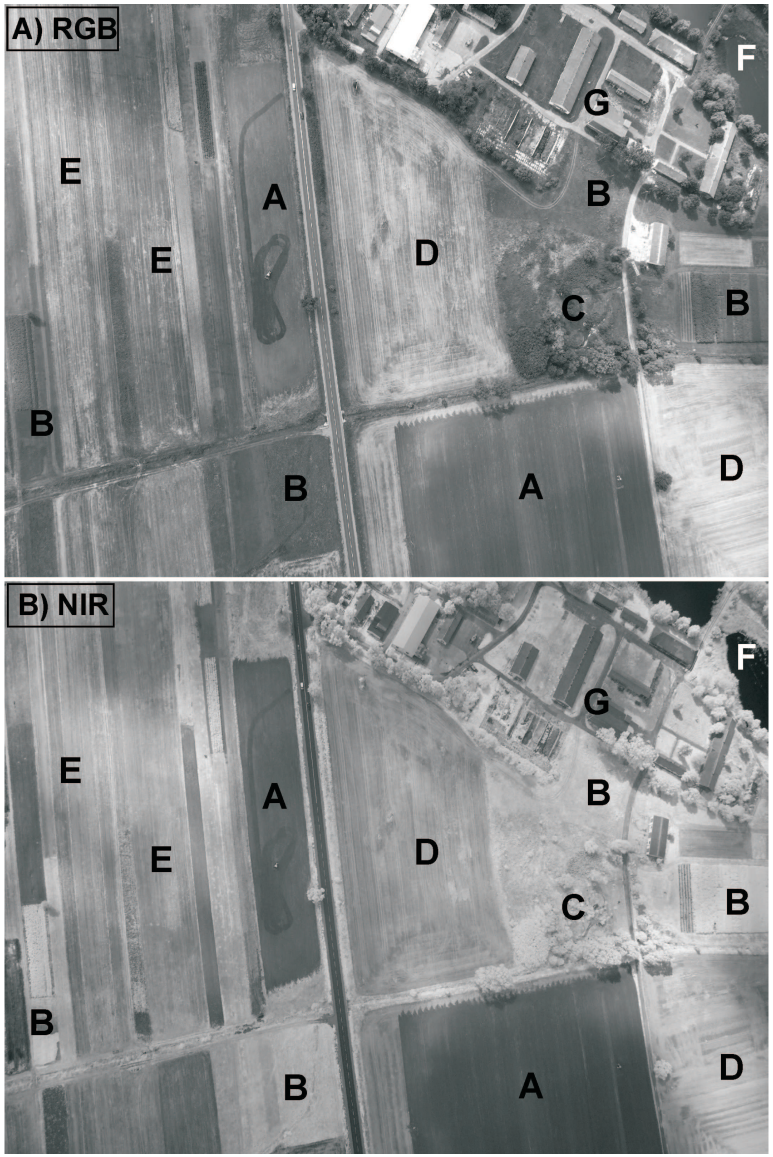

Fig. 2. RGB and NIR aerial photos taken on 30-08-2008.
Description of sites: A – ploughed fields, B – fields with developed vegetation, C – abandoned area, D – fields with no developed vegetation, E – fields with medium developed vegetation, F – water, G – buildings.

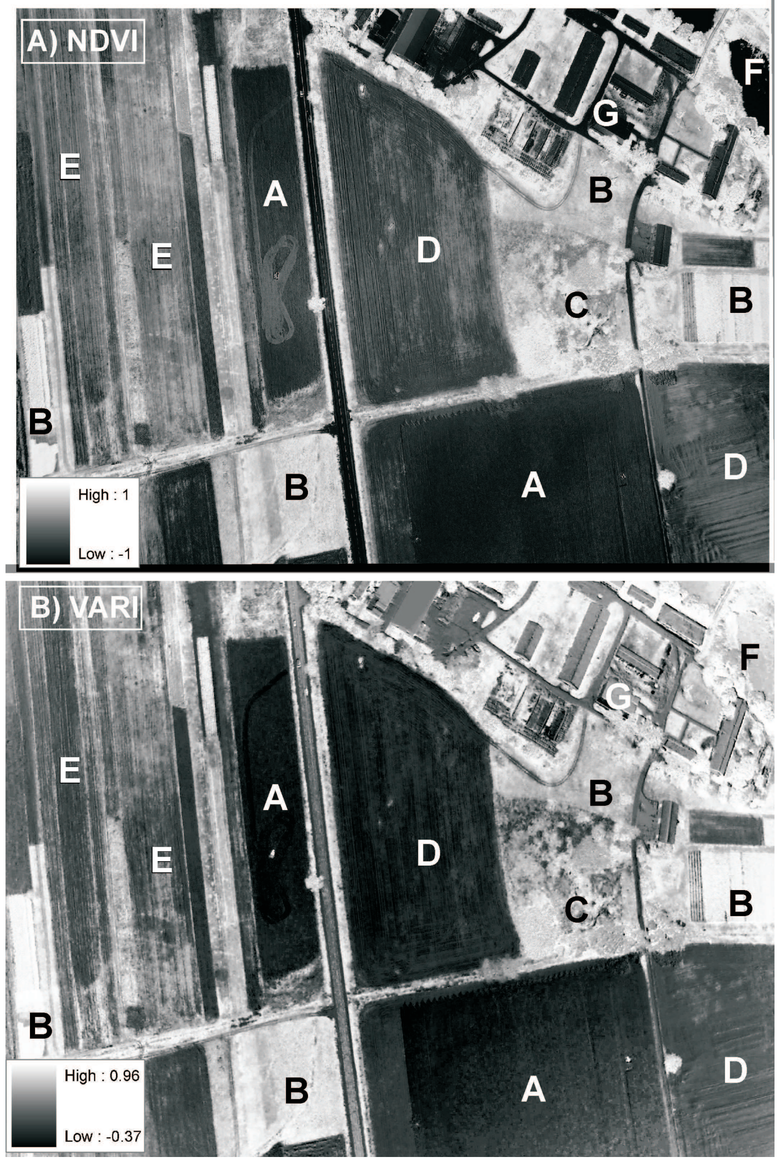

Fig. 3. NDVI and VARI maps based on the photos shown in Figure 2. The sites A,B...G have characteristics according to their descriptions in Figure 2.

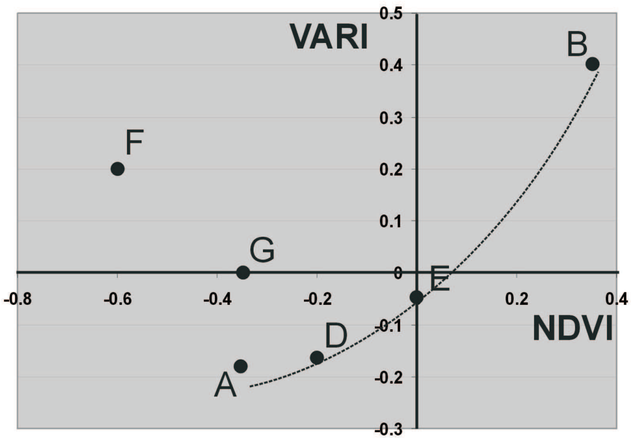

Fig. 4. The relation between NDVI and VARI for the mean (representative) values of chosen zones (see Figure 2 and 3). The dashed line on the diagram shows a positive correlation for A, D, E, B.

The low cost of obtaining high-resolution spatial data constitutes a major advantage of the presented method when applied to assess the diversity of land use structure in surveyed areas. The ability to make the rapid analysis of crop conditions during the growing season is crucial when it is necessary to draw up maps of varied applications of three doses of nitrogen fertilizer and plant protection products. The doses may be established on the bases of NDVI and VARI distribution.

CONCLUSION

1. Digital photography based on the popular CCD matrix offers the opportunity to interpret the image registered in blue, green, red, and near infrared.
2. Analysis of the recorded value of individual RGB and NIR channels allows obtaining indices that characterise the development of vegetation and the land use.
3. Low altitude aerial photography is an efficient way of obtaining remotely sensed data. The main advantages of this method is the possibility to study the vegetation status within short time of acquisition and low cost of obtaining the image.

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Polish Journal of Agronomy
2010, 3, 28–33

Life of game animals in transformed biotopes

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Abstract: One of the conditions of the species continuity is a natural living space (habitat), in which the species achieves its basic needs. Large area of agriculture and forestry monoculture are not conducive to existence of game animals. Permanent devastation of game preserves, windbreaks, liquidation of foraging sites and shelterbelts force the wild animals to feed in the field and forest crops. In modern agrotechnique – the usage of plant protection products deprives the wild species (eg the hare) of forage, on the other hand it causes contamination of food absorbed by animals. Not only does it disorganize the trophic pyramide, but also can cause permanent damage to the organism – environment networks system, which is essential for proper circulation of matter and energy in ecosystems.

The aim of the study is to draw attention to the effects of the changes in the biotypes caused by agriculture and forestry.

key words: habitat, hazard, game animals.

INTRODUCTION

The part played by present-day agriculture and forestry in reducing the population of hunting animals consists in the multidirectional influence on the biotope. This interference takes place on many levels of the biological organization. Changes occurring at the ecosystem, population and individual levels but also at the genetic level are of foremost importance in the methodology of hunting investigations.

The damage done by agriculture to the population of game animals is caused at various stages of the process of the plant production. Intensive farming, both of the arable land and of the forest, most often leads to negative changes in the biocenoses. As the adaptive capabilities of any wild-living species, and not only game animals, become exceeded it is now imperative to ensure sustainability of natural resources. A continuing decline of many economically useful wildlife populations is connected to progressive and often irreversible biotope devastation.

Today, the rapid decline of the partridge or hare populations in our country, is such an overt example of that adverse process. In less than 40 years Polish hunting grounds have lost 96% of the partridges and 89% of the hares.

The biological problem to be solved today are extensive areas under crops, most often under monocultures. As a result of land consolidation, the natural passageways between neighbouring biocenoses are being disrupted and at the same time the ecological niches are disappearing. This is true chiefly of agroecosystems, but not only so. Changes arising in the environment as a consequence of the misguided economic concept of the "management of farming" create a conflict between social groups – farmers and hunters, farmers and beekeepers, farmers, anglers and fishing farms. Inevitably, legal issues appear concerning settlement of the damages caused by animals in agricultural crops and, vice versa, those related to the repair of the damages to the nature and, in particular, to the populations of game animals incurred by agriculture. It should be noted that the damage caused by game animals is generated by the farmers and foresters themselves. Indeed, the introduction of alien species in agricultural crops entails a kind of behavioural changes in native wildlife. Ill-conceived actions result in the change of feeding preferences and interfere with reproductive processes for instance in the wild boars – which are the main perpetrators of damage to crops. The lack of knowledge concerning the biological behaviour of deer population – or its disregard – results in some damage to forest ecosystems. Only the changes in work organization, timing of treatments (late cleanings, early thinning, etc.), can mitigate or even prevent bark-stripping by deer. Many positive examples of the co-existence of the forest and game animals are provided by the work done at

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Received 15 March 2010

the regional forest administration office at Kluczbork, the Opole region (Pypłacz, Pypłacz, 2009). In turn, decline of woodland gallinacean game birds (capercaillie and black grouse) indicates the long-term changes in biotopes of these species, associated with the intensification of forestry that was brought down to the crop production-style management. These processes indicate a far-reaching human interference in the complex biological systems such as a habitat, an ecosystem or a biocenose. We cannot ignore the fact that food surplus obtained in agricultural production is lost as a result of deterioration, as a consequence of incorrect storage. Losses due to improper storage can reach even 40% of the final product. Wheat grain and other agricultural products are then assigned to the production of solid biofuel (pellets, cubes or pucks), burned in central heating furnaces, or fireplaces. Viewed in this context, damage to the agricultural crops caused by hunting animals accounts only for a little fraction of a much larger total.

THE CHANGES OF THE LANDSCAPE SPATIAL STRUCTURE

One of the conditions of continuity of the species is a natural living space (habitat) in which the species will realize its basic needs. The habitat as part of the biosphere is characterized by a specific combination of environmental factors or parameters allowing the functioning of the organism. Living conditions in biotopes are evolving faster and faster as a result of constant human intervention. This is a consequence of the urban and rural sprawl and of the expansion of the road infrastructure, detracting from the areas previously occupied by animals.

Mid-field woodlots, shrublands and game preserves, field borders, ecoton areas, ponds, windbreaks which used to be admixtures in the biocoenotic monotony of the field landscape are now either declining in area or disappearing. It is all too frequently that we see game refuges or little ponds become illegal rubbish dumps for neighbouring villages and settlements. A consequence of those changes for different species of game animals is the altering of their food resource base and of their living conditions. Abundance and distribution of forage base can contribute to the reduction of energy losses associated with searching for food (especially in winter) and to the maintaining of good condition necessary for the proper conduct of physiological processes. In the case of deer, the ecotone may contribute to more even distribution of animals in the area of their existence (Brzuski, Hędrzak, 2002 – acc. to Bobek et al., 1992). It is not possible to ignore the fact that the ecotone areas significantly reduce the damage caused by deer, both in forest and field ecosystems.

The biotope, in the ecological sense, presents a very complex biological system. People interfere in it through different management operations. Wild animals moving between ecosystems become a permanent channel of the flow of the energy and organic matter. Together with the matter they introduce different chemicals, which were carried along with the agricultural practices into circulation in the environment. Under natural conditions, undisturbed by humans wild animals are the link of ecological succession. Carrying seeds and spores between the ecosystems, they integrate these ecosystems. Their role in this process is significant. Knowledge of the participation of game animals in the biocoenotic process allow the functioning of the habitat to be used rationally. The development of wildlife populations and therefore intensity of reproduction and lifespan of individuals, their density, and biological production of populations, which may be used by the host of the hunting-ground, is dependent in the considerable degree on the landscape structure and on the processes that take place therein. The possibility of the compensative use of the ecosystems of different forage supply potential contributes positively to the welfare of game animals. The compensation of food resources occurs when the lack of food in a wild ecosystem is able to be satisfied in the neighbouring ecosystem, such as during the "winters of the century" appearing in our climatic zone every few years. Homogeneous field surfaces (monoculture) must be sufficiently small, if the movement of animals between them takes place every day or even several times a day. The structure of the landscape, the deployment of its refuges and the distance between them, determine the course of the migration routes of animals. Communication routes, fenced fields (electric shepherds) intersect the migration routes, forcing animals to overcome often dangerous barriers (eg highways). Preserving the diversity of ecological niches in the landscape contributes positively to the whole habitat. For example, the presence of mid-field shelterbelts influence on the yield of the crops in the micro-climatic range of its impact thus increasing the yield of cereals by 5–15%, sugar beet by 5–10%, potatoes by about 20% and the yield of vegetables compared to the yield in open areas by as much as 50–70% (Wilusz, Jaworski, 1960).

The diversity of ecological niches is important not only in fields, which gradually become more and more monotonous and poor. Also, in forest areas, the monocultures will benefit from the introduction of even small areas with different species composition (biocenotic game preserves). If all of the different land-use patterns are based on the use of different ecosystems, and their mutual mosaic location creates a landscape made by man then it is important to leave some space for wildlife and hunting management. The transformations of the landscape restrain the existence of permanent passageways, changing the quantitative and spatial relations in the structure of crops, in the age composition of forests, the availability of space. It causes the substantial reduction in the efficiency of the hunting management, farming and forestry. The unification in the use and management of the space is in contradiction to the principles of conservation of nature, including the preserving space for the game animals.

THE REDUCTION OF ANIMALS HOME RANGES

The space of the wild species undergoes a permanent reduction, the process is progressive, and remains in functional dependence on the exponential growth of human population. This is the global phenomenon, very clearly perceptible around the world. Until recently it was estimated that the home range area per individual of a roe deer ranged from 100 to 150 ha, and nowadays it is estimated at around 40 hectares. An example on a local scale is the reduced area of roe deer originally inhabiting an area 143 306 ha in the Opole region (Sporek, Sporek, 2009).

The study was conducted based on 14 field game shooting districts, and 12 hunting forest game shooting districts. The total area of the analysed units, was respectively; 68 097 hectares of meadow/field and 75 209 ha of forest. However, after reducing the surface area (the surface after the exemption referred to in Article 26th of the government law/act of 13th Oct. 1995 „The Hunting Law") was 63 319 ha for the hunting districts in the field and 70 816 ha of the hunting districts in the wood. In absolute numbers, roe deer habitat on the field is reduced by 4 778 ha, and it is a permanent loss of space for this species by 7% compared to the original territory, while the deer forest habitat is reduced by 4 393 ha (about 5.84%). In total, the hunting economy has lost more than 9000 ha in the analysed area. The area of permanent loss of the territory does not include areas not accessible for deer in the interim period – ranging up to a dozen years – is a result of the fencing of forest crops, young tree stands, edges of forests, establishing of electrical shepherds in the fields by farmers. In the analysed area, the share of the forest complexes was 3.1% of the total area (the smallest forest complex was 20 hectares and the largest one – 533 hectares). A controversial issue that remains to be solved are the restrictions to or even a total ban on game shooting in the vicinity of designated hiking trails, bicycle paths or ski-routes. It is mainly concerned with areas which contain elements of tourist infrastructure such as information boards, marked rest stops equipped with seats, etc. which are built close to the existing hunting facilities. Safe hunting is not possible at those sites. More and more often, this situation coincides with the problem of hunting-related damages occurring at those places, and, at the same time, with the claims for loss settlement put forward by local hunter associations. It should also be noted that the hunters' point of view is ignored in the evaluation of local investments as is the opinion of biologists who inform about wildlife migration routes.

THE EFFECTS OF CHEMICAL PLANT PROTECTION IN THE BIOTOPE

To combat harmful factors influencing the economic effects in agriculture and forestry, the wide range of chemicals is used. For example, fungicides are used to combat fungi, insecticides are employed to exterminate insects, rodenticides control rodents, acaricides kill mites, herbicides suppress the growth of weeds, and those bacteria that cause crop diseases are controlled by bactericides. Generally, all these agents are referred to as pesticides or biocides.

Initial achievements obtained by the use of chemicals in the fight against pests and weeds, and as a result, the effective increase in yields – for a long time masked the negative aspects of this method. Only with time it became apparent that, while not being quite perfect, chemical control can be a two-edged weapon.

Attention is drawn to the fact that we know now about 13 million chemical compounds, about 100,000 of them are produced on an industrial scale, and each year approximately another 100–200 thousand new compounds are identified, of which about 2000 are annually placed on the market (Siemiński, 2001).

According to the report of the U.S. Environmental Protection Agency – EPA 1998 (Chemical Hazard Data Availability Study) only 7% of 3,000 chemicals used in significant quantities in the U.S. have been sufficiently tested for their impact on human health and the environment (Siemiński, 2001).

In the vast majority of agrarian activities, the farmers lack knowledge about the effects of chemical interference in the biological system. They are ignorant of the consequences of the impact of active substances and their metabolites in the ecosystem. Even given the optimistic assumption that farmers have full knowledge of the consequences of their own chemical treatments and that those treatments are done exactly according to the agricultural recommendations, as many as 20% of apiaries become poisoned (Banaszkiewicz, Lipiński, 2009). We would not be aware of this were it not for the beekeepers and their organizations and the unique interest of the researchers in those useful insects. But what about other species that are present in any analogous trophic networks? Do really the sprayed toxins act only on the targeted organisms?

The transforming of the environment is the result of rapid and high profit-oriented agricultural activity with minimum inputs of labour. This postulate can be fulfilled under the assumption that 95% of agricultural practices will be made using chemicals. Chemical plant protection products, also known as pesticides, are a small group of toxic substances intentionally introduced into the environment. The aim of this treatment is to combat harmful organisms, A side effect is that, at the same time, they are toxic for the organisms identified by the humans as useful (eg bees) and neutral ones (if there is such a thing as "neutral" in biocenoses). Animals, living in the wild, in the environment treated with plant protection agents, are extremely exposed to toxic substances, because they are located at the top of the pyramid of the trophic network. The negative consequences of the modern agrarian technology do not only concern the game animals existing there, but also endanger the safety of the people's health. We must be aware of the complexity of the biological system in which farmers intervene by introducing the biocides and xenobiotics.

If we ask the question whether modern agriculture does damage in populations of wild game, then we should answer the question: what happens to the biocides and xenobiotics introduced with agrarian treatments into the agroecosystem. The specific question would be: "What is happening with the glyphosate as a herbicidal agent of a broad spectrum of activity after spraying the field? Glyphosate (HOOC-CH_2-CH_2-CH_2-NH-$PO(OH)_2$), is a non-selective herbicide that is absorbed by the leaves of annual, biennial and perennial plants. Incorporation of the glyphosate resistance in the genetically modified crops of oilseed rape is a widely used strategy in modern plant breeding. With this approach, the field may be dusted during the crop growth and development. All other plants are destroyed, but the crop itself remains intact. This technology gives rise to complex environmental consequences associated with this strategy. One of the many environmental consequences is the total elimination of the food base allowing continuity of the existence of such species as the hare. The literature data indicate that this species needs more than a hundred species of plants for the normal development and maintenance of physiological functions including reproduction. The effectiveness of herbicides also effectively reduces and eliminates the population of hares in our fields, depriving them of livelihood ie food.

According to the International Union for Conservation of Nature (IUCN) data, up to 27% of mammal species shows presently a declining trend (33% – lack of information, 32% – stable, 8% – growth). In the Opole region the number of the European hare *Lepus europaus* declined, by more than 80% within 10 years (1994 – 34,753 individuals, 2004 – 5,410 individuals), which, in the light of international criteria for the IUCN classifies this species as critically endangered (Sporek, Weżgowiec-Bagrowicz, 2009). The cause of such a substantial reduction of the number of hares must be sought in the intensification of agriculture and in particular in the use of pesticides. The current register of the Minister of Agriculture and Rural Development (Ministerstwo..., 2008), contains 795 plant protection products approved for use in Poland. The legal act of the European Union is the Council Directive 91/414/EEC of 15 July 1991. A total of 149 plant protection products offered for sale in the Opole region were analyzed based on these acts. It was found that:

– 58 are permitted to be marketed in Poland, although not on the list of the EU,
– 8 are approved for use by the EU directive, but they are absent in the national registry,
– 59 are allowed by both acts,
– 24 are not authorized by both acts.

The effectiveness and mode of action of the biocides is defined by the content of the active substance. The analysed plant protection products consist of 113 active substances present in different concentrations. Active substances contained in plant protection products define the direction of the toxic action (the number of preparations is given in the brackets):

1. very toxic to the aquatic organisms, with long-term adverse effects in the aquatic environment (72),
2. highly toxic to bees (10),
3. toxic to bees (6),
4. limited evidence for carcinogenicity (17),
5. causing hereditary genetic defects (5),
6. impaired fertility (6),
7. deleterious effects on the unborn child (21),
8. possible risk of irreversible changes in health status (2) (Sporek, Weżgowiec-Bagrowicz, 2009).

In the studied group of preparations there are fungicides, herbicides, insecticides, desiccants, growth retardant and seed treatment products (eg for the protection of cereals, oilseed rape, maize, vegetable and fruit crops). The production of the plant protection products in 1990–2007 in Poland increased from 19.7 to 42.7 thousand tonnes, together with a simultaneous decrease of the cultivated area from more than 18.5 millions of hectares in 1990 to 16.2 millions in 2007.

The active ingredients were selected in this group that are carcinogenic and potentially adversely affecting the fertility. These are compounds from the triazoles group – flusilazole, triadimenol, tebuconazole, cyproconazole, epoxiconazole; benzimidazoles – carbendazim, thiophanate-methyl; dinitroaniline derivatives – trifluralin. Triazoles are a group of compounds used in very low doses of 0,015–0,025 kg/ha active against numerous fungal pathogens.

Some chemical plant protection products may cause fertility disorders (compounds based on triazoles, benzimidazoles, dinitroaniline). Fungicides based on triazole derivatives are used, inter alia, in the protection of cereals (rye, spring and winter barley, spring and winter wheat), sugar beet, oilseed rape. It was proved (*in vivo* and *in vitro* on rats) that triazoles (including flusilazole) cause craniofacial and heart abnormalities in the fetus and (Menegola et al. 2001), also induce alterations to the structure of the throat and the fusion of aortic arches (Menegola et al., 2005). Directive 91/414/EEC does not allow marketing and using them (whereas the national registry does), but their sale is growing steadily within the country, and they are the fourth largest group of fungicides used in Opole region. Carbendazim, a compound belonging to the benzimidazoles, widely used in the cultivation of cereals, oilseed rape, sugar beet, causes disorder of the spermatogenesis process of rats (Rajeswary et al., 2007) and causes male infertility of the quails (Aire, 2005). Both the Directive 91/414/EEC and the national register of plant protection products allow for marketing and use of carbendazim. The fungicides preparations based on benzimidazoles reached the peak of the sales in 2005 in the country (Ministerstwo..., 2006). Such fungicides rank second in terms of use in Opole region. Another compound, which is derived from dinitroanilines is also widely used in plant protection. Trifluralin administered to pregnant female rabbits causes anorexia, general emaciation of the body and weight loss. In some cases miscarriage occurs, and after the birth, only some of the young are able to survive (Byrd et al., 1995). Trifluralin is not allowed to be used according to both of these acts. Detailed histological-toxicological studies on the game animals have shown that animals dwelling in the chemically contaminated environment lose weight and are less resistant to pathogens and organic disorders, and also show reduced sexual activity and fertility. This is all the more disturbing because – in contrast to the acute fatal poisoning, which concerns only a certain number of animals – a chronic poisoning, as manifested, inter alia, by the degeneration of the internal organs, infertility, and starvation of the body relates to a much larger number of individuals in the population. It is very frequent that the adverse effects of poisoning do not show up until the next generation.

The populations of small game animals having their food resources systematically chemically treated (hares, partridges, pheasants) are especially at risk. Boteva and co-authors research in Bulgaria (acc. to Borowiec, 1976) showed that number of birds in the pesticide-treated area was 30 times lower than in the control fields. It is only in the season after the treatment that the number of the birds increased, but it was still four times lower than that on the untreated surfaces. The searching for not contaminated food is very difficult or even impossible in a case of the chemical treatment of a wide area. Therefore, we speculated that the adverse genetic and development modification of the warm-blooded organisms, including humans caused by the pesticides, incalculable in size and in consequences, pose a greater threat, than the direct acute toxicity. The possibility of effective intervention on part of the specialists regarding the acceptance of pesticide usage, the concentration of applied chemicals, the size of the treated area, timing and frequency of the chemical treatments, etc., would reduce the negative impact of pesticides on the animals by eliminating the inappropriate chemicalization of their habitats.

SUMMARY

Wild game is an integral component of the natural environment, and therefore the management of their populations should be in accordance with the rules of biology. Progressive changes in the environment caused by human activities led to an imbalance in the ecosystem. Continuous changes in biotopes cause behavioral changes of game animals in their habitat as well as in their food preferences. Despite considerable progress in chemical plant protection there is still a major threat to wild species. It can be assumed that the main cause of this phenomenon is the lack of continuous monitoring of applied pesticides, timing of treatments performed and the quality of the products used.

According to the principle of sustainable development of agriculture and forestry, the natural resources should be used in such a manner and pace that will allow us to preserve and maintain biological diversity and productivity at such a level that it does not lead to the destruction of the trophic network links)

The new mid-fields shelterbelts should be created and the last existing ones should be preserved and maintained because they are often the only chance for the survival of many species in the highly impoverished phytocoenoses. It is therefore necessary to make further efforts and to bear costs of their implementation in agrocoenoses.

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IUNG-PIB – zam. 54/B/11
format A4, 280 egz.