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EFFECT OF 3-PYRIDYLKETOXIME AND ITS QUATERNARY PYRIDINIUM SALTS ON MIZE GERMINATION*

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K e y w o r d s: phytotoxicity, quaternary pyridinium ketoximes, 3-pyridyl ketoxime, maize.

A b s t r a c t

In the present work the impact of the 3-pyridyl ketoxime and its quaternary pyridinium bromides with various alkyl chain lengths on the monocot plant – maize (*Zea mays* L.) was tested. The study was carried out on two systems – hydroponic and soil. After a seven-day period of these experiments, the number of germinated seeds was counted and the length of maize radicle and shoot was also measured. 3-pyridyl ketoxime and pyridinium ketoxime bromides had varying effects on the germination of maize seeds and the development of radicle and shoot. Toxicity of these compounds depended largely on the structure of the compound, and the systems on which the plant was grown. The toxic effects of tested compounds on maize development were higher in hydroponic systems compared with the soil system.

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WPŁYW 3-PIRYDYNOKETOKSYMU ORAZ JEGO CZWARTORZĘDOWYCH SOLI PIRYDYNIOWYCH NA KIEŁKOWANIE KUKURYDZY

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S ł o w a k l u c z o w e: fitotoksyczność, czwartorzędowe bromki pirydynioketoksymów, 3-pirydyloketoksym, kukurydza.

A b s t r a k t

W pracy przedstawiono wpływ oksymu ketonu 3-pirydylowego oraz jego czwartorzędowych bromków zawierających różną długość łańcucha alkilowego na kiełkowanie i początkowy rozwój rośliny jednoliściennej – kukurydzy (*Zea mays* L.). Badania przeprowadzono w dwóch układach – hydroponicznym i glebowym. Po upływie siedmiu dni od założenia doświadczenia zliczono liczbę wykiełkowanych nasion oraz zmierzono długość korzeni i pędów kukurydzy. 3-pirydyloketoksym oraz jego czwartorzędowe bromki pirydyniowe wywierały zróżnicowany wpływ na kiełkowanie nasion kukurydzy, jak i rozwój korzeni i pędów. Toksyczność tych związków zależała w dużym stopniu od struktury związku oraz układu, w którym roślina była uprawiana. Silny efekt zahamowania kiełkowania oraz rozwoju nasion kukurydzy obserwowano w układach hydroponicznych, natomiast mniejszą inhibicję rozwoju nasion podczas upraw kukurydzy w glebie. Prawdopodobnie jest to związane z sorpcją analizowanych związków w matrycy glebowej i mniejszą biodostępnością.

Introduction

Interest in quaternary ammonium salts and ionic liquids is currently very high. The quaternary pyridinium salts can be used in a wide range of applications including pesticides, fungicides and preparations bactericidal, as well as extractants of metals from aqueous solutions (ABELE et al. 2003, VÖRÖS et al. 2014, KONIDARIS et al. 2011, KLIACHYNA et al. 2014, WIESZCZYCKA et al. 2013). Pyridyl ketoximes quaternary derivatives have the ability to reactivate the enzyme acetylcholinesterase, when it is fully inhibited by organophosphorus compounds, including pesticides and chemical warfare agents, and they are of particular pharmacological interest (EYER 2003). With such a broad spectrum of possibilities their usefulness is also certainly associated with the fact that they may enter the environment, both at the production and due to improper management of waste products. Ecotoxicological analysis is one of the essential elements of the assessment of the suitability and risks of chemicals. Many authors tested the dependence of compound structures on the toxicity for different organisms. The results showed that the toxic properties of this group of compounds are mainly dependent on the type of cation.

The modification of the anion in molecules of the ionic liquid usually affected changes in the physico-chemical properties of the compound. It was also noted that a long chain extension in the substituent alkyl pyridinium or imidazolium increases the toxicity of these compounds (CHENG et al. 2009, LATAŁA et al. 2009, PERNAK et al. 2011 and 2012, GRABIŃSKA-SOTA 2011, PETKOVIĆ et al. 2011, MATZKE et al. 2007, CVEJTKO BUBALO et al. 2014, KULACKI and LAMBERTI 2008). Among other things, therefore, a very important part of the research of the newly synthesized compounds is an analysis of their interaction with the environment and living organisms, including analysis of phytotoxicity. Their impact on soil and water is certainly of considerable concern at the time of their disposal.

From the above, the most interesting to study was analyses of the effect of 3-pyridylketoxime and quaternary pyridinium ketoxime salts on germination model monocotyledonous plant – maize (*Zea mays*). In addition, the obtained results will enable an assessment of risk of environmental contamination with such compounds.

Materials and Methods

Chemicals

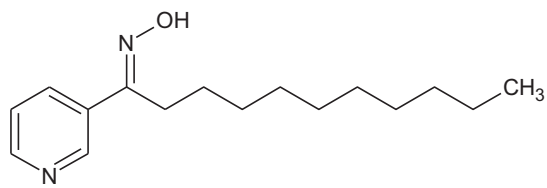
The tested compounds were synthesized on the based methods described by WIESZCZYCKA et al. (2013). Structures of tested compounds were shown at Figure 1. At first 1-(3-pyridyl)-undecane-1-one was synthesized by treating 3-pyridylcaronitrile with decylmagnesium bromide. And next, to the obtained ketone was added hydroxylamine hydrochloride in the presence of sodium carbonate (at pH 7). The synthesized 1-(3-pyridyl)-undecane-1-one oxime was stirred and heated (60°C) for 12h with appropriate alkyl halides (bromide propane or bromide pentane) in ethanol as diluent to give quaternary pyridinium ketoximes (WIESZCZYCKA et al. 2013). The yields of the pyridinium ketoxime bromides were 87 to 89%. The structure and purity of the compounds obtained was confirmed by spectroscopic methods (nuclear magnetic resonance (^{1}H and ^{13}C NMR)).

Treatments and experimental design

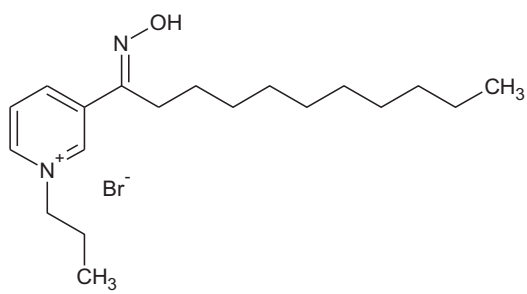
The analysis of impact the 3-pyridyl ketoxime and its quaternary pyridinium ketoximes on germination of maize (*Zea mays* L.) was exanimated in hydroponic and soil systems. The tested compounds were dissolved in meth-

anol and then prepared in the aqueous solutions containing 10, 25, 50, 100, 500 and 1000 mg l^{-1} respectively. The addition of methanol to the samples was not more than 5%. The control samples were made with distilled water. Aqueous solutions of the tested compounds or water were added to the soil and mixed.

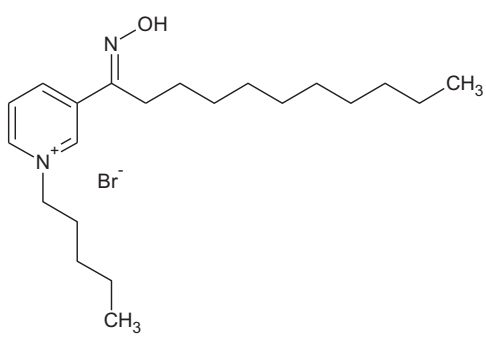
First, investigation of the effect of the pyridylketoxime and its quaternary bromides was carried out in the hydroponic. The maize seeds were placed in Petri plate on a piece of filter paper covered with a thin layer of cotton wool and moistened with 10 ml of water or compounds solution of the appropriate concentration.



1-(3-pirydyl)-undecan-1-one oxime (**3PC10**)



3-[1-(hydroximino)-undecyl]-1-propyl-pyridinium bromide (**3PC10-PrBr**)



3-[1-(hydroximino)-undecyl]-1-penyl-pyridinium bromide (**3PC10-PnBr**)

Fig. 1. The structures of tested compounds

The examination of germination inhibition in the soil system was carried out on plates Phytotoxkit™. On each plate 100g soil was added containing the appropriate concentration of the test substance (0, 10, 25, 50, 100, 500 and 1000 mg kg^{-1} dry weight of soil (d.w.s.)). Ten seeds were placed on each plate. All tests were carried out in 5 replications (5 plates for each concentration of test compounds – analysis for 50 seeds). The prepared samples were left for 7 days at 21°C.

Soil composition

Composition of the soil used in the experiment was as follows (mg kg^{-1} of soil): 81 P, 88 K, 69 Mg, pH 5.92, C organic content of 1.01% (10.1 g kg^{-1} of soil). According to the international classification WRB (*World reference...* 2015), the examined soils should be classified as Phaeozems (Haplic Phaeozems), while according to the FAO Soil Taxonomy (*Soil taxonomy* 1999), as Mollisols (Typic Endoaquolls). The influence of 3-pyridyl ketoxime and its quaternary pyridinium ketoximes was investigated using the phytotoxicity test based on the ISO-11269-2:2012 International Standard (*Soil quality...* ISO-11269-2 2012).

Calculation

After end of the experiments, the number of germinated seeds was counted and the radicle and shoot length were measured. The germination index (GI) was calculation based on the formula (MOSSE et al. 2010):

$$\mathrm{GI} = \left(\frac{G_x}{G_c}\right) \cdot \left(\frac{L_x}{L_c}\right) \cdot 100\ [\%]$$

where:
G_x and G_c represent the number of germinated seeds, respectively: x – sample c – control
L_x and L_c is the length of radicle respectively: x – sample, c – control.

The pyridine ketoximes and its quaternary derivatives were obtained at Poznan University of Technology in Department of Organic Chemistry. The experiments about impact of these compounds on plant germination were also carried out at Poznan University of Technology in 2015.

Statistical analysis

All the experiments were carried out in 5 replications and each collected sample was tested in duplicate. The mean values and standard deviations (SD) of the experimental data were calculated using software Microsoft Excel.

Results

The studies have showed that all tested compounds stimulated radicle growth of maize at the concentration of 10 mg l^{-1} (Figure 2). The increasing of compounds concentration caused the inhibition of radicle growth. It could be observed that the greatest inhibitory effect on radicle growth caused oxime 3PC10. In contrast, all tested substances with concentrations of 500 mg l^{-1} and 1000 mg l^{-1} showed a complete inhibition of the seeds germination processes. The presence of compounds of concentration 10 mg l^{-1} did not inhibit the shoot

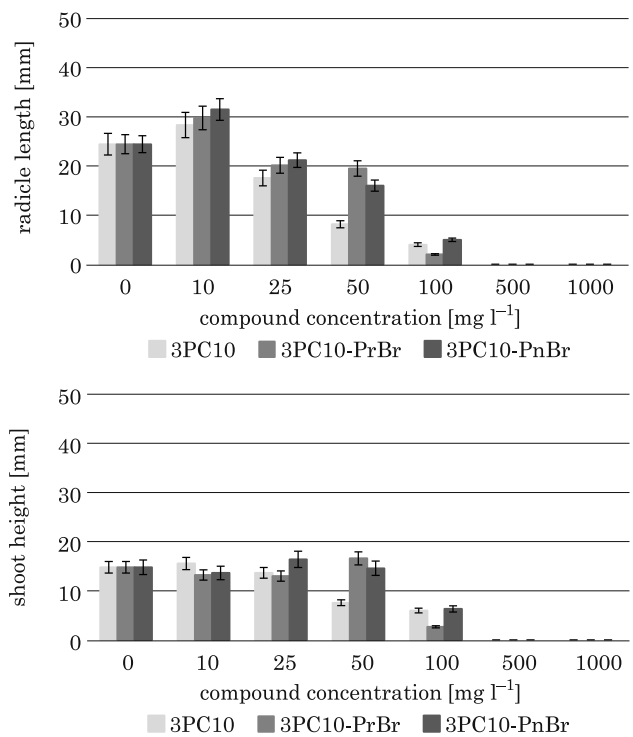


Fig. 2. Effect of 3-pyridylketoxime and its quaternary pyridinium ketoxime salts on the radicle and shoot growth of maize in hydroponic system

growth (Figure 2). Moreover, in the case of oxime 3PC10 growth stimulating properties relative to the control sample without oxime and its derivatives were observed.

This demonstrates the beneficial effects of oxime 3PC10 at the lowest concentration on the seeds germination, and also the further development of the plant. Increasing 3PC10 concentration in the medium to above 50 mg l^{-1} resulted in a significant (50%) decrease in the average shoot length. Slightly less effect on the shoot growth after exposure to 10 mg l^{-1} of 3PC10-PnBr was recorded. However, 3PC10-PnBr stimulated growth of shoot at concentration 25 mg l^{-1}. But when tested, results for 3PC10-PrBr, in the concentration range 10–25 mg l^{-1} had no effect on the shoot growth, but when the concentration increased to 50 mg l^{-1}, the shoot length increased above 15% in comparison to the control. In general, germination and growth inhibition was more pronounced when higher concentrations of oxime and its quaternary bromide were used. The shoot length was decreased for about 50% (3PC10 and 3PC10-PnBr) and 80% (3PC10-PrBr) after exposure to 100 mg l^{-1}. However all compounds used at a concentration 500 mg l^{-1} and higher caused a complete stop of the germination processes of maize seeds (Figure 2).

On the base of the number of seeds germinated and the radicle length compared to controls germination index (formula 1) was calculated and then plotted the dependence of the germination index to the concentration of tested compounds (Figure 3). It could be observed that with a concentration 10 mg l^{-1}, all compounds have a stimulating effect on the maize seeds germination. That confirms the GI value more than 100%. With the increase of the compounds concentration in the solution, germination index decreased. Only in the case of 3PC10-PrBr at a concentration of 50 mg l^{-1} could be observed a slight increase

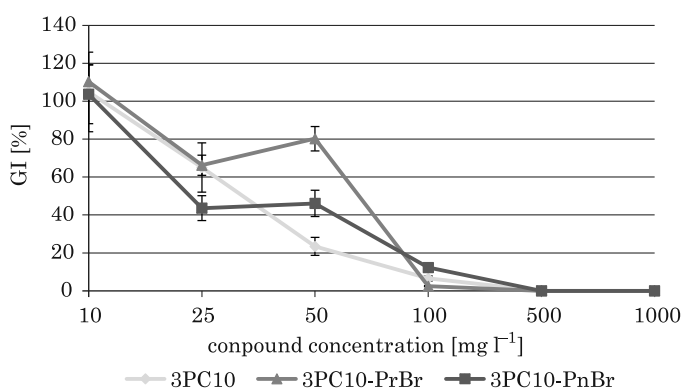


Fig. 3. Dependence of germination index on 3-pyridylketoxime and quaternary pyridinium ketoxime salts concentration in hydroponic systems

in GI values, relative to a concentration of 25 mg l^{-1}. The lowest GI values observed at a concentration of 100 mg l^{-1}. At 500 mg l^{-1} and above the germination process did not occur.

Studies on the effect of 3-pyridyl ketoxime and its quaternary pyridinium ketoxime salts on the maize germination were also performed for soil system. Figure 4 shows the dependence of the radicle and shoot growth on the compounds concentration. As could be seen, these compounds resulted in the varied effect on radicle growth (Figure 4). The application of 3PC10-PrBr at a concentration of 50 mg kg^{-1} d.w.s. stimulated radicle growth about 15% compared to the control, which was not exposed to compounds. However, all tested substances inhibited seeds germination and radicle growth above

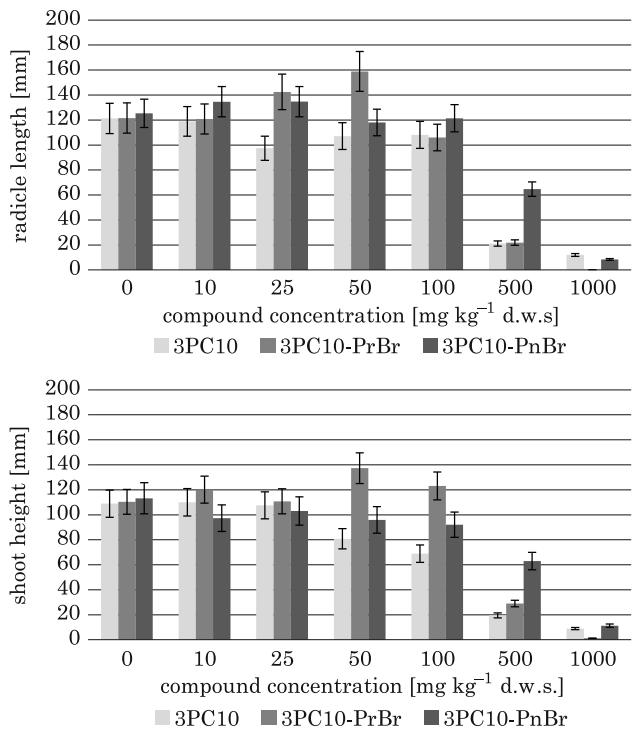


Fig. 4. Effect of 3-pyridylketoxime and quaternary pyridinium ketoxime salts on the radicle and shoot growth in soil system

100 mg kg^{-1} d.w.s, and 500 mg kg^{-1} d.w.s. inhibited radicle growth by about 50% (3PC10-PnBr) and 80% (3PC10 and 3PC10-PrBr). The highest inhibition of radicle growth of seed maize was observed when exposed to 1000 mg kg^{-1} d.w.s. Then the radicle length did not exceed 10% of the radicle length of the control.

During the impact study of the 3-pyridylketoxime and its quaternary salts on the growth and development maize, the shoot growth was also tested (Figure 4). In this case, the increase of oxime 3PC10 and 3PC10-PnBr concentration in the soil resulted in inhibition of the shoot length in all tested compound concentrations. However, the cultivation in a soil containing 50 and 100 mg kg^{-1} d.w.s. of 3PC10-PrBr induced stimulation of shoot growth relative to the control samples watered only with deionized water. Application of 3PC10-PrBr at a concentration of 500 and 1000 mg kg^{-1} d.w.s., as well as for other tested substances, resulted in significant inhibition of the shoot length. The highest inhibition of growth in the aboveground parts was observed for the concentration of 1000 mg kg^{-1} d.w.s.

In the final stage of the study the germination index was determined and then the graphs were plotted in correlation to the germination index to the oxime and quaternary pyridinium ketoxime salts concentration (Figure 5). When analysing Figure 5 it was observed that there was a marked decrease in germination index (GI) above 100 mg kg^{-1} d.w.s. Only in the case of the 3PC10-PrBr concentration of 50 mg kg^{-1} d.w.s. there was a 30% increase of germination index, whether evidenced by GI value exceeding 100%.

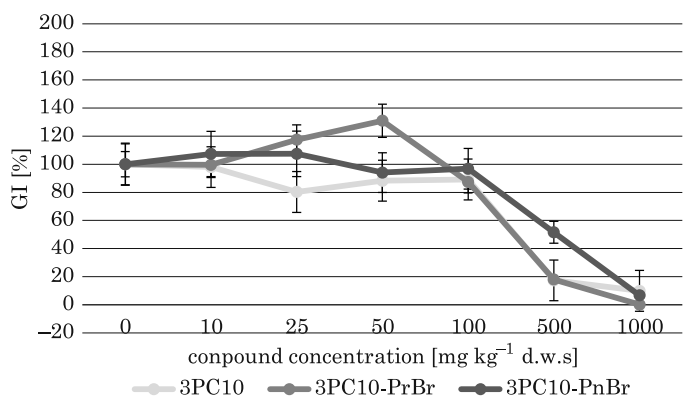


Fig. 5. Dependence of germination index on 3-pyridylketoxime and quaternary pyridinium ketoxime salts concentration

Discussion

In this study the influence of structure pyridine derivatives and alkyl chain length linked to the nitrogen atom of pyridine ring on its toxicity was tested. In general, germination and growth inhibition was more pronounced when a higher concentration of oxime and quaternary pyridinium ketoximes salts were used. Also, once the seeds germinated the inhibition of radicle growth was

more pronounced than growth of shoot, which could be explained by direct contact between compounds and radicle. The study was carried out in two systems, hydroponic and soil. Comparing growth in both systems, it could be seen that 3-pyridylketoxime and quaternary pyridinium ketoxime salts in the soil matrix exhibit less toxic than the effect hydroponic systems. This phenomenon could be caused by the sorption process of chemicals compounds in the soil matrix which may reduce the availability and thus smaller seeds in contact with the stressor agent. However, using the hydroponic system, seeds were exposed to direct contact with the chemical, which may adversely affect the germination of seeds and the subsequent development of the plant. In the literature, toxicity of different ionic liquids and quaternary salts are described, but most of them noted that the ionic liquids toxicity increased with the increase in length of the alkyl chain, which is in accordance with studies on various model organisms (CVEJTKO BUBALO et al. 2014, THUY et al. 2010, MATZKE et al. 2007, STUDZINSKA and BUSZEWSKI 2008). The observed effect of the length chain could be explained by mode of toxic action which takes place through membrane distribution due to the ionic liquids structural similarities to detergent, pesticides and antibiotics (ZHANG et al. 2012, LI et al. 2012). The long alkyl chains in the cation caused the increase the possibility of their interaction with cell membrane phospholipid bilayers and hydrophobic domains of membrane proteins, leading to disruption of membrane physiological functions and in consequence to the greater toxicity (RADOŠEVIČ et al. 2013, CVEJTKO BUBALO et al. 2014). However, in the study about influence of 3-pyridylketoxime and quaternary pyridinium ketoxime salts it was observed that pyridinium ketoxime bromides were less toxic for maize than precursor 3-pyridylketoxime, especially in the hydroponic system. But the analysis of the influence of the alkyl chain length was observed with different results than those described in the literature. Quaternary pyridinium salt with chain contain 3 carbon atoms at concentration 50–100 mg kg^{-1} d.w.s. stimulated shoot and radicle growth. However the compound with pentyl chain at concentration 500 i 1000 mg kg^{-1} d.w.s. was less toxic than compound with propyl chain.

Conclusions

3-pyridylketoxime and quaternary pyridinium ketoxime salts had varying effects on the germination of maize seeds. Phytotoxicity of these compounds depended largely on the structure of the compound, and the substrate on which the plants were grown. Higher toxicity of tested chemicals was observed in hydroponic system compared to the maize grown on the soil. The results

indicate almost ten-fold lower toxicity of tested chemicals in the soil than in the hydroponic system. This was probably due to sorption processes that occur in the soil matrix, while in an aqueous system, seeds were in direct contact with solutions of chemical substances. Generally, in the hydroponic cultivation all the tested compounds inhibited germination of seeds at the concentration of above 100 mg l^{-1} and the concentration which reached 500 mg l^{-1} resulted in complete inhibition of seeds germination. However, at low concentrations, in the range of 10 mg l^{-1} to 25 mg l^{-1}, they stimulated the germination of maize seeds. The toxic concentration of analysed compounds become evident above 100 mg l^{-1}. Less toxicity was indicated at soil cultivation than in hydroponic. The application of these substances at a concentration of 500 mg kg^{-1} d.w.s. resulted in a significant inhibition of radicle and shoot of maize, and the presence of substances with a concentration of 1000 mg kg^{-1} d.w.s. resulted in both the shoot length and radicle length not exceeding 10 mm, which represents about 10% of the shoot length or radicle length of control sample not exposed to chemical agent.

Translated by AUTHORS

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THE EFFECT OF ASAHI SL AND POLYVERSUM WP PREPARATIONS ON THE CHEMICAL COMPOSITION OF STRAWBERRY FRUITS

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A b s t r a c t

Within a three-year cycle of studies, experiments were conducted with Polyversum WP biofungicide and Asahi SL biostimulant used as crop protection products of strawberry fruits of Kent and Senga Sengana varieties. A comparative combination was made of the following fungicides: thiram, iprodione, pyrimethanil and fenhexamid, that were applied accordingly to the strawberry protection program. All protective treatments had no significant effect on dry matter content of the fruits of both strawberry varieties and on the marketable yield in the case of Kent var. fruits. The use of combination of Asahi SL and Polyversum WP preparations resulted in increased contents of vitamin C and total sugars in fruits of Kent var. In the case of Senga Segana var., this treatment had a beneficial effect only on vitamin C content in one year of the study and on the increase in the marketable yield when coupled with synthetic fungicides. The applied crop protection products caused also sporadic changes in the contents of extract, organic acids, polyphenols, anthocyanins and antioxidative capacity of fruits of both strawberry varieties.

WPŁYW PREPARATÓW ASAHI SL I POLYVERSUM WP NA SKŁAD CHEMICZNY OWOCÓW TRUSKAWKI

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S ł o w a k l u c z o w e: biostymulatory, biofungicyd, skład chemiczny, owoce truskawki, pestycydy.

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Abstrakt

W ciągu trzyletniego cyklu badań przeprowadzono doświadczenia z zastosowaniem biofungicydu Polyversum WP oraz biostymulatora Asahi SL w ochronie truskawki odmiany Kent i Senga Sengana. Kombinację porównawczą stanowiły fungicydy tiuram, iprodion, pirymetanil i fenheksamid stosowane zgodnie z programem ochrony truskawek. Wszystkie zabiegi ochronne nie miały istotnego wpływu na zawartość suchej masy w owocach obu badanych odmian truskawki oraz na wysokość plonu handlowego w odmianie Kent. W kombinacji z łącznym zastosowaniem preparatu Asahi SL i Polyversum WP obserwowano zwiększenie zawartości witaminy C i cukrów ogółem w owocach odmiany Kent. W przypadku odmiany Senga Segana widoczny był jedynie korzystny wpływ tego zabiegu na zawartość witaminy C w jednym roku badań oraz wzrost wysokości plonu handlowego w kombinacji z użyciem syntetycznych fungicydów. Stosowane środki ochrony roślin były również przyczyną sporadycznych zmian w zawartości ekstraktu, kwasów organicznych, polifenoli, antocyjanów i zdolności przeciwutleniającej owoców obu badanych odmian truskawek.

Introduction

Fruits of strawberry (*Fragaria* × ananassa Duch.) are appreciated by consumers for their high nutritional and health-promoting value and for their unique flavor and color (GIAMPIERI et al. 2012, MAHMOOD et al. 2012). This urges the search and implementation of novel solutions in crop protection to ensure the expected quality of yield, without residues of harmful chemical substances (CRERENTE-CAMPO et al. 2012). Therefore, the use of biostimulants, i.e. chemical compounds that enhance the growth and development of plants, is becoming increasingly common in the horticulture production. Their uptake is through roots or leaves of plants (BATTACHARYYA et al. 2015). They have a positive effect on the amount and quality of the yield as well as stimulate the natural immunity of plants to drought, frosts and soil salinity (ESITKEN and PIRLAK 2002). One of such stimulants is Asahi SL preparation which contains phenolic compounds that positively affect biochemical processes proceeding in a plant (PRZYBYSZ et al. 2014). Its effects are especially noticeable under conditions of plants exposure to stress induced by abiotic environmental conditions (BASAK and MIKOS-BIELAK 2008, KOZAK et al. 2008, KSIĘŻAK 2008, KOCIRA et al. 2015). Apart from biostimulants, a growing interest is observed in the sustainable horticulture in useful fungal species that aid the development and protection of plants against pathogens. The use of appropriate microorganisms as mycorrhitic symbionts may also contribute to the reduction of mineral fertilization (AZCÓN-AGUILAR and BAREA 1997). The Polyversum WP (*Pythium oligandrum*) biofungicide is usually applied for the protection of strawberries against *Botrytis cinerea* pathogen which induces gray mold (MESZKA and BIELENIN 2010). Under favorable weather conditions (high temperature of air and high precipitation), this disease may cause significant losses in the marketable yield of fruits. The non-parasitic fungus *Pythium*

oligandrum shows the capability of inducing direct pressure against plant pathogens and simultaneously of promoting the synthesis of phenolic compounds and lignin in the cellular membrane of plants and – by this means – of protecting the host against infection and pathogen spreading (REKANOVIC et al. 2007). In addition, it has a beneficial effect upon phosphorus metabolism and contents of saccharides in plants.

This study was aimed at determining the effect of Asahi SL and Polyversum WP preparations on the chemical composition of fruits of the analyzed strawberry varieties having various resistance to gray mold (*Botryris cinerea)*.

Material and Methods

The three-year field experiment (2010–2012) was carried out at the Experimental Garden of the University of Warmia and Mazury in Olsztyn. Strawberries were planted on plots with the area 5.1 m^2 in 30 x 80 x 100 cm spacing. The experiment was conducted in 4 replications. Experimental plots were as follows:

I) Control, without crop protection

II) Polyversum WP

III) Asahi SL

IV) Asahi SL + Polyversum WP

V) thiram (Thiram Granuflo 80 WG) + iprodione (Rovral Flo 255 SC) + pyrimethanil (Mythos 300 SC) + fenheksamid (Teldor 500 SC).

Detailed data concerning doses and terms of application of the preparations were provided in Table 1. The treatments were applied from the beginning of strawberry vegetation, following agrotechnical guidelines.

Samples of non-damaged fruits free of any symptoms of fungal infection were collected to determine the effect of the analyzed preparations on their quality. Laboratory analyses of strawberries harvested in the full fruiting season included determinations of contents of:

– dry matter (with the gravimetric method – *Fruit and vegetable...* PN-90/A-75101/03),

– extract (with the refractometric method – *Fruit and vegetable...* PN-EN 12143:2000),

– organic acids (with the titration method – *Fruit and vegetable...* PN-90/A-75101/04),

– total sugars (with the Lane-Eynon method – *Fruit and vegetable...* PN-90/A-75101/07),

– vitamin C (with the spectrophotometric method – *Foodstuffs...* PN-A-04019),

Table 1

Application terms and dosages of plant protection products in studied objects

Treatments	Plant protection products	Dosage	Year of investigation and dates of treatments		
			2010	2011	2012
I	control	–	–	–	–
II	Polyversum WP	100 g ha^{-1}	07.04; 11.05; 21.05; 01.06; 08.06	15.04; 10.05; 16.05; 23.05; 30.05; 07.06	18.04; 05.05; 09.05; 14.05; 18.05; 29.05
III	Asahi SL	0.5 l ha^{-1}	13.04; 11.05; 17.05	18.04; 10.05; 23.05	24.04; 05.05; 18.05
IV	Asahi SL + Polyversum WP	0.5 l ha^{-1}	13.04; 11.05	18.04; 10.05	24.04; 05.05
		100 g ha^{-1}	21.05; 01.06; 08.06	16.05; 23.05; 30.05; 07.06	09.05; 14.05; 18.05; 29.05
V	Thiram Granuflo 80 WG	4 kg ha^{-1}	11.05; 02.06	10.05; 30.05	05.05; 18.05
	Rovral Flo 255 SC	5 l ha^{-1}	17.05	16.05	09.05
	Mythos 300 SC	2.5 l ha^{-1}	24.05	23.05	13.05
	Teldor 500 SC	1.5 l ha^{-1}	09.06	05.06	29.05

– polyphenols (with the spectrophotometric method – SHAHIDI and NACZK 1995),

– anthocyanins (with the spectrophotometric method – WROLSTAD 1974),

– antioxidative capability (with the spectrophotometric method – MOURE et al. 2001).

Results obtained for the chemical composition of strawberry fruits were subjected to the two-way ANOVA analysis (first factor – protective treatment, second factor – variety), with the use of Statistica 10 software. The significance of differences between mean values was evaluated using Duncan's test at $P = 0.05$.

Results

Weather conditions noted in the vegetative season (IV–VII) during the years of 2010–2012 were usually favorable for the growth and development of plants (Table 2). In the first two years of the study, a significantly lower sum of precipitation was recorded in April compared to the multi-year data, which could have a negative effect on plant development during vegetation. Drought occurred also in the first decade of May in the third year of the study, which could affect the setting of strawberry fruits. In the successive period, namely during strawberry fruiting, the sum of precipitation and air temperatures in all study years did not differ from the average values recorded in the years 1961–2000.

In the analyzed period, the marketable yield (Table 3) of both strawberry varieties was primarily affected by variety-specific factors. Differences were noticeable mainly in the second and third year of fruiting, when a significant increase was determined in the yield, in favor of Senga Sengana variety. Exceptionally low yields of strawberry fruits in the first year of the study (2010) resulted from the delayed term of planting. The quality of fruits of both varieties was high, especially owing to a low pathogen pressure in the vegetative season. Analyses demonstrated an increase in contents of dry matter, extract and polyphenols in two study years in fruits of Kent var., whereas an opposite correlation was found in the case of organic acids and antioxidative capability of the fruits. Over the three-year study period, analyses demonstrated a higher content of anthocyanins in fruits of Senga Sengana var. The second and third year of the study were also characterized by higher contents of total sugars and vitamin C in fruits of Kent var., however statistically significant differences were demonstrated only in the second year. In turn, higher contents of sugars and extract in fruits of Senga Sengana var. were determined only in the first year of the study.

Table 2

Weather conditions in strawberry during 3-year vegetation periods (April – July) years in Olsztyn (Poland)

	Temperature [°C]												
	Year	2010				2011				2012			
	Month	April	May	June	July	April	May	June	July	April	May	June	July
Decade	1	6.5	10.8	18.2	20.0	8.1	8.7	18.8	17.1	2.2	13.1	12.2	21.6
	2	8.4	12.2	15.5	23.3	6.3	14.2	15.4	19.2	7.2	12.0	16.5	15.5
	3	9.3	12.9	15.6	20.1	12.9	16.2	16.9	17.6	14.1	14.8	16.4	19.9
	Average	8.1	12.0	16.4	21.1	9.1	13.0	17.0	18.0	7.8	13.3	15.0	19.0
Multiyear (1961–2000)		7.0	12.5	15.8	17.2	7.0	12.5	15.8	17.2	7.0	12.5	15.8	17.2
	precipitation [mm]												
Decade	1	4.8	25.4	25.3	31.3	9.1	10.8	39.9	124.8	20.5	0.8	33.8	76.7
	2	3.2	46.1	36.0	6.2	12.2	32.5	30.7	36.4	32.2	48.6	18.5	32.1
	3	10.2	60.4	23.5	42.9	1.2	7.8	11.1	41.6	20.4	2.3	50.9	12.2
	Sum	18.2	131.9	84.8	80.4	22.5	51.1	81.7	202.8	73.1	51.7	103.2	121
Multiyear (1961–2000)		35.4	57.6	69.5	81.6	35.4	57.6	69.5	81.6	35.4	57.6	69.5	81.6

Table 3

Marketable yield, dry mass and antioxidants capacity in strawberry fruits under plant protection products treatments

Cultivar and treatment		Yield [t ha^{-1}]				Dry mass [%]				DPPH [µmol DPPH 1 mg^{-1} phenolic compound]			
		2010	2011	2012	Mean	2010	2011	2012	Mean	2010	2011	2012	Mean
Kent	I*	$1.32^{a–c}$	10.46^{bc}	9.15^{ab}	6.98^{A}	10.83^{ab}	$9.27^{a–c}$	8.66^{bc}	9.59^{A}	13.58^{ab}	13.91^{bc}	13.39^{b}	13.62^{A}
	II*	$1.41^{a–c}$	$10.17^{a–c}$	10.18^{b}	7.25^{A}	11.34^{b}	10.13^{c}	$8.08^{a–c}$	9.85^{A}	$14.95^{a–}$c	14.93^{d}	10.35^{a}	13.41^{A}
	III*	$1.35^{a–c}$	9.02^{ab}	7.67^{a}	6.02^{A}	11.24^{ab}	9.71^{bc}	8.86^{c}	9.94^{A}	$14.43^{a–c}$	13.04^{a}	10.57^{a}	12.68^{A}
	IV*	1.26^{ab}	10.86^{c}	7.14^{a}	6.52^{A}	11.21^{ab}	$9.25^{a–c}$	$8.33^{a–c}$	9.60^{A}	13.14^{a}	12.98^{a}	13.04^{b}	13.05^{A}
	V*	1.60^{c}	8.58^{a}	9.49^{ab}	6.56^{A}	11.15^{ab}	$9.23^{a–c}$	$7.85^{a–c}$	9.40^{A}	$14.51^{a–c}$	$14.08^{b–d}$	10.36^{a}	12.98^{A}
	Mean	1.39^{A}	9.82^{A}	8.73^{A}	–	11.15^{B}	9.52^{B}	8.36^{A}	–	14.12^{A}	13.79^{A}	11.54^{A}	–
Senga Sengana	I*	$1.36^{a–c}$	13.28^{d}	19.99^{c}	11.55^{A}	10.20^{a}	$8.78^{a–c}$	7.66^{ab}	8.88^{A}	17.53^{de}	14.49^{cd}	14.09^{b}	15.37^{A}
	II*	1.50^{bc}	14.17^{d}	20.53^{c}	12.07^{A}	10.64^{ab}	8.10^{ab}	$8.23^{a–c}$	8.99^{A}	18.30^{de}	14.47^{cd}	12.93^{b}	15.23^{A}
	III*	$1.42^{a–c}$	14.34^{d}	21.06^{c}	12.27^{A}	10.41^{ab}	8.06^{ab}	$8.01^{a–c}$	8.83^{A}	16.45^{cd}	$13.70^{a–c}$	14.13^{b}	14.76^{A}
	IV*	1.18^{a}	10.84^{c}	20.07^{c}	10.69^{A}	10.93^{ab}	8.05^{ab}	$8.08^{a–c}$	9.02^{A}	$15.83^{b–d}$	13.45^{ab}	20.04^{c}	16.44^{A}
	V*	1.58^{c}	20.24^{e}	26.16^{d}	15.99^{A}	10.55^{ab}	7.92^{a}	7.33^{a}	8.57^{A}	19.25^{e}	$14.21^{b–d}$	12.12^{ab}	15.20^{A}
	Mean	1.41^{A}	14.57^{B}	21.56^{B}	–	10.55^{A}	8.18^{A}	7.86^{A}	–	17.47^{B}	14.06^{A}	14.66^{B}	–

Explanations: the same letters in column marks averages which do not differ between at $\alpha = 0.05$ significance level.
* Description of plant protection products treatments as in Table 1.

Table 4

Changes in extract, organic acids and total sugar content in strawberry fruits under plant protection products treatments

Cultivar and treatment		Extract [brix]				Total sugar [%]				Organic acids [%]			
		2010	2011	2012	Mean	2010	2011	2012	Mean	2010	2011	2012	Mean
Kent	I*	11.5^{b}	9.67^{bc}	$8.42^{a–c}$	9.86^{A}	6.32^{c}	5.75^{c}	6.22^{b}	6.10^{A}	1.13^{b}	0.72^{ab}	0.85^{ab}	0.90^{A}
	II*	9.67^{a}	10.00^{cd}	$8.17^{a–c}$	9.47^{A}	6.45^{cd}	6.06^{cd}	5.90^{b}	6.14^{A}	1.16^{b}	0.70^{a}	0.89^{ab}	0.92^{A}
	III*	10.00^{a}	10.33^{de}	8.67^{bc}	9.67^{A}	6.53^{de}	6.06^{cd}	5.96^{b}	6.18^{A}	1.14^{b}	0.68^{a}	0.89^{ab}	0.90^{A}
	IV*	11.83^{b}	10.67^{e}	8.92^{c}	10.47^{A}	6.64^{de}	6.34^{d}	6.15^{b}	6.38^{A}	1.18^{bc}	0.78^{b}	0.98^{b}	0.98^{A}
	V*	9.50^{a}	9.67^{bc}	$8.50^{a–c}$	9.23^{A}	6.07^{b}	6.11^{cd}	5.90^{b}	6.03^{A}	1.01^{a}	0.71^{a}	0.87^{ab}	0.86^{A}
	Mean	10.50^{A}	10.07^{B}	8.54^{B}	–	6.40^{A}	6.04^{B}	6.02^{A}	–	1.12^{A}	0.72^{A}	0.90^{A}	–
Senga Sengana	I*	11.67^{b}	8.83^{a}	8.08^{ab}	9.53^{A}	7.62^{f}	4.87^{b}	4.91^{a}	5.80^{A}	1.20^{bc}	0.94^{d}	0.89^{ab}	1.01^{A}
	II*	11.67^{b}	8.67^{a}	8.08^{ab}	9.47^{A}	7.97^{g}	4.82^{ab}	4.84^{a}	5.88^{A}	1.21^{bc}	0.85^{c}	0.84^{a}	0.97^{A}
	III*	12.00^{b}	8.83^{a}	$8.25^{a–c}$	9.69^{A}	6.70^{e}	4.92^{b}	4.91^{a}	6.84^{A}	1.26^{bc}	0.86^{c}	0.89^{ab}	1.00^{A}
	IV*	11.83^{b}	8.17^{ab}	7.83^{a}	9.28^{A}	5.68^{a}	4.70^{ab}	5.00^{a}	5.13^{A}	1.29^{c}	0.86^{c}	0.94^{ab}	1.03^{A}
	V*	12.00^{b}	8.67^{a}	$8.17^{a–c}$	9.63^{A}	5.81^{a}	4.34^{a}	4.76^{a}	4.97^{A}	1.18^{bc}	0.92^{cd}	0.92^{ab}	1.01^{A}
	Mean	11.83^{B}	8.64^{A}	8.09^{A}	–	6.76^{A}	4.73^{A}	5.68^{A}	–	1.23^{B}	0.89^{B}	0.90^{A}	–

Explanations: the same as in Table 3.

Table 5

Changes in vitamin C, polyphenols and anthocyanins content in strawberry fruits under plant protection products treatments

Cultivar and treatment		Vitamin C [mg 100 g^{-1}]				Polyphenols [mg 100 g^{-1}]				Anthocyanins [mg 100 g^{-1}]			
		2010	2011	2012	Mean	2010	2011	2012	Mean	2010	2011	2012	Mean
Kent	I*	53.51^{a}	64.62^{b}	59.41^{bc}	59.18^{A}	1151.03^{b}	1334.84$^{b–d}$	841.97^{ab}	1109.28^{A}	46.14^{a}	38.56^{a}	31.74^{bc}	38.81^{A}
	II*	74.11$^{b–d}$	73.63^{c}	73.55^{d}	73.76^{AB}	1034.71^{b}	1243.59^{ab}	1006.68^{e}	1094.99^{A}	48.19^{a}	44.69^{ab}	35.42^{c}	42.77^{AB}
	III*	83.12$^{b–d}$	74.06^{c}	55.35^{b}	70.84^{AB}	1145.66^{b}	1426.93^{d}	1355.81^{g}	1309.47^{A}	56.13^{ab}	43.58^{ab}	25.09^{a}	41.60^{A}
	IV*	84.77^{d}	82.52^{d}	81.59^{e}	82.96^{B}	1190.49^{b}	1406.08^{cd}	1210.22^{f}	1268.93^{A}	56.57^{ab}	54.32^{bc}	36.15^{c}	49.01^{AB}
	V*	80.18$^{b–d}$	73.36^{c}	66.56^{cd}	73.34^{AB}	1107.40^{b}	1353.32^{cd}	1248.22^{f}	1236.30^{A}	42.22^{a}	54.49^{bc}	26.73^{ab}	41.15^{A}
	Mean	75.14^{A}	73.65^{B}	67.28^{A}	–	1125.86^{B}	1352.95^{A}	1132.58^{B}	–	49.85^{A}	47.13^{A}	31.01^{A}	–
Senga Sengana	I*	70.22^{bc}	55.33^{a}	40.46^{a}	55.34^{A}	787.59^{a}	1221.05^{a}	914.94^{cd}	974.53^{A}	60.99^{ab}	54.35^{bc}	51.77^{f}	55.70^{AB}
	II*	81.21$^{b–d}$	55.89^{a}	45.27^{a}	60.79^{A}	758.84^{a}	1211.60^{a}	940.31^{d}	970.25^{A}	59.45^{ab}	61.51^{cd}	49.95^{ef}	56.97^{AB}
	III*	73.87$^{b–d}$	57.62^{a}	68.88^{d}	66.79^{AB}	857.34^{a}	1313.32$^{a–c}$	811.47^{a}	993.89^{A}	67.96^{ab}	71.17^{de}	44.67^{de}	61.27^{AB}
	IV*	83.73^{cd}	55.34^{a}	57.53^{b}	65.55^{A}B	881.82^{a}	1352.93^{cd}	875.35^{bc}	1036.39^{A}	70.06^{ab}	81.44^{e}	42.63^{d}	64.71^{B}
	V*	69.53^{b}	53.47^{a}	69.34^{d}	64.11^{AB}	747.14^{a}	1214.52^{a}	853.81^{ab}	938.47^{A}	85.69^{b}	58.87^{c}	48.01$^{d–f}$	53.71^{AB}
	Mean	75.71^{A}	55.54^{A}	56.29^{A}	–	806.54^{A}	1262.49^{A}	879.08^{A}	–	62.53^{B}	65.47^{B}	47.40^{B}	–

Explanations: the same as in Table 3.

The use of Asahi SL and Polyversum WP preparations had no effect on the marketable yield of fruits of both strawberry varieties. In turn, an increase in its value was found on the plot of Senga Sengana var. with fungicide protection (by 52% in the second and by 30% in the third year of the study). This effect was due to high susceptibility of this variety to infestation with gray mold – *Botrytis cinerea*. The applied preparations did not contribute to any noticeable changes in dry matter content (Table 3) and organic acids content in fruits of both strawberry varieties (Table 4). Sporadic changes were observed in the content of polyphenols (Table 5) and anthocyanins as well as in antioxidative capability (Table 3) in fruits of both strawberry varieties. Changes in contents of the extract and total sugars (Table 4) were noted in two years of the study in fruits of Kent var. from the plots treated with Asahi SL + Polyversum WP preparations. This variant of crop protection allowed also achieving a significant increase in vitamin C content in fruits of this variety. The analyzed preparations caused sporadic fluctuations in the chemical composition of strawberry fruits of Senga Sengana var.

Discussion

Plant genotype and cultivation conditions have a key impact on fruit quality (GÜNDÜZ and ÖZDEMIR 2014). Strawberry plants reach their yield-bearing potential in the second and third year of fruiting (KARP and STARAST 2002). Results of experiments conducted by many authors (PIŠTEKOVÁ et al. 2011, BOČEK et al. 2012, SZOT et al. 2014) confirm no direct effect of using crop protection products on the yield of strawberry fruits. In turn, many research works indicate a significant effect of the cropping system on changes in the chemical composition of strawberry fruits (ESITKEN et al. 2010, ROCHALSKA et al. 2011, FERNANDES et al. 2012, KOVAČEVIĆ et al. 2015). Investigations conducted by ESITKEN and PIRLAK (2002) point to the accumulation of greater quantities of macro- and microelements in leaves of strawberries treated with biostimulants. The use of growth and development stimulants in horticulture was found to increase crop yield (KOCIRA et al. 2015) and contents of vitamin C, polyphenols, anthocyanins and sugars (GHONAME et al. 2009; SZOT et al. 2014) as well as organic acids (GRAJKOWSKI and OCHMIAN 2007). Some other studies demonstrate, however, no effect of biostimulants on contents of dry matter and organic acids (KRAWIEC 2008), extract (SZOT et al. 2014) as well as vitamin C, polyphenols and anthocyanins (ROUSSOS et al. 2009).

Conclusions

1. The Asahi SL and Polyversum WP preparations had no significant effect on the size of the marketable yield and on contents of dry matter and organic acids in strawberry fruits of Kent and Senga Sengana varieties. The noticeable differences were caused by variety-specific factors.

2. The study demonstrated that the use of the Asahi SL biostimulant and the Polyversum WP biofungicide in one combination caused increased contents of vitamin C and total sugars in fruits of Kent var.

3. Intensive fungicide protection had only a sporadic positive effect on the chemical composition of strawberry fruits.

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THE PERFORMANCE OF GERMAN SHEPHERD AND BELGIAN SHEPHERD MALINOIS DOGS IN OBEDIENCE, OBSTACLE COURSE, DEFENSE AND TRACKING TESTS

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K e y w o r d s: police dogs, German Shepherd, Belgian Shepherd Malinois, patrol-tracking dogs, patrol dogs, tracking dogs.

A b s t r a c t

In Poland and other countries, the two breeds that are most commonly trained for police operations are the German Shepherd (GS) and the Belgian Shepherd Malinois (BSM). The purchase, training and maintenance of police dogs are very expensive. Appropriate preselection can lower those costs. Therefore, the aim of the study was to compare GS and BSM dogs based on their performance in obedience, obstacle, defense and tracking tests. The study was performed on 222 dogs which participated in patrol-tracking training, patrol training and tracking training at the Kennel of the Police Training Center in Sułkowice. In many exercises: stay in obedience, stair climbing, balance beam, wooden wall and an obstacle course in obstacle completion, pursuit with a muzzle, pursuit without a muzzle and guarding detainees in defense, as well as field search, indoor search and vocalization in tracking, BSM were scored significantly better than GS.

PORÓWNANIE OWCZARKÓW NIEMIECKICH I OWCZARKÓW BELGIJSKICH MALINOIS W ZAKRESIE POSŁUSZEŃSTWA, POKONYWANIA PRZESZKÓD, OBRONY I TROPIENIA

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Abstrakt

W Polsce i na świecie dwiema najczęściej szkolonymi rasami na psy patrolowo-tropiące, patrolowe i tropiące są owczarki niemieckie (GS) oraz owczarki belgijskie malinois (BSM). Zakup, szkolenie oraz utrzymanie psów policyjnych jest bardzo drogie. Odpowiednia preselekcja zwierząt może obniżyć te koszty. Celem pracy było porównanie GS i BSM oparte na wynikach zwierząt w testach posłuszeństwa, pokonywaniu toru przeszkód, obronie i tropieniu. Badaniami objęto 222 psy, które uczestniczyły w kursach na psa patrolowo-tropiącego, patrolowego lub tropiącego odbywających się w Zakładzie Kynologii Policyjnej Centrum Szkolenia Policji w Sułkowicach. W wielu ćwiczeniach: zostawanie w posłuszeństwie, schody, kładka, drewniana ściana i pokonywanie grupy przeszkód, pościg w kagańcu, pościg bez kagańca i konwojowanie osób w obronie, a także przeszukiwanie terenu, przeszukiwanie pomieszczeń i wokalizacja w tropieniu, BSM osiągnęły statystycznie istotnie wyższe wyniki niż GS.

Introduction

In the past, shepherd dogs assisted shepherds in managing a flock or a herd of animals. Today, shepherd dogs are more often used as defense dogs and military working dogs (COURREAU and LANGLOIS 2004). In Poland and other countries, the two breeds that are most commonly trained as operational-patrol dogs, patrol dogs and tracking dogs for police operations are the German Shepherd (GS) and the Belgian Shepherd Malinois (BSM). This reflects, in part, the overal trends in dog breeding in Poland (STRYCHALSKI and GUGOŁEK 2010). Dogs that are crosses between the two breeds or Dutch Shepherds are far less frequently used in police work (SINN et al. 2010, WALASEK 2012). GS and the BSM are easy to train, and they are widely available from breeders and vendors. These breeds are also scent-trained to detect substances such as narcotics and explosives. However, Labradors, Springer Spaniels, German Shorthaired Pointers and other hunting breeds are increasingly used in scent training (WARREN 2013, BRADSHAW 2015).

Dogs have to be properly and regularly trained for top performance. Patrol-tracking dogs should be trained for both defense and scent detection (tracking). Patrol dogs and tracking dogs are trained for the same skills: patrol dogs are trained for defense, and tracking dogs – for scent detection. They are also trained for obedience and obstacle completion. The selection of dogs that are best suited for training, the choice of handlers with the desired qualities and proper training are essential for the effective performance of police dogs (BRADSHAW 2015). The purchase, training and maintenance of police dogs are very expensive. Appropriate preselection can lower those costs. Due to lack of standardization in tests designed to evaluate dog behavior, scientific knowledge about the behavioral differences between breeds and their predispositions for specific tasks remains modest (GOODLOE and BORCHELT 1998, MURPHY 1998, DIEDERICH and GIFFROY 2006).

In view of the above, the aim of the current study was to compare German Shepherd and Belgian Shepherd Malinois dogs based on their performance in obedience, obstacle course, defense and tracking tests.

Materials and Methods

The study was performed on 222 dogs, aged 1 to 2 years,which participated in patrol-tracking training, patrol training and tracking training (Table 1). The animals were qualified for training based on the results of tests carried out by certified evaluators. Training courses were held at the Kennel of the Police Training Center in Sułkowice in 2014 and 2015. All dogs were obtained from breeders certified by the Polish Kennel Club (ZKwP), a member of Federation Cynologique Internationale (FCI), the international federation of kennel clubs.

Table 1

Dogs which participated in patrol-tracking training, patrol training and tracking training

Specification	Number of dogs	Breeds	
		GS	BSM
Patrol-tracking training	156	126	30
Patrol training	27	20	7
Tracking-training	39	33	6
Total	222	179	43

GS – German Shepherd; BSM – Belgian Shepherd Malinois

Dogs participating in all three courses were evaluated for obedience and obstacle completion. Obedience tests involved the following exercises: heel-work (the dog walks close to the handler's leg), turns (the dog maintains its position throughout the turn), stand (the dog maintains a standing position), sit, down, stay (while the handler walks away), recall (the dog is recalled from a stay position and sits by the handler's left leg) and stop (while moving). The obstacle completion test included the following exercises: stair climbing (open-work stairs – Figure 1), balance beam (trapezoid), tunnel, wooden wall and an obstacle course (various obstacles arranged in line). Dogs participating in patrol-tracking training were also evaluated in defense and tracking categories. The defense test included the following exercises: pursuit with a muzzle, pursuit without a muzzle, protecting the handler, guarding detainees, assisting in identifying a suspect, assisting in frisking a suspect. The tracking test included the following exercises: tracking human scent, field search (to locate a person), indoor search (to locate a person) and vocalization (to indicate that

the searched person has been found). Dogs participating in patrol training did not perform tracking exercises, whereas dogs participating in tracking training did not perform defense exercises.

Fig. 1. Openwork stairs in the Police Training Center in Sułkowice

The animals were trained by the same persons. Tests were conducted under standard conditions by certified examiners from the Kennel of the Police Training Center in Sułkowice. Examination grades obtained by each dog were entered into Training Progress Reports. Dogs were evaluated during each of the 4 training stages. Mean scores were computed for each exercise, and the results were used to determine mean scores for every training category. Dogs were evaluated subjectively by examiners, on a scale of 2 to 5 points (2 points – fail, 5 points – highest grade).

Data were expressed as means ± standard deviation (SD). The results were processed statistically using the least squares method in the GLM procedure. The results were compared with the use of the $Y_{ijk} = \mu + \alpha_i + \beta_j + \alpha_i\beta_j + \varepsilon_{ijk}$ model, where μ is the overall average, α_i is the effect of breed, β_j is the effect of sex, $\alpha_i\beta_j$ is the interaction effect between breed and sex, and εijk is random error. Analyses did not reveal significant effects of sex or significant interactions between fixed effects, therefore,the relevant data were not presented. Calculations were performedin the Statistica (StatSoft, Inc. 2010) program.

Results

The results scored in obedience tests are presented in Table 2. It should be noted that BSM received higher grades in all exercises, but significant differences between the two breeds were determined only in the "stay" exercise (4.16 ± 0.45 BSM vs. 3.96 ± 0.50 GS, $p = 0.019$). BSM also scored higher in the overall obedience rating, but significant differences were observed only at the trend level ($p = 0.053$).

Table 2

Scores (pts) obtained by dogs from the discipline of obedience (mean ± SD)

Exercises	Breeds		*P*-value
	GS	BSM	
Heelwork	3.82 ± 0.51	3.91 ± 0.52	0.298
Turns	3.70 ± 0.52	3.82 ± 0.52	0.174
Stand	3.73 ± 0.57	3.91 ± 0.53	0.054
Sit	4.07 ± 0.51	4.22 ± 0.49	0.091
Down	3.98 ± 0.47	4.05 ± 0.55	0.386
Stay	3.96 ± 0.50	4.16 ± 0.45	**0.019**
Recall	3.52 ± 0.61	3.60 ± 0.60	0.403
Stop	3.75 ± 0.55	3.92 ± 0.53	0.060
Total	3.82 ± 0.41	3.95 ± 0.39	0.053

SD – standard deviation; GS – German Shepherd; BSM – Belgian Shepherd Malinois

Significant differences between the evaluated breeds were noted in obstacle completion (Table 3). Overall, GS scored 3.77 ± 0.41 points, and BSM – 4.00 ± 0.36 points ($p < 0.001$). The breeds differed significantly in the number of points scored in stair climbing, balance beam, wall and obstacle course tests. Similarities were noted only in the tunnel test, although BSM also scored somewhat higher in this category.

Table 3

Scores (pts) obtained by dogs from the discipline of obstacle completion (mean ± SD)

Exercises	Breeds		*P*-value
	GS	BSM	
Stair climbing	3.84 ± 0.46	3.99 ± 0.38	**0.047**
Balance beam	3.66 ± 0.49	3.82 ± 0.40	**0.044**
Tunnel	3.89 ± 0.51	4.02 ± 0.41	0.105
Wooden wall	3.60 ± 0.57	4.00 ± 0.47	**<0.001**
Obstacle course	3.88 ± 0.53	4.20 ± 0.51	**<0.001**
Total	3.77 ± 0.41	4.00 ± 0.36	**<0.001**

SD – standard deviation; GS – German Shepherd; BSM – Belgian Shepherd Malinois

The results of defense tests are presented in Table 4. Overall, BSM scored 4.01 ± 0.37 points and GS 3.86 ± 0.35 points, and the differences between the breeds were statistically significant. BSM scored significantly higher in the following trials: pursuit in a muzzle, pursuit without a muzzle, and guarding detainees. At the trend level, BSM also scored somewhat higher in protecting the handler (4.20 ± 0.57 BSM vs. 4.01 ± 0.61 GS, $p = 0.096$, statistical trend). No significant differences between the analyzed breeds werenoted in the suspect identification test ($p = 0.614$) or the frisking test ($p = 0.209$).

Table 4

Scores (pts) obtained by dogs from the discipline of defense (mean ± SD)

Exercises	Breeds		*P*-value
	GS	BSM	
Pursuit with a muzzle	3.78 ± 0.46	4.00 ± 0.42	**0.009**
Pursuit without a muzzle	3.98 ± 0.41	4.18 ± 0.38	**0.006**
Protecting the handler	4.01 ± 0.61	4.20 ± 0.57	0.096
Guarding detainees	3.83 ± 0.38	4.05 ± 0.45	**0.003**
Assisting in identifying a suspect	3.81 ± 0.47	3.86 ± 0.52	0.614
Assisting in frisking a suspect	3.70 ± 0.50	3.82 ± 0.52	0.209
Total	3.86 ± 0.35	4.01 ± 0.37	**0.021**

SD – standard deviation; GS – German Shepherd; BSM – Belgian Shepherd Malinois

In the tracking category, BSM scored a total of 3.99 ± 0.44 points, and GS – only 3.76 ± 0.47 points ($p = 0.003$). Both breeds obtained similar scores in human scent tracking, whereas BSM scored significantly more points in field search (4.15 ± 0.49), indoor search (4.22 ± 0.47) and vocalization (4.75 ± 0.39) than GS (3.89 ± 0.51, 3.90 ± 0.55 and 4.06 ± 0.52, respectively).

Overall, GS did not outperform BSM in any of the evaluated categories (obedience, obstacle completion, defense and tracking) or individual tests/exercises.

Table 5

Scores (pts) obtained by dogs from the discipline of tracking (mean ± SD)

Exercises	Breeds		*P*-value
	GS	BSM	
Tracking human scent	3.40 ± 0.70	3.44 ± 0.69	0.726
Field search	3.89 ± 0.51	4.15 ± 0.49	**0.002**
Indoor search	3.90 ± 0.55	4.22 ± 0.47	**0.002**
Vocalization	4.06 ± 0.52	4.75 ± 0.39	**0.006**
Total	3.76 ± 0.47	3.99 ± 0.44	**0.003**

SD – standard deviation; GS – German Shepherd; BSM – Belgian Shepherd Malinois

Discussion and Conclusions

Empirical research targeting the behavioral characteristics of dog breeds is generally scarce because comprehensive statistical data are difficult and costly to collect (WEISS and GREENBERG 1997, MEHRKAM and WYNNE 2014, STRYCHALSKI et al. 2015). For this reason, most studies comparing the trainability of various dog breeds rely on surveys and other indirect assessment methods (COREN 1994, HELTON 2010, GARDIÁNOVÁ et al. 2013). The results of our study clearly indicate that BSM are more suited for police work as patrol-tracking dogs, patrol dogs and tracking dogs than GS. BSM scored higher in all evaluated categories.

In a study by GARDIÁNOVÁ et al. (2013) who analyzed the results of FCI-IPO world championships, BSM also outperformed GS. BSM scored a higher number of points in each evaluated category (obedience, defense and scent tracking). BSM were more gifted than GS, and the greatest differences between the two breeds were reported in obedience and defense categories. In the evaluated years, BSM significantly outperformed GS in the tracking category only in 2003. The IPO disciplines are similar to police training categories, but police dogs are additionally required to complete an obstacle course. Our study revealed that BSM scored highly significantly more points than GS in obstacle and tracking tests, and significantly more points than GS in defense tests. Significant differences in obedience scores were not noted between the analyzed breeds.

According to GARDIÁNOVÁ et al. (2013), GS respond better to a change of handler and are more focused on the performed tasks. GS are widely trained for outdoor activities, services and home tasks. However, BSM are more enthusiastic workers. They are more active in training, they work harder and learn faster than GS, which is why they are the preferred breed for sports training. Therefore, it can be assumed that test scores are largely determined by BSM's willingness to work. This trait cannot be measured directly, but our experience with dogs suggests that BSM are much more willing to work than GS. However, for a new handler, GS is usually a better choice than BSM because it has better habits and a more balanced character (GARDIÁNOVÁ et al. 2013).

Dog breeds differ not only in appearance, but also in working intelligence and trainability (ROONEY and BRADSHAW 2004). COREN (1994) has developed the most comprehensive scale for ranking breeds by trainability. In Coren's scale, GS rank third, whereas BSM occupy a distant 22nd position. GS score top grades in the first category, and BSM score lowgrades in the second category. These results indicate that GS are able to comprehend a new command already after several repetitions (1 to 4), and that they perform a task at first command

in more than 95% of cases. According to Coren, BSM understand the meaning of the command after 5 to 15 repetitions, and correctly perform the task at first command in 85% of cases. Coren's methodologyhas been debated (COPPINGER and COPPINGER 2001), but most researchers agree with the ranking positions occupied by individual breeds (DAVIS and CHEEKE 1998, MIKLÓSI 2009, STRYCHALSKI et al. 2015). However, Coren's ranking was first published 20 years ago, and the positions occupied by GS and BSM should be revised due to evolutionary changes in different breeds and modern training techniques. This observation is confirmed by the results of the present study. It should also be noted that the number of GS used by border guards and customs officers in Germany, the breed's country of origin, decreased by 70% in just one decade (GERRITSEN and HAAK 2007).

The results of this study and published data do not indicate that GS is no longer suitable for police work. According to many authors, GS is a highly desirable breed for less experienced dog handlers (GERRITSEN and HAAK 2007, SINN et al. 2010, ALLSOPP 2012, GARDIÁNOVÁ et al. 2013). Beginners quickly achieve the desired results, even when they make more training mistakes than their more experience colleagues. BSM can score higher in tests if they are handled by more experienced police officers. BSM are more eager to work and are more impulsive than GS, therefore, more experienced handlers and longer training are needed to control them. BSM seem to be better suited for police work if placed under the care of appropriate handlers.

Summarizing results obtained in our study, BSM outperformed GS in obedience, obstacle, defense and tracking tests.

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GRILLED *VERSUS* FIRE-ROASTED SAUSAGE – THE CONTENT OF POLYCYCLIC AROMATIC HYDROCARBONS AND HEALTH SAFETY

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K e y w o r d s: sausage, polycyclic aromatic hydrocarbons, grill, campfire, health.

A b s t r a c t

The concentrations of polycyclic aromatic hydrocarbons (PAHs) were determined in grilled sausage and fire-roasted sausage, and the results were compared with statutory threshold levels. Sausages were grilled over charcoal and roasted over a campfire. Sausage was heated until the temperature in the geometric center of the sample reached 75°C. Nine PAH compounds were identified by high-performance liquid chromatography with fluorescence detection (HPLC-FLD) using an internal calibration standard. Thermal processing increased the content of PAHs in the analyzed samples, and significantly higher levels of contamination were noted in fire-roasted sausage than in grilled sausage. In grilled sausage, the content of BaP and Σ 4 PAHs (BaA, BaP, CHR, BbF) did not exceed the maximum permissible levels of 5 and 30 μg kg^{-1}, respectively, whereas in fire-roasted sausage, the concentrations of the above compounds exceeded maximum levels several fold and reached 18 and 73 μg kg^{-1}, respectively. The results of this study indicate that fire-roasted sausage poses a significant risk of exposure to PAHs.

KIEŁBASA GRILLOWANA *VERSUS* OPIEKANA W OGNIU – ZAWARTOŚĆ WIELOPIERŚCIENIOWYCH WĘGLOWODORÓW AROMATYCZNYCH A BEZPIECZEŃSTWO ZDROWOTNE

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S ł o w a k l u c z o w e: kiełbasa, wielopierścieniowe węglowodory aromatyczne, grill, ognisko, zdrowie.

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Abstrakt

Celem pracy było określenie zanieczyszczenia związkami z grupy WWA grillowanej i opiekanej w ogniu kiełbasy oraz ocena tego zanieczyszczenia w odniesieniu do obowiązujących regulacji prawnych. Obróbkę termiczną prowadzono na grillu węglowym oraz w ognisku. Surowce ogrzewano do uzyskania temperatury 75°C w centrum geometrycznym próbek. Oznaczenie 9 związków z grupy WWA przeprowadzono metodą wzorca wewnętrznego, techniką HPLC/FLD. Obróbka termiczna kiełbasy skutkowała zwiększeniem zawartości związków z grupy WWA w produktach, przy czym zanieczyszczenie kiełbasy z ogniska było znacząco większe w porównaniu z produktami grillowanymi. W grillowanej kiełbasie zawartość BaP i Σ 4 WWA (BaA, BaP, CHR, BbF) nie przekroczyła dopuszczalnych poziomów maksymalnych 5 i 30 μg kg^{-1}, natomiast w kiełbasie opiekanej w ogniu zawartość tych związków wielokrotnie przekraczała dopuszczalne poziomy, osiągając wartości odpowiednio 18 i 73 μg kg^{-1}. Kiełbasa opiekana w ogniu stanowi więc istotne źródło narażenia zdrowotnego na związki WWA.

Introduction

Processed meat products, including grilled meats, contain polycyclic aromatic hydrocarbons (PAHs), and their consumption could pose a health risk for humans. Polycyclic aromatic hydrocarbons with more than 4 aromatic rings are known as heavy polyarenes which are characterized by greater stability and higher toxicity than light polyarenes (JANOSZKA 2011, FARHADIAN et al. 2010). In vivo studies performed on laboratory animals revealed that PAHs exert genotoxic and mutagenic effects on somatic cells. The metabolic activation of PAHs to epoxide intermediates is responsible for the mutagenic and carcinogenic properties of those compounds. The resulting diol epoxides form covalent bonds with cellular macromolecules, including DNA, which could lead to DNA replication errors and mutations (BENFORD et al. 2010, BENIGNI and PASSERINI 2002, JANOSZKA 2011, JÄGERSTAD and SKOG 2005). The International Agency for Research on Cancer (IARC) has classified PAHs as substances probably carcinogenic for humans (group 2A) or possibly carcinogenic for humans (group 2B) (IARC 2010, JANOSZKA 2011). The maximum permissible levels of PAHs in selected foodstuffs, including smoked and grilled meat products, have been defined to guarantee consumer safety based on the frequency of consumption of different food products (Commission Regulation (EU) No 835/2011). Fire-roasted sausage is rarely sold in retail, and it is prepared individually by consumers in spring and summer.

In charcoal barbecues, food is heated by hot air with a temperature of 150–320°C (CIEMNIAK 2007, DYREMARK et al. 1995, ŠIMKO 2002). In grilled foods, smoke from incomplete fuel combustion and compounds produced during pyrolysis of organic substances are the main sources of contamination with PAHs (DYREMARK et al. 1995, EFSA 2008, FARHADIAN et al. 2011, JÄGERSTAD and SKOG 2005, REINIK et al. 2007). Sausages are also popularly roasted over a campfire. The wood burning process is relatively unstable, and wood is

thermally decomposed during open fire burning (with unlimited availability of atmospheric oxygen), thermolysis (under anaerobic conditions) or pyrolysis (with limited availability of atmospheric oxygen). At temperatures higher than 425–450°C during pyrolysis or 800°C during open fire burning, roasted products become highly contaminated with PAHs. The rate of combustion can be controlled only by modifying the availability of oxygen (DOLATOWSKI et al. 2014).

The aim of this study was to determine the concentrations of polycyclic aromatic hydrocarbons in grilled and fire-roasted medium-ground sausages, and to compare the results with the statutory maximum limits of PAHs.

Materials and Methods

Materials

The experiment was performed on cooked and smoked medium-ground sausages which were grilled on charcoal or roasted over a campfire. Sausages were purchased in a chain store based on the personnel's ranking of the most popular barbecue products in the summer season. The purchased products had the following composition (based on label data): sausage 1 (S_1) – pork (68%), water, beef (5%), potato starch, pork fat, salt, soybean protein, pork protein, spices and additives; sausage 2 (S_2) – pork (60%), water, salt, pork collagen, hydrolyzed corn protein, spices and additives. Sausage samples of approximately 100 g were grilled on charcoal and roasted over a campfire.

Thermal processing

Sausages were grilled on charcoal in the Outdoor 83521T barbecue. The cooking grid was suspended 0.2 m above the flames, and grid diameter was 0.5 m. Charcoal briquettes (3.5 kg) were used as fuel. The average grilling temperature was 247°C ± 16. Fire-roasted sausages were prepared in pyramid-shape campfires when most wood had turned to charcoal. Campfire 1 (CF1) was built of beech wood, and campfire 2 (CF2) was made of beech and debarked birch wood. Sausages were roasted away from direct flame, at a distance where temperature did not exceed 350°C. Sausages were roasted until the temperature inside the geometric center of each sample reached 75°C, and the time required to achieve the above temperature was determined experimentally (n = 9). The average grilling time was 11 minutes, and the average fire-roasting time was 8 minutes. Temperature was measured with the Hanna

Instrument HI 92804 C thermometer and temperature probes (HI 766 B, HI 762 PWL). Samples in duplicate ($n = 2$) of S_1 and S_2 (raw material: sausages 1 and 2), GS1 and GS2 (grilled sausages 1 and 2), GST1 and GST2 (sausages 1 and 2 grilled on aluminum trays), CF11 and CF21 (sausage 1 roasted in campfire 1 and 2), CF12 and CF22 (sausage 2 roasted in campfire 1 and 2) were analyzed to determine the content of PAHs ($n = 3$).

Polycyclic aromatic hydrocarbons – analytical procedure

The following reagents and standards were used in the PAH analysis: acetonitrile (ACN), dichloromethane (DCM), chloroform, methanol (HPLC Labscan, Dublin, Ireland), fluoranthene, pyrene, benz[*a*]anthracene (BaA), chrysene (CHR), benzo[*b*]fluoranthene (BbF), benzo[*k*]fluoranthene (BkF), benzo[*a*]pyrene (BaP), dibenz[*a,h*]anthracene (DhA), benzo[*ghi*]perylene (BghiP) in acetonitrile – 10 ng μl^{-1} (Ehrenstorfor GmbH, Germany). The remaining reagents were of analytical grade.

The analyses were carried out based on the method described by WĘGRZYN et al. (2005) where the lipid fraction was extracted and PAHs were separated by preparative size-exclusion chromatography (SEC). Quantitative and qualitative analyses of PAHs were performed by reversed-phase high-performance liquid chromatography with fluorescence detection (HPLC-FLD; stationary phase – non-polar, mobile phase – water, acetonitrile).

Homogenized samples of 1 g were placed in glass test tubes, and the internal standard and 1.5 ml of methanol were added. Samples were shaken in a vortex mixer for 1 min, and they were combined with 3 ml of chloroform and 1.5 ml of water. The mixture was shaken for 3 min and centrifuged for 10 min at 10,000 rpm. The chloroform solution containing the PAH lipid fraction was passed through grade 4 Whatman filter paper into a 10 ml test tube. The samples were extracted with 3 ml of chloroform, shaken in a vortex mixer for 3 min and centrifuged for 10 min at 10,000 rpm. The chloroform fraction was passed through filter paper. Chloroform fractions were combined and evaporated to dryness under nitrogen in a water bath (40°C). The residue left after evaporation was dissolved in 4 ml of dichloromethane. The extracts were purified by SEC with the mobile phase (DCM) at a flow rate of 1 ml min^{-1}. Measurements in the UV detector were performed at $\lambda = 254$ nm. Dichloromethane extracts of 400 µl were purified. Eluent was directed to the fraction collector and it was evaporated until dryness under nitrogen in a water bath (40°C). Dry residues were dissolved in 200 µl of ACN. The samples were applied onto a chromatographic column in the HPLC-FLD system. Chromatographic separation was performed with the water/acetonitrile mobile

phase in the gradient elution mode. Samples were applied to columns with 50:50 water : acetonitrile. Acetonitrile concentration was increased to 100% within 20 min, it was maintained for 15 min and returned to the initial level. The mobile phase had a flow rate of 0.8 ml min^{-1} and temperature of 25°C. The volume of the samples applied to the column was 20 µl. The column was heated at 25°C in a thermostat. The fluorescence detector was calibrated based on the retention times of benzo[*b*]chrysene and benzo[*a*]pyrene (KUBIAK et al. 2011, WĘGRZYN et al. 2005). The applied analytical procedure fulfilled the requirements of Commission Regulation (EC) No. 333/2007 of 28 March 2007. The limit of detection (LOD, µg kg^{-1}), limit of quantification (LOQ, µg kg^{-1}) and recovery [%] were determined at: 0.2/0.4/102 for fluoranthene, 0.2/0.4/96 for pyrene, 0.2/0.4/101 for BaA, 0.1/0.3/78 for CHR, 0.1/0.3/98 for BbF, 0.1/0.2/104 for BkF, 0.1/0.3/90 for DhA, 0.1/0.3/76 for BghiP, and 0.1/0.2/102 for BaP, respectively. The concentrations of PAHs in untreated sausages (raw material) and heat-treated sausages (final products) were used in a comparative analysis.

The results were expressed by means ± standard deviation, which were calculated in a spreadsheet (Excel). The significance of differences between mean PAH concentrations was determined by one-way ANOVA at $p \leq 0.05$.

Results and Discussion

The concentrations of the analyzed PAHs in untreated and thermally processed sausages are presented in Table 1 for sausage 1 and Table 2 for sausage 2. As expected, untreated sausage (cooked and smoked) was contaminated with PAHs from the smoking process. The concentrations of PAHs in sausage 1 (S_1) and sausage 2 (S_2) were comparable at 28.9 and 28.13 µg kg^{-1}, respectively. The quality of the examined sausages, relative to the PAH content of smoked meat products, evaluated based on the content of BaP and Σ 4 PAHs (BaA, BaP, CHR, BbF), fulfilled the requirements of Commission Regulation (EU) No. 835/2011 of 19 August 2011. The concentrations of four PAHs in the analyzed sausages did not exceed 12 µg kg^{-1}, whereas BaP content was below the method detection limit at 0.2 µg kg^{-1}.

The contamination levels of smoked sausage determined in this study are consistent with the results reported by other authors. KUBIAK et al. (2011) analyzed 466 samples of smoked meat products and observed that BaP concentrations in sausages characterized by various degrees of grinding did not exceed the maximum level of 5 µg kg^{-1}. In a study by CIECIERSKA et al. (2011), the average content of BaP in the outer and inner layers of smoked medium-ground sausages was determined at 0.89 and 0.32 µg kg^{-1}, respective-

ly. Total contamination, expressed by the concentrations of 15 PAHs which are classified as potentially carcinogenic by the Scientific Committee on Food of the European Food Safety Authority (SCF-EFSA), was determined at 38.27 μg kg^{-1} in outer layers. The concentrations of 4 PAHs (BaA, BaP, CHR, BbF) in the analyzed samples of smoked medium-ground sausage exceeded the maximum level of 12 μg kg^{-1} only in outer layers (17.49 μg kg^{-1}), whereas significantly lower levels (2.84 μg kg^{-1}) were found in inner layers (CIECIERSKA et al. 2011). According to the cited authors, the observed variations in the PAH content of smoked meat products can be attributed mainly to differences in the applied smoking methods.

Table 1

The content of PAHs in raw material and the final product – sausage 1

PAHs	Experimental material									
	S_1		GS1		GST1		CF11		CF21	
	μg kg^{-1}	SD	μg kg^{-1}	SD	μg kg^{-1}	SD	μg kg^{-1}	SD	μg kg^{-1}	SD
Fluoranthene	12.5^{A}	0.29	14.19^{A}	1.32	10.73^{B}	0.44	58.22^{C}	2.35	36.77^{D}	2.42
Pyrene	15.07^{A}	0.39	17.26^{A}	1.77	13.14^{B}	0.38	68.56^{C}	1.78	40.35^{D}	2.09
Benz[*a*]anthracene	0.81^{A}	0.07	1.66^{B}	0.09	0.84^{A}	0.03	19.04^{C}	0.77	12.03^{D}	0.17
Chrysene	0.52^{A}	0.03	1.46^{B}	0.13	0.68^{C}	0.02	16.35^{D}	0.36	10.49^{E}	0.26
Benzo[*b*]fluoranthene	blm	–	1.49^{A}	0.18	0.7^{B}	0.02	19.36^{C}	0.09	15.71^{D}	0.12
Benzo[*k*]fluoranthene	blm	–	0.57^{A}	0.02	0.31^{B}	0.01	7.29^{C}	0.08	5.11^{D}	0.05
Benzo[*a*]pyrene	**blm**	**–**	**1.51^{A}**	**0.07**	**0.99^{B}**	**0.02**	**18.72^{C}**	**0.9**	**12.6^{D}**	**0.13**
Dibenz[*a,h*]anthracene	blm	–	blm	–	blm	–	0.81^{A}	0.04	0.63^{B}	0.04
Benzo[*g,h,i*]perylene	blm	–	1.00^{A}	0.11	0.62^{B}	0.02	11.69^{C}	0.27	8.31^{D}	0.46
Σ 4 PAHs	**1.33^{A}**	**0.1**	**6.12^{B}**	**0.47**	**3.21^{C}**	**0.09**	**73.47^{D}**	**2.12**	**50.83^{E}**	**0.68**
Σ PAHs	**28.9^{A}**	**0.78**	**39.14^{B}**	**3.69**	**28.01^{A}**	**0.94**	**220.04^{C}**	**6.64**	**142^{D}**	**5.74**

Explanations: S_1 – sausage 1, GS1 – grilled sausage 1, GST1 – sausage 1 grilled on aluminum trays, CF11 – sausage 1 roasted in campfire 1, CF21 – sausage 1 roasted in campfire 2;
Σ PAHs – the sum of fluoranthene, pyrene, benz[*a*]anthracene, chrysene, benzo[*b*]fluoranthene, benzo[*k*]fluoranthene, benzo[*a*]pyrene, dibenz[*a,h*]anthracene, benzo[*g,h,i*]perylene;
Σ 4 PAHs – the sum of benz[*a*]anthracene, benzo[*a*]pyrene, chrysene and benzo[*b*]fluoranthene;
A, B... – means in rows denoted by different superscript letters differ significantly at $p \leq 0.05$;
SD – standard deviation ($n = 6$);
blm – below the quantification limit of the method.

The contamination of sausage 1 (S_1) increased after thermal processing and was determined at 39.14 μg kg^{-1} in grilled sausage (GS1), 220.04 μg kg^{-1} in sausage roasted in campfire 1 (CF11) and 142 μg kg^{-1} in sausage roasted in campfire 2 (CF12).

Grilling increased the concentrations of the analyzed PAHs by more than 35% relative to initial levels, but the content of BaP and Σ 4 PAHs did not

exceed the maximum levels for grilled meat products which are set at 5 and 30 μg kg^{-1}, respectively. Sausages grilled on aluminum trays were characterized by practically unchanged total PAH content and higher levels of BaA, BaP, CHR and BbF, but the concentrations of those 4 PAHs did not exceed maximum levels.

The contamination of grilled products with PAHs is determined by various factors, including the chemical composition of processed material, time and temperature of grilling, and the applied grilling method (CIEMNIAK 2007, WIĘK et al. 2013). Regardless of the processed material and grilling method, the content of PAHs generally does not exceed 5 μg kg^{-1} in sausage. In a study by LARSSON et al. (1983), BaP concentrations did not exceed 0.2–0.3 μg kg^{-1} in Frankfurter sausages cooked on an electric grill, and they were below 1 μg kg^{-1} in sausages grilled over charcoal. In this study, the BaP content of grilled sausage was determined at 1.51 μg kg^{-1} for sausage 1 and 1.58 μg kg^{-1} for sausage 2. Similar values were reported by MOTTIER et al. (2000) in whose study, BaP concentrations in lamb sausage reached 0.32–2.81 μg kg^{-1}. In the grilling process, not all fuel components are completely combusted during pyrolysis, and volatile products, including PAHs, are deposited on grilled food (DYREMARK et al. 1995, WIĘK et al. 2013). According to researchers, the contamination of grilled foods with PAHs can be reduced through the use of physical barriers such as aluminum trays (CIEMNIAK 2007, TKACZ et al. 2012). TKACZ et al. (2012) reported that aluminum trays can reduce PAH contamination by more than 70% and BaP levels by up to 80%.

A dramatic increase in PAH levels was noted in fire-roasted sausage 1. Contamination levels increased nearly 8-fold in sausage roasted in campfire 1 and nearly 5-fold in sausage roasted in campfire 2. The BaP content of the above samples was determined at 18.72 and 12.6 μg kg^{-1}, respectively, and the content of Σ 4 PAHs – at 73.47 and 50.83 μg kg^{-1}, respectively. Similar changes were observed in sausage 2 (S_2) after fire roasting (Table 2). Thermal processing increased the concentrations of PAHs in the analyzed products, and the contamination levels noted in fire-roasted sausage were significantly higher than in grilled sausage.

The maximum levels of PAHs set by Commission Regulation (EC) No. 835/2011 of 19 August 2011 apply only to meat and meat products that have undergone a heat treatment process known to potentially result in the formation of PAHs, i.e. only grilling and barbecuing. The concentrations of BaP and Σ 4 PAHs, which are particularly hazardous for consumer health, in fire-roasted sausage significantly exceeded the maximum levels for grilled products.

There are scant published data on the contamination of fire-roasted sausage with PAHs. Most studies focus on the health risks associated with the

Table 2

The content of PAHs in raw material and the final product – sausage 2

PAHs	Experimental material									
	S_2		GS1		GST1		CF11		CF21	
	μg kg^{-1}	SD	μg kg^{-1}	SD	μg kg^{-1}	SD	μg kg^{-1}	SD	μg kg^{-1}	SD
Fluoranthene	12.21^{A}	0.21	15.51^{B}	0.94	11.17^{C}	0.31	55.88^{D}	1.66	39.19^{E}	1.72
Pyrene	14.69^{A}	0.27	19.03^{B}	1.18	13.78^{A}	0.27	66.78^{C}	1.26	42.44^{D}	1.48
Benz[*a*]anthracene	0.74^{A}	0.05	1.75^{B}	0.06	0.87^{A}	0.02	18.27^{C}	0.56	12.20^{D}	0.12
Chrysene	0.49^{A}	0.02	1.59^{B}	0.09	0.67^{C}	0.01	16.00^{D}	0.25	10.75^{E}	0.18
Benzo[*b*]fluoranthene	blm	–	1.67^{A}	0.12	0.68^{B}	0.01	19.45^{C}	0.06	15.83^{D}	0.09
Benzo[*k*]fluoranthene	blm	–	0.60^{A}	0.01	0.32^{B}	0.01	7.21^{C}	0.06	5.16^{D}	0.03
Benzo[*a*]pyrene	**blm**	**–**	$\mathbf{1.58^{A}}$	**0.05**	$\mathbf{0.98^{B}}$	**0.01**	$\mathbf{17.81^{C}}$	**0.64**	$\mathbf{12.73^{D}}$	**0.09**
Dibenz[*a,h*]anthracene	blm	–	blm	–	blm	–	0.85^{A}	0.03	0.67^{B}	0.02
Benzo[*g,h,i*]perylene	blm	–	1.10^{A}	0.07	0.63^{B}	0.01	11.96^{C}	0.19	7.86^{D}	0.32
Σ 4 PAHs	$\mathbf{1.23^{A}}$	**0.07**	$\mathbf{6.59^{B}}$	**0.32**	$\mathbf{3.2^{C}}$	**0.05**	$\mathbf{71.53^{D}}$	**1.51**	$\mathbf{51.51^{E}}$	**0.48**
Σ PAHs	$\mathbf{28.13^{A}}$	**0.55**	$\mathbf{42.83^{B}}$	**2.52**	$\mathbf{29.1^{A}}$	**0.65**	$\mathbf{214.21^{C}}$	**4.71**	$\mathbf{146.83^{D}}$	**4.05**

Explanations: S_2 – sausage 2, GS2 – grilled sausage 2, GST2 – sausage 2 grilled on aluminum trays, CF12 – sausage 2 roasted in campfire 1, CF22 – sausage 2 roasted in campfire 2;
Σ PAHs – the sum of fluoranthene, pyrene, benz[*a*]anthracene, chrysene, benzo[*b*]fluoranthene, benzo[*k*]fluoranthene, benzo[*a*]pyrene, dibenz[*a,h*]anthracene, benzo[*g,h,i*]perylene;
Σ 4 PAHs – the sum of benz[*a*]anthracene, benzo[*a*]pyrene, chrysene and benzo[*b*]fluoranthene;
A, B... – means in rows denoted by different superscript letters differ significantly at $p \leq 0.05$;
SD – standard deviation ($n = 6$);
blm – below the quantification limit of the method.

consumption of smoked and grilled meat products which are generally characterized by high levels of PAHs. In this study, fire-roasted sausage was significantly more contaminated with PAHs than grilled sausage. Similar results were reported by LARSSON et al. (1983) who observed that roasting over an open fire can dramatically increase the BaP content of sausage to more than 50 μg kg^{-1}. In our study, BaP concentrations in fire-roasted sausage did not exceed 20 μg kg^{-1}, probably because the analyzed samples were roasted farther away from the flames. In meat products roasted over an open fire, high contamination with PAHs can be attributed to the high temperature of the wood burning process, which increases from an ignition temperature of 300–350°C to 800°C or even 1100°C (DOLATOWSKI et al. 2014).

The presence of dibenz[*a,h*]anthracene was also noted in fire-roasted sausage. This compound considered to be five times more toxic than BaP – the toxicity equivalency factor (TEF) is 1 for BaP and 5 for DhA. Dibenz[*a,h*]anthracene was not found in grilled samples.

According to the SCF-EFSA, the content of 15 PAHs in processed food should be monitored to minimize potential health hazards for consumers. The

above list does not include fluoranthene and pyrene which have been placed on the list of 16 PAHs classified as priority pollutants by the US Environmental Protection Agency (Us EPA 1984). In the present study, the percentage of fluoranthene and pyrene in the total PAH content of grilled sausage was high in the range of 54–95%, but samples with a higher total PAH content were characterized by lower concentrations of fluoranthene and pyrene.

Conclusions

The following conclusions were formulated based on the results of this study:

– fire-roasted sausage is a significant source of potentially toxic PAHs;

– the PAH content of grilled sausage did not exceed the statutory maximum limits for meat products;

– the use of aluminum trays contributed to a significant decrease in the contamination of grilled sausage with PAHs;

– the contamination of fire-roasted sausage with PAHs varied significantly depending on the type of wood;

– the maximum permissible levels have not been set for contaminants in fire-roasted sausage, which is prepared individually by consumers; therefore, the potential health hazards associated with the consumption of fire-roasted sausage should be communicated to the public in scientific and popular science literature.

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THE EFFECT OF DIETS WITH DRIED BEET PULP ON GROWTH INDICATORS IN ARCTIC FOXES (*Vulpes lagopus* L.)

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K e y w o r d s: arctic fox, dried sugar beet pulp, grains, body weight, daily gains.

A b s t r a k t

The aim of the study was to determine the effect of feed ration with a variable content of carbohydrates on the production performance of blue arctic fox. The research included 60 blue arctic foxes which were divided into two groups: experimental (30 individuals) and control (30 individuals). The experimental group, compared to the control group, was fed with a ration with a higher level of carbohydrates, whose source were ground grains (ground barley and wheat) as well as dried beet pulp. The experiment lasted from weaning of the pups until the day when the foxes were slaughtered (29 week). Based on control weighing (at the beginning of the experiment, at 17 and 29 weeks of age) the average body weight was calculated (in kg), as well as the total (in kg) and daily (in g) body weight gain. The average final body weight of foxes in both groups was similar, and ranged from 15.67 kg (control group) to 16.20 kg (experimental group). Males were characterized by a slightly higher body weight than females (however, these differences were not confirmed statistically). In the experimental group the total and daily body weight gains during growth were higher, and during the fur growth period they were lower compared with the control group. The use of rations with an increased proportion of carbohydrates in the arctic fox nutrition, whose source were extruded ground grains and dried beet pulp, did not negatively affect on selected of the production performance of foxes over the whole experimental period compared with the control group.

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WPŁYW DIET Z UDZIAŁEM SUSZONYCH WYSŁODKÓW BURACZANYCH NA WSKAŹNIKI WZROSTU LISÓW POLARNYCH (*Vulpes lagopus* L.)

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S ł o w a k l u c z o w e: lis polarny, suszone wysłodki buraczane, śruty zbożowe, masa ciała, przyrosty dobowe.

A b s t r a k t

Celem badania było określenie wpływu dawki pokarmowej o zróżnicowanej zawartości węglowodanów na efekty produkcyjne lisa polarnego niebieskiego. Badaniami objęto 60 osobników lisa polarnego niebieskiego, które podzielono na dwie grupy: doświadczalną (30 sztuk) i kontrolną (30 sztuk). Grupa doświadczalna, w porównaniu z grupą kontrolną, była żywiona dawką pokarmową o wyższym poziomie węglowodanów, których źródłem były śruty zbożowe (jęczmienna i pszenna) oraz suszone wysłodki buraczane. Doświadczenie trwało od momentu odsadzenia szczeniąt do dnia uśmiercenia lisów (29 tydzień). Na podstawie kontrolnych ważeń (na początku doświadczenia, w 17. i 29. tygodniu życia) obliczono średnią masę ciała [kg], całkowity [kg] i dobowy [g] przyrost masy ciała. Średnia końcowa masa ciała lisów w obu grupach była podobna i wynosiła od 15,67 kg (grupa kontrolna) do 16,20 kg (grupa doświadczalna). Samce charakteryzowały się nieznacznie wyższą masą ciała niż samice (różnic tych nie potwierdzono jednak statystycznie). W grupie doświadczalnej całkowite i dobowe przyrosty masy ciała w okresie wzrostu były wyższe, a w okresie kształtowania się okrywy włosowej niższe w porównaniu z grupą kontrolną. Wykorzystanie w żywieniu lisów polarnych dawek pokarmowych ze zwiększonym udziałem węglowodanów, których źródłem były ekstrudowane śruty zbożowe i suszone wysłodki buraczane, w porównaniu z grupą kontrolną nie wpłynęło negatywnie na wybrane wyniki produkcyjne lisów w całym okresie doświadczenia.

Introduction

Optimal balancing of the ration for carnivorous fur-bearing animals always constitutes a great challenge for a breeder, as this is one of the most important factors determining health condition of these animals, their growth, size of production, and quality of the obtained pelts. Foxes are typical carnivorous animals whose digestive system is adapted to digest food that is high in protein and energy. Therefore, in breeding practice the main dietary components for them are fish and post-slaughter products of meat industry. However, it should be highlighted that a relatively cheap and easily available substitute of energy for carnivorous fur-bearing animals may be some groups of carbohydrates, mainly simple sugars and starch (AHLSTRØM et al. 2003, LAERKE et al. 2004, GUGOŁEK et al. 2014). The above mentioned authors state that the process of domestication of these animals, compared with their wild ancestors, significantly increased the level of carbohydrate use. The research of AHLSTRØM et al.

(2003) indicated a higher ability to digest carbohydrates in the feed (including starch and glucose) in blue arctic foxes than in foxes living in the wild. On the other hand, KORHONEN and NIEMELÄ (2014) found that farm-raised foxes digested carbohydrates better than minks. Carbohydrates constitute not only a cheap source of energy for animal's body but they also play a significant role in fat conversion – they are precursors of producing oxaloacetic acid which takes part in oxidation and synthesis of fatty acids in the liver. Deficiency or lack of carbohydrates in the diet of carnivorous fur-bearing animals leads to metabolic disorders which may result in acetonaemia (*Zalecenia żywieniowe*... 2011). In order to avoid mistakes in nutrition, minimal percentage proportion of energy should be observed in the total amount of metabolic energy (EM) in the ration. According to NRC (1982) and *Normy żywienia*... (1994), minimal proportion of energy from carbohydrates should be 12% and the maximal one 20% EM of the diet, irrespective of the nutrition period. The latest Polish nutrition recommendations for fur-bearing animals (*Zalecenia żywieniowe*... 2011) state that proportion of energy from carbohydrates in rations for farm-raised foxes and minks should be within a range from 20% EM.

In breeding practice, the most frequent source of carbohydrates in rations for carnivorous fur-bearing animals are ground grains of barley and wheat. Although alternative carbohydrate sources to cereal plants (e.g.: potatoes, potato pulp, Jerusalem artichoke, distillers dried grains with solubles DDGS, beet pulp) were also tested, yet with a conclusion that they may be used in limited amounts (RUVIENEN-WATTI et al. 2000, ZAKRZEWSKA-CZARNOGÓRSKA and BRZOZOWSKI 2000, LAERKE et al. 2004, LISITSKAYA et al. 2011).

The aim of the research was to determine if dried beet pulp may be used in the diet of arctic foxes (*Vulpes lagopus* L.) as an additional source of carbohydrates, apart from ground grains, without a negative effect on the production performance.

Material and Methods

The experiment was carried out on a fox farm in the Kuyavian-Pomeranian Voivodship. 60 blue arctic foxes were selected for studies after weaning at 10 weeks of age. The pups were divided into two nutrition groups, each including 30 foxes (15 ♀: 15 ♂). The foxes were kept in individual cages (0.6 m^2 per fox) in a shed system, providing them with a continuous access to water. Two types of diet were used in fox nutrition over the period of growth and development (July-September) as well as over the fur growth period (October-November). The feed was given once a day at the same time. For the whole experimental period, from weaning until the slaughter day, the foxes had ad libitum access

Table 1

Components [%] and energy value of fox diets

Item	Growth period		Fur growth period	
	control group	experimental group	control group	experimental group
Whole flounder	12.0	12.0	10.0	10.0
Whole cod	10.0	10.0	2.0	2.0
Bones	10.0	10.0	10.0	10.0
Various poultry wastes	11.0	6.0	16.0	9.5
Poultry meat	12.0	12.0	8.0	8.0
Poultry viscera	25.0	25.0	30.0	30.0
Fish meal	1.0	1.0	2.0	2.0
Blood meal	1.0	1.0	1.0	1.0
Meat and bone meal	3.5	3.5	4.0	4.0
Animal fat	3.5	2.5	5.0	4.0
Dried beet pulp	0.0	3.0	0.0	4.0
Extruded cereals (barley/wheat)	11.0	14.0	12.0	15.5
In total	100.0	100.0	100.0	100.0
Vitamin and mineral supplement*	+	+	+	+
Metabolic energy [MJ kg^{-1}]	7.03	7.05	7.74	7.85
Energy share [%] from:				
protein	35.72	35.70	30.80	30.63
fat	52.11	45.00	57.14	49.37
carbohydrates	12.17	19.30	12.06	20.00

* commercial vitamin and mineral supplement was added to diets in quantities recommended by the producer

to feed. In rations for foxes from the control group, the source of carbohydrates were cereal plants (extruded barley and wheat in a proportion of 1:1). The experimental animals were fed with a ration to which, apart from extruded cereals, dried beet pulp was added. All diets were balanced according to nutrition recommendations for raising foxes (NRC, 1982, Normy żywienia... 1994, *Zalecenia żywieniowe*... 2011). The content of protein, fat and carbohydrates in the feed was determined with the use of a full-spectrum Diode Array NIR Analyzer 7200, at the laboratory of the National Association of Breeders and Producers of Fur-Bearing Animals, Tarnowo Podgórne. Energy value of tested diets was evaluated based on chemical ingredients and energy equivalents adapted from *Zalecenia żywieniowe*... (2011). The main components of the tested diets (Table 1) were products of animal and fish origin. The proportion of plant feeds in control diets over the period of growth and development as well as over the fur growth period oscillated on the level of 11 and 12%, and it was lower than in the experimental diets by 3 and 3.5%, respectively. In rations

for control foxes the source of carbohydrates were extruded ground grains such as: ground barley and ground wheat, used in a proportion of 1:1. However, apart from ground grains, dried beet pulp was additionally introduced to the experimental diets. Energy value of the rations in particular experimental periods and percentage proportion of EM from protein in their total energy value were comparable. Percentage proportion of EM from carbohydrates compared with the total energy value of the diet of control foxes oscillated on the level of about 12%, and was lower by over 7% compared with rations for the experimental foxes. Inverse dependences were found compared with the percentage proportion of EM from fat.

During research, body weight of foxes was controlled individually three times (at the beginning of the experiment, at 17 and 29 weeks of age) with an accuracy to 0.1 kg. The average body weight was calculated [kg], as well as the average total body weight gain [kg], and the average daily body weight gain [g].

The results were subjected to statistical analysis using program Statistica (StatSoft Inc. 2010). An arithmetic mean ($\bar{x}$) was calculated as well as standard deviation (SD). To evaluate the effect of nutrition and sex, fixed effects model (GLM) was used, as well as a two-way analysis of variance with the use of cross classification with an interaction. The model included the following effects: diet and sex.

$$y_{ijk} = \mu + a_i + B_j + (aB)_{ij} + e_{ijk}$$

where:

y_{ijk} – value of the studied trait (body weight, total gains, daily gains)
a_i – the effect of i^{th} diet,
B_j – the effect of factor B (sex) of j^{th} sex,
$(aB)_{ij}$ – interactions between factors,
e_{ijk} – random error.

Results

From the data presented in Table 2 it follows that at the beginning of the experiment, an average body weight of pups in both groups was equal and oscillated on the level of 3.80 kg. At 17 weeks of age, both males and females fed with a diet with a proportion of ground grains and dried beet pulp (experimental group) were heavier ($P \leq 0.01$) than foxes getting a ration in which the source of carbohydrates were only ground grains (control group), by 17 and 20%, respectively. However, no significant differences were indicated between the groups in an average body weight of whole fox populations. On the

Table 2

Indicators of growth rate in foxes

Item	Statistical measures	Feeding groups					
		control			experimental		
		♀	♂	♀ + ♂	♀	♂	♀ + ♂
Body weight [kg]							
10 weeks	$\bar{x}$	3.77	3.84	3.81	3.57	3.99	3.78
	SD	0.31	0.30	0.30	0.46	0.68	0.61
17 weeks	$\bar{x}$	9.89^{A}	10.09^{B}	9.99	11.55^{A}	12.15^{B}	11.85
	SD	1.91	1.05	1.52	1.37	1.68	1.54
29 weeks	$\bar{x}$	15.35	15.99	15.67	15.49	16.90	16.20
	SD	1.50	1.05	1.31	1.92	2.25	2.18
Total body weight gain [kg]							
10–17 weeks	$\bar{x}$	6.12^{a}	6.25^{B}	6.18^{C}	7.97^{a}	8.16^{B}	8.07^{C}
	SD	1.70	0.98	1.36	1.13	1.54	1.33
18–29 weeks	$\bar{x}$	5.47^{A}	5.90^{b}	5.68^{C}	3.94^{A}	4.75^{b}	4.35^{C}
	SD	1.30	0.56	1.01	1.01	2.00	1.61
10–29 weeks	$\bar{x}$	11.58	12.15	11.86	11.91	12.91	12.42
	SD	1.32	1.05	1.20	1.66	1.85	1.80
Daily body weight gain [g]							
10–17 weeks	$\bar{x}$	98.71^{A}	100.80^{B}	99.68^{C}	128.60^{A}	131.61^{B}	130.11^{C}
	SD	27.40	15.83	22.02	18.15	24.89	21.46
18–29 weeks	$\bar{x}$	71.04^{A}	76.62^{b}	73.77^{C}	51.17^{A}	61.69^{b}	56.49^{C}
	SD	16.30	7.02	12.64	13.11	26.01	20.95
10–29 weeks	$\bar{x}$	83.31	87.41	85.32	85.71	92.87	89.33
	SD	9.41	7.47	8.60	11.92	13.29	12.94

Explanations: *aa*, *bb* – means (in rows) differ significantly at $P \leq 0.05$; *AA*, *BB*, *CC* – means (in rows) differ significantly at $P \leq 0.01$

slaughter day (29 weeks of age) arctic foxes from the experimental group reached a similar body weight to foxes from the control group.

In the period of growth and development the total body weight gains of foxes (Table 2) fed with a ration with a proportion of ground grains and dried beet pulp were higher by 30% ($P \leq 0.01$) compared with the control group. In the fur growth period, it was indicated that, compared with the control group, diets with a maximal proportion of energy from carbohydrates significantly decreased ($P \leq 0.05$ and $P \leq 0.01$) the total gains in males and females by 28 and 19%, respectively, and by 23% in the whole fox population. The total body weight gains for the whole experimental period in both groups were comparable.

Growth rate of arctic foxes (Table 2) fed with rations with a maximal proportion of energy from carbohydrates between 10 and 17 weeks of age was on average by about 30% higher ($P \leq 0.01$) than growth rate in control foxes. In the fur growth period, an inverse dependence was indicated. Higher variation

in daily body weight gains was observed between females (28%) than between males (19%) of the compared groups. Average daily body weight gains of the whole population of foxes fed with rations with a high proportion of carbohydrates were lower by 23% ($P \leq 0.01$) compared with the population of control foxes. Average daily body weight gains in foxes over the whole experimental period in the control and experimental group were comparable. The rate of fox growth between 18 and 29 weeks of age was lower than between 10 and 17 weeks of age, over twice as low in the group fed with a diet with approximately 20% proportion of energy from carbohydrates, and over 1.4-fold in the control group. Males were characterized by a slightly better production performance than females, however the occurring differences were not confirmed statistically.

Discussion

An important aspect of nutrition of farmed fur-bearing carnivorous animals is an appropriate proportion of plant material in the diet which should oscillate from several to a dozen or so percent (LOREK et. al. 2002, GUGOŁEK et. al. 2012). In our studies, the proportion of plant material in the component composition of diets was a factor varying fox nutrition, and depending on the nutrition period in the control group oscillated on the level of 11–12%, and in the experimental group on the level of 17 and 19.5%. Introducing a higher amount of plant components into the experimental diet than in the control diet, in the form of extruded ground grains (ground barley and ground wheat) and dried beet pulp, increased % proportion of EM from carbohydrates in the total energy value of rations from 12 to about 20%. From the available literature (ZAKRZEWSKA-CZARNOGÓRSKA and BRZOZOWSKI 2000, AHLSTRØM et al. 2003, LAERKE et al. 2004, GUGOŁEK et al. 2007, GUGOŁEK et. al. 2012, KORHONER and NIEMELÄ 2014) it follows that the degree of plant feed utilization by carnivorous animals is diversified and depends, among other things, on carbohydrate groups occurring in them, on animal species and technological treatment before feeding. Among various carbohydrate groups, simple sugars and starch subjected to thermal treatment are the most bioavailable for carnivorous animals. Therefore, in breeding practice the most popular plant component of diets for fur-bearing carnivorous animals are extruded ground grains, mainly ground barley and ground wheat. The studies of KORHONEN and NIEMELÄ (2014) indicated that the process of extruding barley grain significantly increased digestibility of carbohydrates in diets of farmed minks and foxes, from 45–52% to 57–60% and 65–73%, respectively, with an approximately 16% proportion of this cereal in the ration structure. Moreover,

farm-raised foxes digested carbohydrates better than minks. ZAKRZEWSKA-CZARNOGÓRSKA and BRZOZOWSKI (2000) indicated a favorable effect of enzymatic preparations added to diets with a 21% proportion of ground grains on digestibility of nutrients in red foxes. The cited authors state that easily digestible and highly bioavailable feed guarantees good production performance in foxes, proper animal size and quality of their pelts. Introducing dried beet pulp into rations as an additional, apart from extruded ground grains, source of energy significantly increased body weight of arctic foxes at 17 weeks of age, however it did not vary the final body weight of animals compared with the foxes fed with a diet with a proportion of ground wheat and ground barley. It should be highlighted that average body weights of foxes both at 17 (6.18 and 8.07 kg) as well as at 29 weeks of age (15.67 and 16.20 kg) were higher compared with the results of research of other authors (LOREK et al. 2002, GUGOŁEK et. al. 2004, GUGOŁEK et al. 2007, GUGOŁEK et al. 2012, NOWICKI et al. 2013, PRZYSIECKI et al. 2013). GUGOŁEK et. al. (2007) and GUGOŁEK et. al. (2012) states that for arctic foxes raised under Polish production conditions, the final body weight oscillated on the level of 10–11.5 kg. In our studies, feeding with rations with plant carbohydrate feeds (ground grains and dried beet pulp) on the level of 17% favorably affected the total and daily body weight gains in foxes over the period of growth compared with the foxes fed with a diet with 11% proportion of carbohydrates. Inverse dependencies were observed in the fur growth period, in which experimental foxes were fed with rations with a proportion of carbohydrate feeds on the level of about 20%. It was indicated in author's research results that body weight gains in males between 10 and 17 weeks of age and between 18 and 29 weeks of age were higher than in females regardless of the type of diet. The research of PRZYSIECKI et. al. (2013) indicated similar results. The analyzed production performance for the whole experimental period in both groups was similar but higher from 37 to 42%, compared with the results presented by PRZYSIECKI et al. (2010). Literature data state that carbohydrates contained in plant feeds not only provide animal body with energy but also affect physical and chemical properties and functioning of the digestive system (LOREK et al. 2001, LAERKE et al. 2004, GUGOŁEK et al. 2012, KORHONER and NIEMELÄ 2014). LAERKE et al. (2004) indicated that diets with a proportion of beet pulp and corn starch on the level of 4.40 and 11.31%, respectively, increased minks' ability to fix water from the digesta, and decreased digestibility of nutrients compared with diets in which the source of carbohydrates were such cereal products as expanded wheat and oat flakes.

Conclusion

The use of rations with an increased proportion of carbohydrates in arctic fox nutrition whose source were extruded ground grains and dried beet pulp, compared with the control group which was fed with a diet supplemented with only extruded ground grains:

– increased body weight of foxes at 17 weeks of age but did not vary this parameter in the period before slaughter;

– favorably affected total and daily body weight gains in foxes in the period of growth, however it decreased analyzed production indicators in foxes in the fur growth period;

– did not impact analyzed production parameters for the whole experimental period.

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EFFECTS OF NEONICOTINOID INSECTICIDE ACETAMIPRID ON SWIMMING VELOCITY, HEART RATE AND THORACIC LIMB MOVEMENT OF *DAPHNIA MAGNA*

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K e y w o r d s: acetaprimid, neonicotinoid, Daphnia, behaviour, crustacean physiology.

A b s t r a c t

Little is known on the effects of nicotinoid pesticides on behavioural and physiological parameters of microcrustaceans. The aim of the study was to determine the effects of three concentrations (25, 50 and 100 mg l^{-1}) of neonicotinoid insecticide Mospilan 20 SP (containing 20% of the active ingredient acetamiprid) on swimming velocity and physiological parameters such as heart rate and thoracic limb activity in *Daphnia magna*. The results showed that acetamiprid induced concentration-dependent inhibition of swimming velocity and thoracic limb activity after 2 hours of the exposure. The insecticide depressed the heart rate at 100 mg l^{-1} after 24 hours of the exposure, however stimulation was noted at 25 and 50 mg l^{-1}. The study suggests that neonicotinoids may alter on behavioural and physiological parameters in *Daphnia magna* thereby increasing susceptibility of these animals to higher predator pressure.

WPŁYW INSEKTYCYDU NEONIKOTYNOWEGO ACETAMIPRIDU NA ZMIANY PRĘDKOŚCI PŁYWANIA, BICIA SERCA I RUCHU ODNÓŻY TUŁOWIOWYCH U *DAPHNIA MAGNA*

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S ł o w a k l u c z o w e: acetamiprid, neonikotynoid, Daphnia, behawior, fizjologia skorupiaków.

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Abstrakt

Niewiele wiadomo na temat oddziaływania neonikotynoidów na behawioralne i fizjologiczne parametry mikroskorupiaków. Celem badań było określenie wpływu trzech koncentracji (25, 50 i 100 mg l^{-1}) neonikotynoidowego insektycydu Mospilan 20 SP (20% acetamipridu) na szybkość pływania i parametry fizjologiczne, takie jak: częstotliwość skurczów serca i aktywność odnóży brzusznych u *Daphnia magna*. Wykazano, że acetamiprid wywołuje zależne od koncentracji obniżenie szybkości pływania i aktywności odnóży brzusznych po 2 godzinach ekspozycji. Insektycyd w stężeniu 100 mg l^{-1} po 24-godzinnej ekspozycji obniża częstotliwość skurczów serca, jednak w stężeniach 25 and 50 mg l^{-1} zanotowano efekt stymulujący. Badania sugeują, iż neonikotynoidy mogą powodować zmiany parametrów behawioralnych i fizjologicznych u *Daphnia magna,* a tym samym zwiększać podatność tych zwierząt na presję ze strony drapieżników.

Introduction

Neonicotinoids are widely used water-soluble synthetic insecticides targetting pest insects on a wide range of crops (ELBERT et al. 2008) including compunds such as acetamiprid, clothianidin, imidacloprid, nitenpyram, nithiazine, thiacloprid, thiamethoxam. These molecules are very efficient insecticides able to bind and interact with acetylcholine receptors located in neurons of central nervous system in target insect causing paralysis leading to death (MATSUDA et al. 2001). Although their toxicity is known be restricted only to pest insects many controversies have emerged recently since they were shown to affect also non-target animals. It was shown that beneficial insects such as honey bees (SCHNEIDER et 2012, WILLIAMSON et al. 2014) and wild bees (WHITEHORN et al. 2012) exposed to these insecticides developed various dysfunctions, in foraging activity and reproduction (CHRISTEN et al. 2016) which, in a consequence resulted in a decreased pollination, lower reproduction and finally increased colony mortality due to a lack of food (MOMMAERTS et al. 2010). Neonicotinoids were also found to induce toxic changes in earthworms (ZHANG et al. 2017) and were linked to increased bird mortality associated with a reduction of insect populations (HALLMAN et al. 2014). Although acetamiprid is considered as safe ad not cancerogenic for humans, some cases of accidental human poisoning with multiorgan dysfunctions and erectile dysfunctions in males exposed to these insecticides were also described (IMAMURA et al. 2010, KUMAR et al. 2013, KAUR et al. 2015, XING et al. 2016).

Although neonicotinoids are susceptible to photoderadation (TISLER et al. 2009) and microbial biotransformation, they are considered as environmentally persistent in soil and water environment (LA et al. 2013) and may pose a risk to aquatic invertebrates after their transfer from soil to the aquatic environment (ARMBRUST and PEELER 2002). Toxic effect of neonicotinoids were documented in Ephemeroptera, Trichoptera and snails such as *Lymnea stagnalis*, however different sensitivity exists in these animals (ROESSINK et al.

2013, MORRISSEY et al. 2015, VEHOVSZKY et al. 2015). Some authors showed that ostracods, amphipods, mayfly nymphs and migde larvae are affected to these pesticides (SANCHEZ-BAYO et al. 2016). Although zooplanktonic organisms are susceptible to neonicotinoids they present different level of sensitivity (HAYASAKA et al. 2012). Lethal and sublethal exposure to these pesticides may impair predator-prey relationship and ecological balance in the aquatic ecosystems. For example, daphnids, important filter feeders subjected to neonicotinoids may develop disturbances in feeding which in a consequence may alter the food chain relationships.

Acetamprid (Figure 1) is one of the most widely used neonicotinoid (MORISSEY et al. 2015, YANG et al. 2016). The insecticide is persistent in water and may reach concentrations up to 41 μg l^{-1} (PPDB 2017). A number of studies indicate that Cladocera seem to be one of the most resistant invertebrates to neonicotinoids, however they present a wide range of sensitivity with LC50 (Lethal Concentration) from 4100 to >1000 000 μg l^{-1} (MORRISEY et al. 2015). LC50 for thiacloprid was estimated to be 4400 μg l^{-1} in *Daphnia magna* after 24-h exposure (BEKETOV and LIESS 2008) and LC50 (48 h) for acetamiprid was estimated to be 49.8 mg l^{-1}. Current knowledge on the effects of neonicotinoids od *Daphnia magna* is limited to mortality or immobilisation but very little is known on other parameters such as swimming behaviour and physiology. Therefore, the aim of the present study was to determine the influence of persistent neonicotionid formulation mospilan (20% acetamiprid) on swimming velocity and physiological parameters: heart rate and thoracic limb activity in *Daphnia magna*.

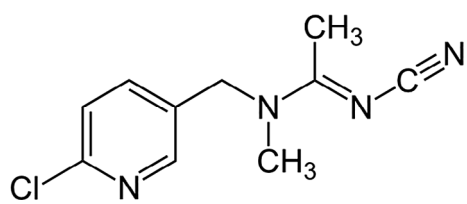

Fig. 1. Chemical structure of acetamiprid

Material and Methods

Culture method

D. magna were cultured in our laboratory in a continuous parthenogenetic reproduction for several generations in 6 L tanks with 5 L of aerated *Daphnia* culture medium under light: dark period of 16 h:8 h. Daphnia culture medium

was prepared according to American Society of Testing and Materials standards (*Standard practice for...* 1986) with a temperature of 23±1°C. The number of cultured daphnids was about 30 animals per liter. Daphnia mothers were fed once daily with a few drops of powdered Spirulina (2 mg l^{-1}) per tank and supplemented with a few drops per tank of 10 mg l^{-1} stock suspension of baker's yeast. Neonates from fifth-sixth generation ≤ 24 h old were used in the study and were not fed 24 h before and during the exposure to the neonicotinoid.

Chemicals and experimental design

Mospilan 20 SP formulation (20% acetamiprid) was used in the form of the commercially available product in Poland. The insecticide was diluted in Daphnia culture medium to reach concentrations of 25, 50 and 100 mg l^{-1}, selected of the basis of toxicological data for acetamiprid (Material Safety Data Sheet). Swimming velocity, heart rate and thoracic limb movement frequency was determined after 2, 24, 48 and 72 hours of the exposure. The control daphnids were kept in clean medium only.

Determination of survival rate

Survival rate of the animals in each experimemtal group and the control was determined after 2, 24, 48, 72 h of the exposure. Daphnids were treated as dead when no heart beat was noted during examination under a light microscope.

Determination of swimming behaviour

Swimming velocity of *D. magna* neonates were determined after 2, 24, 48 and 72 hours according to the experimental setup previously described by SHIMIZU et al. (2002) and BOWNIK et al., (2015) with some modifications. 10 daphnids were placed in the observation culture dish of 55 mm diameter containing 6 ml of Mospilan 20 SP solution. Swimming behaviour of the animals was video recorded for a minimum of 1 min (with a speed of 30 frames per second) with a digital camera Nikon D3100 mounted on a stand over the dish and the recorded video was processed with motion analysis software, Tracker®, version 4.82. Vertical movement of Daphnia was negligible because of very small depth of the solution present in the observation dish. The video

file with the recorded trajectories of swimming *D. magna* was analyzed by a frame-by-frame method with Tracker®. Briefly, by clicking with the cursor on Daphnia image in separate frames, the trail left by a single *Daphnia* (interpreted by the program as a mass point) and mean velocity (v) expressed in millimeters per second (millimeters per second) was measured. As the crustaceans moved in the observation dish virtually in two dimensions swimming behavior analysis was based on the trajectory represented by x and y coordinates. The velocities of ten daphnids calculated by the software were plotted in the separate graphs which were then superimposed. Mean velocity of the experimental group was obtained by statistical calculation of all measurements taken for each individual animal.

Heart rate and thoracic limb activity

Microscopic measurements of physiological parameters: heart rate and thoracic limb movement were performed at 2, 24, 48 and 72 hour of the experiment. A single daphnid was transferred in a 50 μl droplet of appropriate experimental solution to a microscope slide. After the individual daphnid was transported for the analysis, the excess of the medium was aspirated. The microscopic view of the examined daphnid was recorded for more than 1 min (with a speed of 30 frames per second) with a digital camera Nikon D3100 mounted on a light microscope. The magnification (30–100×) and camera resolution allowed to perform the analysis with a good visibility of the heart and thoracic limbs. Heart rate and thoracic limb movement were analyzed with multimedia player with a frame-by-frame method and separate heart contractions were counted for 1 min. Thoracic limb movement was also determined by a frame-by-frame video analysis with the multimedia player and the separate movements (beats) of the limbs were counted for 1 min.

Statistical analysis

The results are shown as means ± standard deviation (SD). Statistical analyses were performed using Develve software. All data were assessed for homogeneity of variance for ANOVA assumptions. Data were analysed using ANOVA followed by Tukey's test to find differences between means. The level of significance was set to be at least $p < 0.05$.

Results

Survival rate

The results showed (Figure 2) that survival rate of daphnids exposed to the insecticide at 100 mg l^{-1} was reduced to 80% after 48 hours, however no alive animals were noted after 72 hours. Less reduced survival rates (to 50% and 40%) at 72 hour were observed at 25 and 50 mg l^{-1} of the neonicotinoid, respectively.

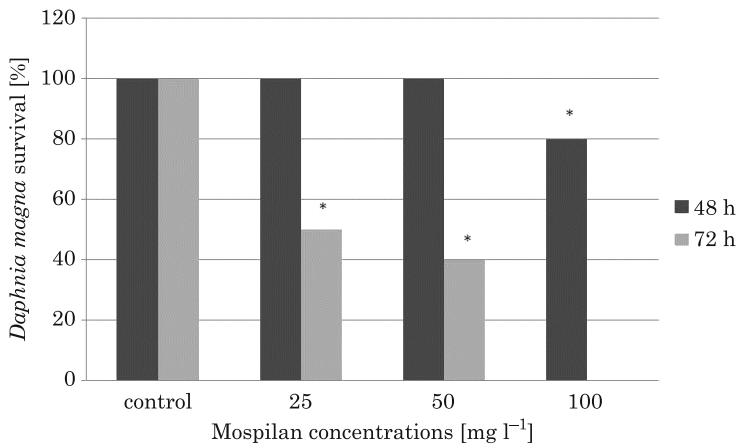

Fig. 2. Survival of *Daphnia magna* subjected to mospilan 20 SP (20% acetamiprid): $n = 10$; * – statistical significance between the experimental and control groups $p < 0.05$

Swimming velocity

The most significant reduction of swimming velocity was noted at 100 mg l^{-1}. The Figure 3 and graph images of velocity (Figure 4) obtained from Tracker® software demonstrate that this parameter was significantly inibited after 2-hour exposure (1.06 ± 0.6 cm s^{-1}) when compared to the control (4.98 ± 0.5 cm s^{-1}). More depressed swimming speed was observed after 24 hours (0.68 ± 0.7 cm s^{-1}). A significant reduction of the parameter at 2 hour was also observed at 50 mg l^{-1} (1.73 ± 0.4 cm s^{-1}), however motile daphnids were noted after 48 hours (0.93 ± 0.6 cm s^{-1}). The neonicotinoid at 25 mg l^{-1} induced only slight depression of swimming velocity (3.82 ± 0.5 cm s^{-1} and 2.33 ± 0.6 cm s^{-1} after 2 and 72 hours, respectively).

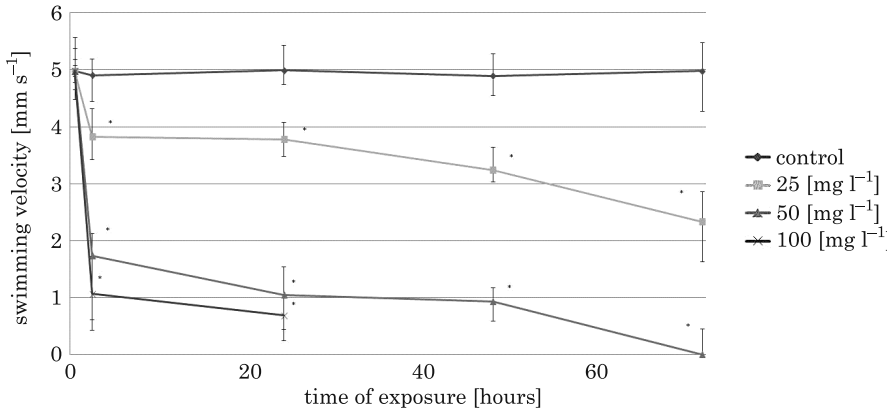

Fig. 3. Swimming velocity of *Daphnia magna* exposed to Mospilan 20 SP. The results are presented as means ± SD; $n = 10$; * – statistical significance between the experimental and control groups $p < 0.05$

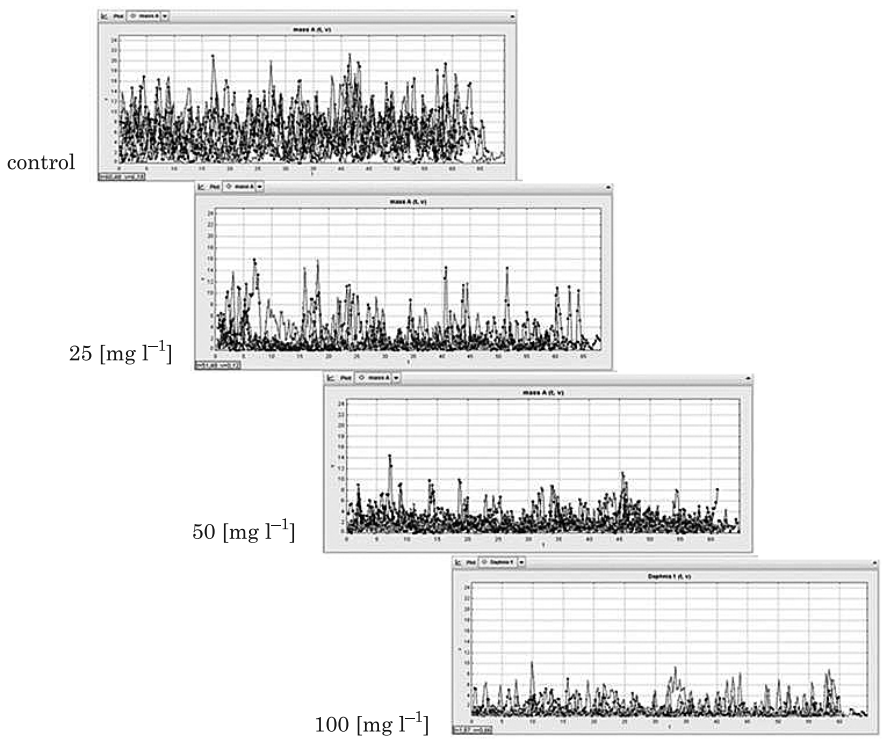

Fig. 4. Exemplary amplitudes of swimming velocity of 10 *Daphnia magna* exposed for 2 hours to different concentrations of neonicotinoid insecticide Mospilan 20 SP. Data from 10 individuals were obtained from the frame-by-frame- analysis in Tracker® software. The graphs present the superimposed amplitudes swimming velocities of 10 animals

Heart rate

The most depressed heart rate was noted after 48 hours in daphnids treated with 100 mg l^{-1} (132 ± 40 bpm) – Figure 5. The animals exposed to the neonicotinoid at 25 mg l^{-1} showed stimulated heart rate in comparison to the control (445 ± 25 bpm). The highest values were noted after 24 (538 ± 20 bpm) and 48 hours (502 ± 32 bpm), however it decreased to 380 ± 55 bpm after 72 hours. Very similar stimulatory effects were also noted at 50 mg l^{-1}, however at 72 hour the reduction of heart rate was more significant (300 ± 45 bpm). All the experimental animals showed no heart arrhythmia (data not shown).

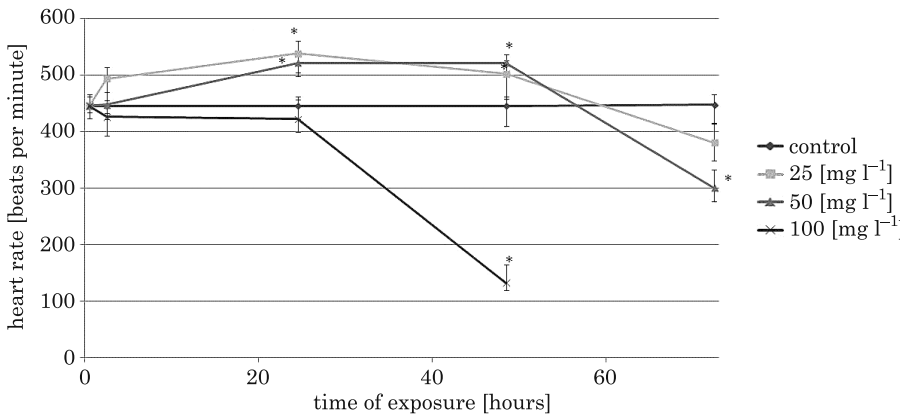

Fig. 5. Heart rate of *Daphnia magna* exposed to Mospilan 20 SP. The results are presented as means ± SD; $n = 10$; * – statistical significance between the experimental and control groups $p < 0.05$

Thoracic limb activity

Daphnia exposed to all tested concentrations of the neonicotinoid showed a time- and concentration-dependent decrease of thoracic limb beat frequency (Figure 6.). The highest inhibition of this activity was observed at 100 mg l^{-1} after 48 hours (12 ± 6 bpm) of treatment, however a significant reduction also appeared after 2 hours (204 ± 46 bpm) when compared to the control (357 ± 25 bpm). Lower concentrations of the insecticide also induced supressory effect and after 48 hours of the exposure the limb activity was 224 ± 55 and 280 ± 30 bpm at 50 and 25 mg l^{-1}, respectively. The parameter was more inhibited after 72 hours and was 180 ± 33 and 220 ± 45 bpm at 50 and 25 mg l^{-1}, respectively.

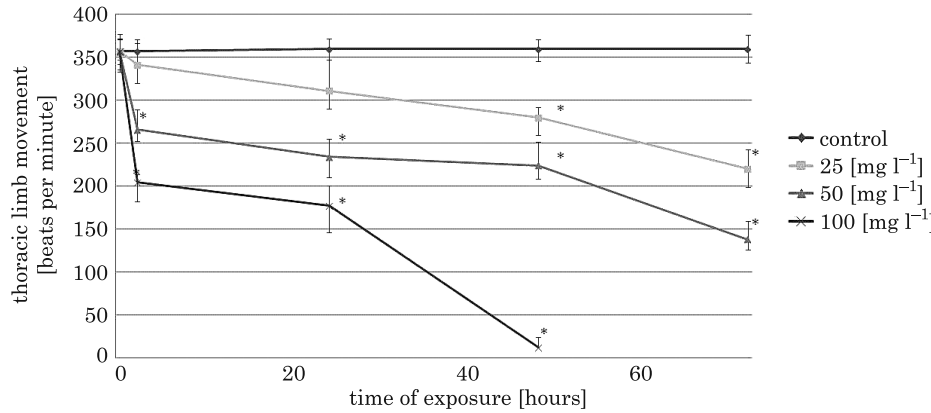

Fig. 6. Thoracic limb activity of *Daphnia magna* exposed to Mospilan 20 SP. The results are presented as means ± SD; $n = 10$; * – statistical significance between the experimental and control groups $p < 0.05$

Discussion

Survival rate or immobilisation of *Daphnia magna* are common endpoints used for determination of toxicity induced by various compounds. They are easy for to detection without special equipment or advanced training with a naked eye. However these parameters may not be suitable as biomarkers in assessment of sublethal effects in sensitive systems of exposed animals such as nervous system. Currently, more sensitive parameters as swimming activity and physiological activity of *Daphnia* such as heart rate or thoracic limb activity are becoming more frequently used early biomarkers required for more detailed information on toxic effects in aquatic invertebrates (BOWNIK et al. 2015, BOWNIK and STĘPNIEWSKA 2015). This is the first study showing that neonicotinoids may affect nervous system of *Daphnia magna* resulting in changes in swimming behaviour and physiological activity.

Survival rate

Our study confirmed that acetamiprid is moderately toxic compound to *Daphnia magna*. The insecticide induced time- and concentration dependent reduction of survival rate which is in agreement with the LC50 value estimated for acetamiprid (49.8 mg L^{-1} after 48 h). These cladocerans are more resistant to this insecticide when compared to LC50 values for other aquatic invertebrates such as *Ephemeroptera* (3.9 μg l^{-1}), *Trichoptera* (6.9 μg l^{-1}), *Chironomus dilutus* (9.3 μg l^{-1}) (LEBLANC et al. 20120, ROESSINK et al. 2013). Although

environmental concentrations of acetamiprid should not be expected to reach LC50 values, its high persistence, and time-cumulative toxicity to invertebrates (RONDEAU et al. 2014) may pose a risk of intoxication to sensitive cladoceran species.

Swimming behaviour

Swimming behaviour is a complex endpoint used for assessment of toxicity in aquatic invertebrates and vertebrates. It can be easily monitored with the use of frame-by-frame video analysis and its impairments may be clearly detected (SHIMIZU et al. 2002, ZEIN et al. 2014, BOWNIK et al. 2015). Our study indicated that although *Daphnia* could survive at high levels of acetamiprid these animals responded after 2 hours with inhibition of swimming velocity. A quick response indicates that *Daphnia* neuromuscular system is sensitive to neonicotinoids. The inhibition of motility is a consequence of interaction of acetamiprid with nicotine receptors leading to inhibition of neuronal transmission and depression of swimming velocity. On the contrary, acetamprid-induced stimulation of locomotor activity in honey bees (EL HASSANI et al. 2008). This may suggest that nicotinic receptors in *Daphnia* and honey-bees have different structure or different mechanisms of neuronal signal transduction. Our study also indicated that, signalling through nicotinic receptors seems to be an important pathway in neuromotor activity in *Daphnia magna*.

Heart rate

Microscopic observation of *Daphnia* heart activity is a simple method used for determination of effects induced by various chemicals (VILLEGAS-NAVARRO et al. 2003, BOWNIK et al. 2015). Our study showed that acetamprid induced concentration- and time- dependent chronotropic effect. Interestingly, stimulation of heart rate was noted at 25 mg l^{-1} and 50 mg l^{-1} of the neonicotinoids, on the other hand, negative chronotropic effect was noted at its highest concentration (100 mg l^{-1}). Although no results on the effects of neonicotinoids on cladoceran heart activity can be available, our findings are similar to those obtained by BAYLOR (1942), who found stimulatory of higher concentrations or inhibitory effects at lower levels of nicotine on heart rate of *Daphnia magna*. Various responses in different species of aquatic invertebrates may also be induced towards another ligand of the nicotinic receptor, acetylcholine. It was found that although the molecule inhibited heart rate in *Daphnia* (BAYLOR

1942), its positive chronotropic effect was found in decapods (DAVENPORT 1941). These opposite reactions of various invertebrate species indicate that different physiological mechanisms are involved in heart responses to neuroleptic molecules.

Thoracic limb activity

We found that acetamiprid inhibited activity of thoracic legs in a concentration- and time-dependent manner which probably suggests inhibitory action of the neonicotinoid on daphnid cholinergic system. Thoracic limbs play an important role in cladoceran feeding and ventilation (SEIDL et al. 2002) and since their activity is modulated by various chemicals they may serve as a sensitive physiological endpoint in ecotoxicology. However, little is known on the influence of neurotoxicants. The nicotinoid-induced inhibition of thoracic limbs may in a consequence cause feeding problems and ventilation disturbances leading to anoxia.

Concluding remarks

The present study revealed that acetamiprid is a potent neuromodulator altering behavioural and physiological parameters of *Daphnia magna*. High persistence in water and the ability to induce cumulative toxicity suggests that this neonicotinoid may interact with nicotinic receptors and thereby affect nervous system of sensitive species of cladocerans which may result in behavioural and physiological changes. Our results also indicate that parameters such as swimming velocity, heart rate and thoracic limb activity may be treated as sensitive, early biomarkers of neurotoxicity in cladocerans.

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γ-LACTONES WITH POTENTIAL BIOLOGICAL ACTIVITY

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K e y w o r d s: natural products, lactones, biological activity.

A b s t r a c t

γ-Lactones are well known organic compounds. They can be isolated from many different plants, e.g. from Asteraceae (*Compositae*) family. They are used as active ingredients of various medicinal herbs and exhibit a variety of biological activities, i.e. antiviral, antibacterial, anticancer, antimalarial, anti-inflammatory and others. Among described compounds vernolide **(7)** is characterized by high antimicrobial activity; epoxy compounds like glaucolide (**13**) and hirsutinolide (**24**, **25**, **26**) possess high anticancer activity. Other noteworthy γ-lactone is vernopicrin (**14**) that shows antimalarial activity. Some of the γ-lactones possess interesting aromatic and flavon properties, which make them desirable ingredients in perfumery and food industry. Further studies to determine relationship between properties and complex composition are caused by diversity of structures and potential activities of γ-lactones. It can be used to design new analogues with interesting pharmacological, flavon and olfactory properties. This paper reviews selected studies concerning γ-lactones and their various biological activities.

γ-LAKTONY O POTENCJALNEJ AKTYWNOŚCI BIOLOGICZNEJ

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S ł o w a k l u c z o w e: produkty naturalne, laktony, aktywność biologiczna.

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Abstrakt

γ-Laktony to grupa związków organicznych. Mogą być wyizolowane z wielu roślin, np. z rodziny Asteraceae (*Compositae*). Są używane jako związki aktywne różnych ziół leczniczych i wykazują aktywność biologiczną, tj. przeciwwirusową, przeciwbakteryjną, przeciwnowotworową, przeciwmalaryczną, przeciwzapalną i inną. Wśród opisanych związków wernolid **(7)** charakteryzuje się wysoką aktywnością przeciwbakteryjną, a związki epoksydowe, takie jak glaukolid (**13**) i hirsutinolid (**24**, **25**, **26**), mają wysoką aktywność przeciwnowotworową. Innym godnym uwagi γ-laktonem jest wernopryna (**14**), która wykazuje aktywność przeciwmalaryczną. Niektóre z γ-laktonów mają interesujące właściwości zapachowe, które czynią z nich pożądane składniki w przemyśle perfumeryjnym i spożywczym. Badania określające zależność między właściwościami oraz złożoną budową związków są wynikiem aktywności biologicznej γ-laktonów. Związki te mogą mieć zastosowanie w projektowaniu nowych analogów o interesujących właściwościach farmakologicznych i zapachowych. W pracy omówiono wybrane badania dotyczące γ-laktonów i ich aktywności biologicznej.

Introduction

Lactones are widely distributed in nature. Their number is estimated to be up to more than 3000. They exhibit interesting and useful biological activities. There is a variety of anticancer, antibacterial and antifungal properties of lactones described in the literature. Much research, which appears understandable, refers antitumor and cytotoxic activity lactones. They are run from the end of the 60s of last century, when the KUPCHAN et al. (1969) started real "hunt" for lactones in plants (KUPCHAN et al. 1969, HANSON et al. 1970). In the literature one can find such reports, which estimate sesquiterpene lactones, as one of the largest classes of natural compounds (FRANCK 1979).

The estimation of the enantiomeric distribution of chiral compounds found in fruits and essential oils has become increasingly important in determining the origin of natural and processed food. The method depends in part on knowledge of the enantiomeric distributions found in nature and also on the changes that may occur with the extraction and processing methods used (SUGINOME et al. 1995) Chiral lactones are known as important flavour compounds and are distributed in nature. A group of compounds that may occur naturally in the racemic form are δ-lactones. Their enantiomeric distribution in many fruits has been extensively studied and summarized (DIAB et al. 2007). It varies among different fruit species and depends on their origin.

Lactones are one of the well-known classes of natural products. They originate from many natural sources. Their structure varies widely as they contain ring of 4 up to 60 members. During past years many researches have been carried out to determine the synthesis and structure of lactones. Many of them are on different steps of medicinal trials. Lactones are also fragrant compounds and because of that they are used in fine and functional perfumery.

γ-Lactones found use in food industry. One of them that is commonly used is γ-nonalactone (**1**) – Figure 1. It has characteristic creamy, sweet, coconut scent (Brenna et al. 2003).

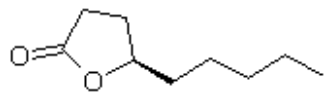

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Fig. 1. Structures of γ-nonalactone

Vast number of natural and synthetic compounds poses a lactone moiety. They show very diversified biological properties. These are the reasons for an extensive interest in this class of compounds. One of the most important branches of industry that uses lactones is pharmaceutics.

Material and Methods

Antimicrobial activity

Cheilocostus speciosus is cultivated mostly in Asia for medicinal use or as an ornamental. It is also popular in Ayurveda because of its pharmacological properties. DURAIPANDIYAN et al. (2012) investigated crude hexane, chloroform and methanol extracts from rhizomes of *C. speciosus* for antimicrobial activities. Several species of bacteria (*Staphylococcus aureus, Staphylococcus epidermidis, Bacillus subtilis*) and fungi (*Aspergillus niger, Candida albicans*) were tested using Minimum Inhibitory Concentration (MIC) method. In microbiology, the minimum inhibitory concentration (MIC) is the lowest concentration of a chemical that prevents visible growth of a bacterium (in other words, at which it has bacteriostatic activity), whereas the minimum bactericidal concentration (MBC) is the concentration that results in microbial death. Results are showed in Table 1 and Table 2. The study revealed that hexane extract comprises two sesquiterpenoid compounds – eremanthin (**2**) and costunolide (**3**) – Figure 2. Both of them show fungi inhibition but no antibacterial activity.

ÖZÇELIK et al. (2009) isolated three guaianolide-type sesquiterpene lactones (SLs) from Meditenranean flowering plant – *Centaurea solstitialis* – centaurepensin (**4**) (chlorohyssopifolin A), chorojanerin (**5**) and 13-acetylsolstitialin A (**6**) – Figure 3. These three compounds were tested against *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Aspergillus niger* using microdilution method. Results are shown in Table 1.

Table 1

Comparison of antibacterial activities of sesquiterpenoid compounds

Compound	Compound MIC [μg ml^{-1}]				
	Gramm-positive			Gramm-negative	
	Staphylococcus aureus	*Staphylococcus epidermidis*	*Bacillus subtilis*	*Escherichia coli*	*Pseudomonas aeruginosa*
Hexane extract from *Cheilocostus speciosus*	1250	625	1250	nd.	nd.
Chloroform extract from *Cheilocostus speciosus*	625	312	> 5000	nd.	nd.
Methanol extract from *Cheilocostus speciosus*	625	625	1250	na.	na.
Centaurepensin (4)	16	nd.	nd.	128	128
Chlorojanerin (5)	16	nd.	nd.	128	128
13-acetyl solstitialin A (6)	16	nd.	nd.	128	128
Vernolide (7)	500	500	nd.	na.	na.
Vermeerin (8)	10	nd.	nd.	na.	na.
11β,13-dihydro cnicin (9)	nd.	0.4	0.2	0.2	nd.
11β.13-dihydro-19-desoxycnicin (10)	nd.	0.4	0.2	0.2	nd.
8α-*O*-(4-acetoxy-5-hydro xyangeloyl)-11β,13-dihydro cnicin (11)	nd.	0.4	0.2	0.3	nd.
Eudesmanolides from *Centaurea pullata*	nd.	0.4	0.2	0.4	nd.

Explanations: nd. – no data; na. – no activity
Source: own study based on Duraipandiyan et al. 2012, Özçelik et al. 2009, Erasto et al. 2006, Fortuna et al. 2011, Djeddi et al. 2007, Toyang et al. 2013

Table 2

Comparison of antifungal activities of sesquiterpenoid compounds

Compound	Compound MIC [μg ml^{-1}]	
	Aspergillus niger	*Candida albicans*
Hexane extract from *Cheilocostus speciosus*	500	> 1000
Chloroform extract from *Cheilocostus speciosus*	> 1000	> 1000
Methanol extract from *Cheilocostus speciosus*	> 1000	> 1000
Eremanthin (2)	125	> 250
Costunolide (3)	250	> 250
Centaurepensin (4)	nd.	64
Chlorojanerin (5)	nd.	64
13-acetylsolstitialin A (6)	nd.	64
Vermeerin (8)	na.	na.
11*β*,13-dihydrocnicin (9)	0.2	nd.
11*β*,13-dihydro-19-desoxycnicin (10)	0.2	nd.
8*α*-*O*-(4-acetoxy-5-hydroxyangeloyl)-11*β*,13-dihydrocnicin (11)	0.2	nd.
Eudesmanolides from *Centaurea pullata*	0.2	nd.

Source: own study based on DURAIPANDIYAN et al. 2012, TOYANG et al. 2013

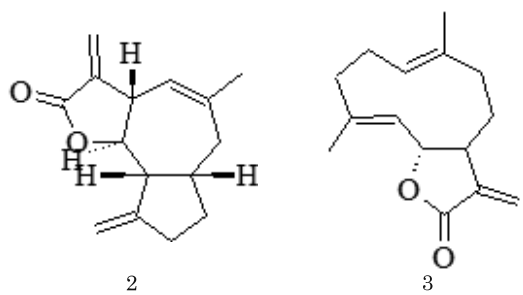

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Fig. 2. Structures of eremanthin (**2**) and costunolide (**3**)

Vernonia amygdalina is a tree growing in South Africa. Leaves of *V. amygdalina* are commonly used to treat dysentery, hepatitis, gastrointestinal problems and venereal diseases. Vernolide (**7**), a sesquiterpene lactone, was isolated from leaves by ERASTO et al. (2006) – Figure 4. Antimicrobial activity of vernolide (**7**) against two *Staphylococcus* strains was assayed by agar dilution method.

Vermeerin (**8**) is a dilactone sesquiterpene compound obtained from aerial parts of Bolivian plant – *Hymenoxys robusta*, a species of Asteraceae family (FORTUNA et al. 2011) – Figure 5. Only vermeerin (**8**), among other lactones isolated from *H. robusta*, is active against *Staphylococcus aureus*. The results also suggested that described dilactone could be used in development of medicines against *S. aureus* infections as it is not toxic to THP-1; human monocytic leukemia cell line.

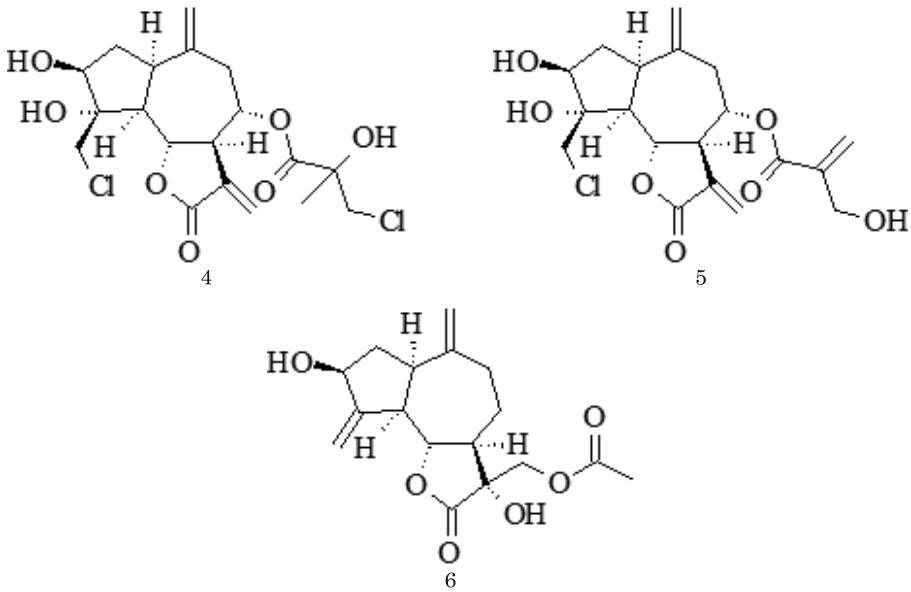

Fig. 3. Structures of centaurepensin (**4**), chlorojanerin (**5**) and 13-acetylsolstitialin A (**6**)

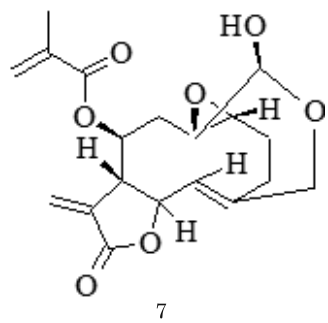

Fig. 4. Structure of vernolide (**7**)

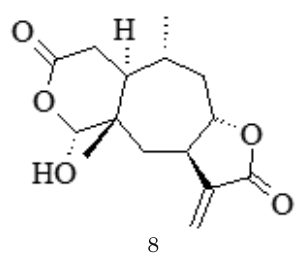

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Fig. 5. Structure of vermeerin (**8**)

Centaurea pullata is biennial plant growing in Western Europe and North Africa, commonly used in Algeria as a seasoning. DJEDDI et al. (2012) isolated five SLs from aerial parts of *C. pullata* – 11*β*,13-dihydrocnicin (**9**), 11*β*,13-dihydro-19-desoxycnicin (**10**), 8*α*-*O*-(4-acetoxy-5-hydroxyangeloyl)-11*β*,13-dihydrocnicin (**11**), 8*αO*-(4-hydroxy-2-methylenebutanoyloxy)-11*β*,13-dihydrosonchucar-polide (**12**), and 8*α*-*O*-(4-hydroxy-2-methylenebutanoyloxy)-11*β*,13-dihydro-4-*epi*-sonchucarpolide (**13**). Compounds **9** and **10** are major sesquiterpene lactones present in described plant. Compound **11** is a germacranolide, while compounds **12** and **13** are eudesmanolides (Figure 6).

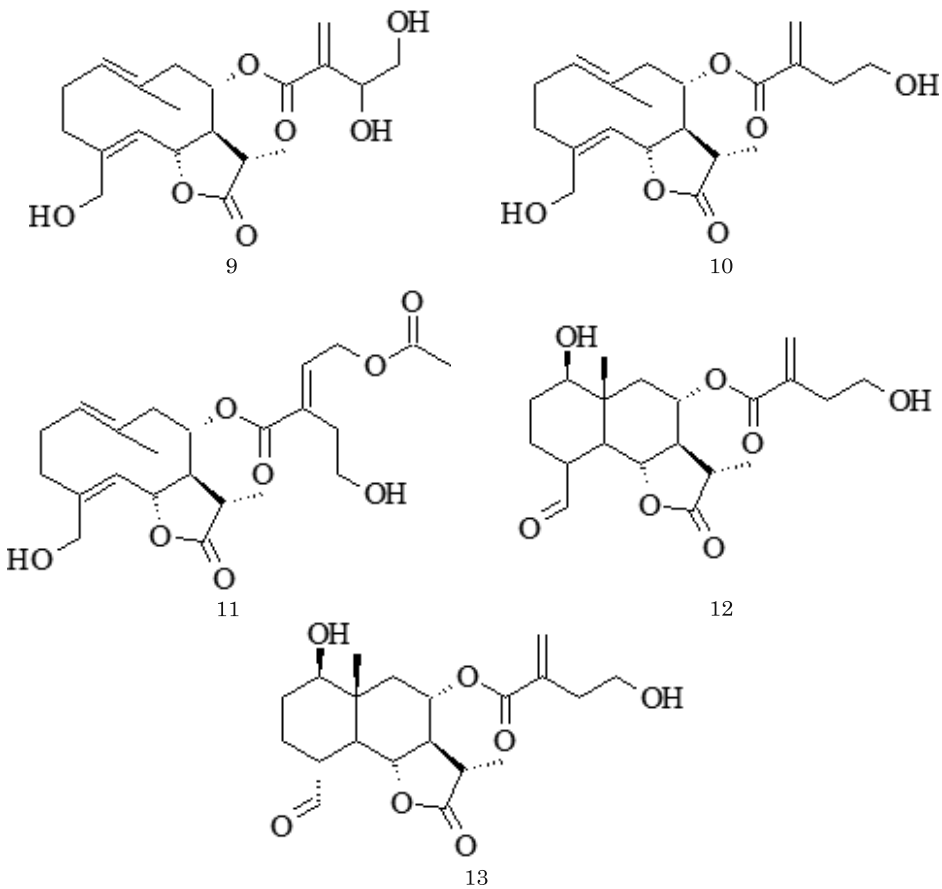


Fig. 6. Structures of compounds obtained from *Centaurea pullata*

As presented in Table 1 and Table 2, all tested compounds showed different levels of antibacterial activity. Vermeerin (**8**) was the most active constituent against *S. aureus*, however there was no influence on Gram-negative bacteria.

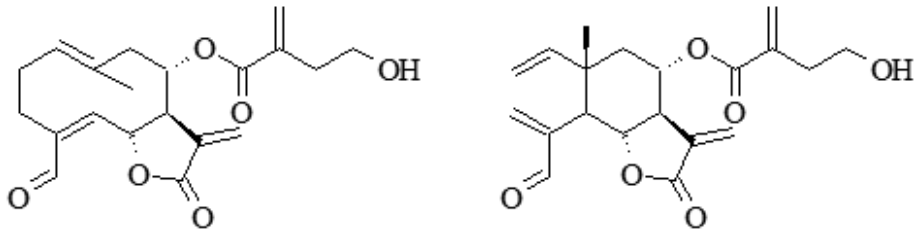
Activity against *S. aureus* of compounds from *C. solstitialis* was similar to vermeerin (**8**), with minimum inhibitory concentration at 16 μg ml^{-1}. Vernolide (**7**) showed medium level of inhibition – 500 μg ml^{-1}. Extracts from *C. speciosus* revealed lowermost level of *S. aureus* inhibition. Considering inhibitory effect on *S. epidermidis* and *B. subtillis,* the strongest inhibitors are constituents of *C. pullata* extract. Hexane, chloroform and methanol extracts from *C. speciosus* are needed in the highest amount to suppress Gram-positive bacteria. Against Gram-positive bacteria centaurepensin (**4**), chorojanerin (**5**) and 13-acetylsolstitialin A (**6**) showed the same level of activity. The strongest inhibitory effect had 11β,13-dihydrocnicin (**9**) and 11β,13-dihydro-19-desoxycnicin (**10**), while vermeerin (**8**), vernolide (**7**) and methanol extract from *C. speciosus* were not active at all. In general compounds obtained from *C. pullata* show the highest activity against both Gram-positive and Gram--negative bacteria.

That compounds obtained from *C. pullata* significantly inhibited the growth of *A. niger*. Isolated eremanthin (**2**) and costunolide (**3**) showed better inhibition than crude extracts from *C. speciosus*. The best against *C. albicans* are compounds from *C. speciosus*. As seen in Table 2, centaurepensin (**4**), chorojanerin (**5**) and 13-acetylsolstitialin A (**6**) are sixteen times stronger grow inhibitors than eremanthin (**2**), costunolide (**3**) and crude extracts from *C. speciosus*, respectively. Vermeerin (**8**) showed no antifungal activity.

Activity against bacteria and fungi is associated with presence of α-methylene-γ-lactone or α-methyl-γ-lactone groups in sesquiterpene lactones. As well polarity of solvents has an impact on passing through the microbial cell walls (DURAIPANDIYAN et al. 2012, TOYANG et al. 2013).

Antimalarial activity

Vernonia guineensis Benth from Asteraceae family is a plant growing in Africa. Preparations from leaves and tubers are commonly used in folk medicine as painkillers, laxatives, antidotes and to treat dysentery, diarrhea and venereal diseases. There are two sesquiterpene lactones present in leaf extract – vernopicrin (**14**) and vernomelitensin (**15**) – Figure 7. Toyang (TOYANG et al. 2013) examined these two compounds for antimalarial activity. Vernopicrin and vernomelitensin (**15**) were tested against two *Plasmodium falciparum* lines: chloroquine (CQ)-sensitive (Hb3) and CQ-resistant (Dd2). SYBR Green I (SG is an asymmetrical cyanine dye used as a nucleic acid stain in molecular biology)-based DNA detection method (GRAZIOSE et al. 2011) was used to measure the 50% inhibitory concentrations (IC_{50}). Results are shown in Table 3.



14 15

Fig. 7. Structures of vernopicrin (**14**) and vernomelitensin (**15**)

Table 3

Comparison of antimalarial activities of sesquiterpenoid compounds

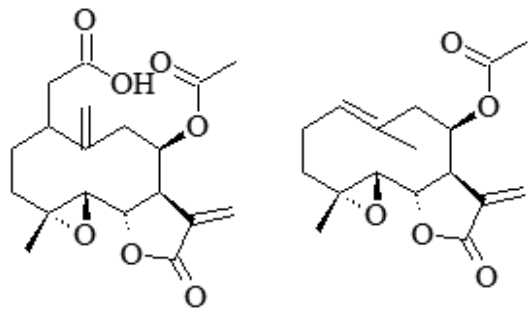
Compound	Antiplasmodial activity IC_{50} [μg ml^{-1}]	
	Plasmodium falciparum line	
	CQ-resistant	CQ-sensitive
Vernopicrin (14)	0.807[a]	0.614[c]
Vernomelitensin (15)	0.569[a]	0.472[c]
Peroxyferolide (16)	4.3[a]	6.2[d]
Lipiferolide (17)	2.3[a]	1.8[d]
Dichloromethane extract from *Oncosiphon piluliferum*	3.1[b]	2.6[d]
Vernolide (7)	nd.	1.86[d]
Vernodalin (18)	nd.	0.52[d]
Tatridin A (19)	nd.	4.4[d]
Tanachin (20)	nd.	2.6[d]
Desacetyl-β-cyclopyrethrosin (21)	nd.	0.4[d]
Sivasinolide	nd.	0.5[d]
Chloroquine	0.07[a]	0.009[c] / 0.01[d]

Explanations: [a] – Dd2 strain, [b] – K1 strain, [c] – Hb3 strain, [d] – D10 strain
Source: own study based on TOYANG et al. 2013, CHUKWUJEKWU et al. 2009, PILLAY et al. 2007, BUSKUH et al. 2010).

Liriodendron tulipifera L., customarily called tulip tree, is a plant from Magnoliaceae family, native to North America. In ethnomedicine the bark from the tulip tree has been used as an antipyretic or stimulant agent. The bark was also used as a replacement to quinine. Accordingly to this fact, antimalarial activity of *L. tulipifera* extract was examined by GRAZIOSE et al. (2011). The researchers isolated two SLs – peroxyferolide (**16**) and lipiferolide (**17**) from the leaves of tulip tree (Figure 8). Two *P. falciparum* lines: chloroquine-sensitive (D10) and the Dd2 were treated with these compounds. Results are shown in Table 3.

Vernonia colorata is another species from *Vernonia* genus containing biologically active lactones. In Africa, leaves and roots of *V. colorata* are used in medicine to treat skin eruptions, anemia, paralysis, epilepsy, fever, gastrointestinal and heart diseases. Vernolide (**7**), described earlier, and vernodalin (**19**)

are sesquiterpene compounds isolated from acetone leaf extract from *V. colorata* by CHUKWUJEKWU et al. (2009) – Figure 9. After purification, the antimalarial activity was assayed using the D10 *P. falciparum* strain (Table 3).



16 17

Fig. 8. Structures of peroxyferolide (**16**) and lipiferolide (**17**)

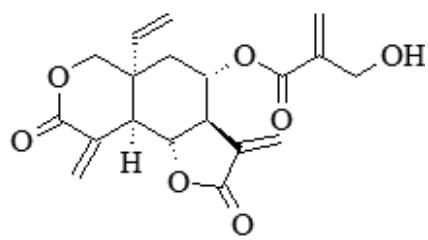


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Fig. 9. Structure of vernodalin (**18**)

Oncosiphon piluliferum, commonly known as globe chamomile, come from North America, but the infusions and decoctions are used worldwide. Medicinal applications include treating fever, typhoid and measles. PILLAY et al. (2010) studied the dichloromethane extract from the *O. piluliferum* aerial parts. The bioassay-guided fractionation process revealed presence of the germacranolide and eudesmanolide type lactones in the extract. The germacranolides are tatridin A (**19**) and tanachin (**20**), whereas the eudesmanolides are desacetyl-β-cyclopyrethrosin (**21**) and sivasinolide (**22**) – Figure 10. Crude dichloromethane extract and isolated compounds were tested against the D10 and CQ-resistant K1 *P. falciparum* strains (Table 3).

The tested compounds showed different levels of antiplasmodial activity, as presented in Table 3. The most active compound against CQ-resistant strain was vernomelitensin (**15**), whereas desacetyl-β-cyclopyrethrosin (**21**) was the strongest against CQ-sensitive strain. Vernomelitensin (**15**) and sivasinolide (**22**)

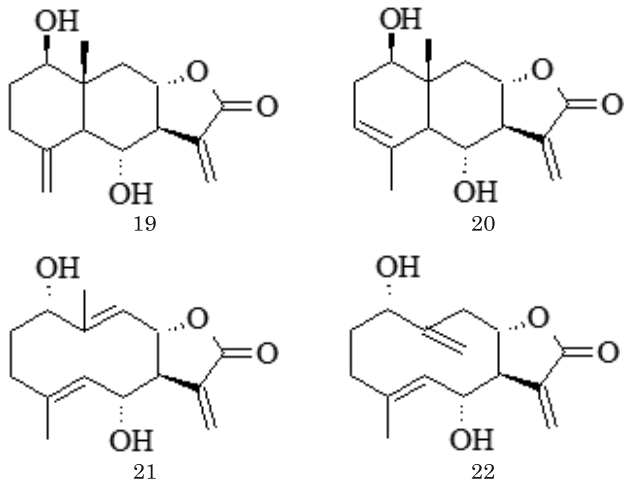


Fig. 10. Structures of tatridin A (**19**), tanachin (**20**), desacetyl-β-cyclopyrethrosin (**21**) and sivasinolide (**22**)

showed nearly the same activity against CQ-sensitive strain as desacetyl-β-cyclopyrethrosin (**21**). Three compounds from *Vernonia* genus had similar level of antimalarial activity. Crude extract from *O. piluliferum,* tatridin A (**19**), tanachin (**20**), lipiferolide (**17**) and vernolide (**7**) revealed medium activity level. Peroxyferolide (**16**) was the weakest compound against both strains. Unfortunately, none of the compounds was as active as chloroquine. Further studies of these compounds are required to assess the safety and efficiency of application *in vivo*, and determine possible use as alternative to traditional antimalarial substances.

Cytotoxic activity

Alcohol extracts from Brazilian plant from Asteraceae family, *Vernonia scorpioides*, are commonly used to treat skin disorders, such as ulcers and chronic wounds. BUSKUHL et al. (2010) isolated four sesquiterpene lactones, one glaucolide (**23**) and three hirsutinolides (**24**, **25**, **26**), from fresh flowers and leaves of *V. scorpioides* (Figure 11). The cytotoxicity of these compounds was tested against cervical cancer cell line – HeLa (a cell type in an immortal cell line) using MTT assay (is a colorimetric assay for assessing cell metabolic activity) to determine IC50 – Table 4 – (ASHOUR et al. 2012).

Bupleurum marginatum, member of Apiaceae family, is a flowering plant growing in the Northern Hemisphere. *B. marginatum* and other *Bupleurum*

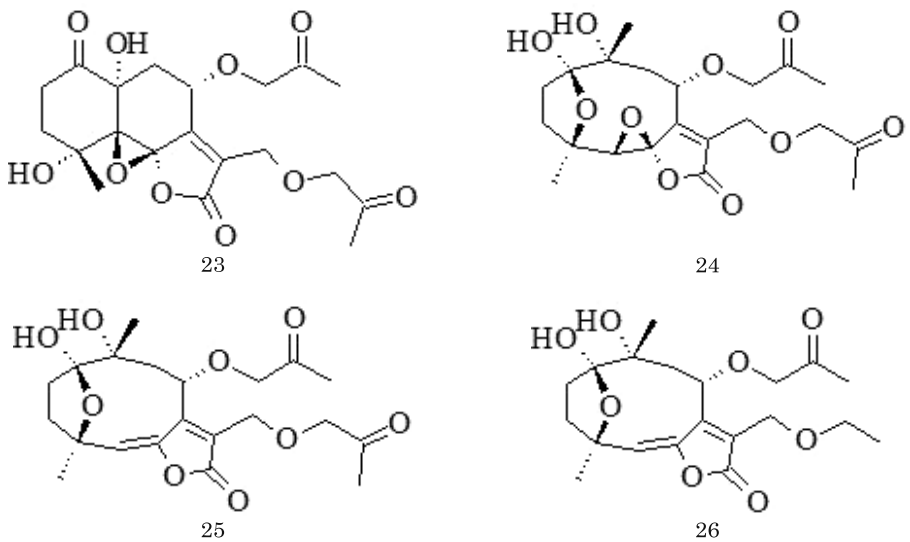


Fig. 11. Structures of *V. scorpioides* lactones

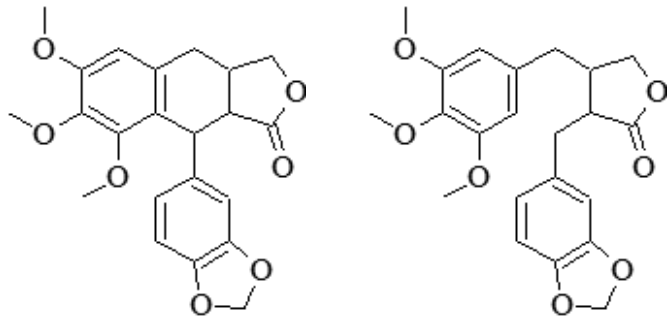
Table 4

Comparison of cytotoxic activity of sesquiterpenoid compounds against HeLa cells

Compound	HeLa IC_{50} [μM]
Glaucolide (23)	2.1 ± 1.8
Hirsutinolide (24)	3.3 ± 4.0
Hirsutinolide (25)	>100
Hirsutinolide (26)	58.5 ± 15
Marginatoxin (27)	16.90 ± 1.23
(3,4-Dimethoxybenzyl)-2-(3, 4-methylenedioxybenzyl) butyrolactone (28)	41.30 ± 6.58

Explanations: IC_{50} was determined after 24 hour incubation
Source: own study based on HUSSAIN et al. 1993, TOYANG et al. 2013

species have been used in traditional Asian medicine, as a drug for malaria, common cold, gastrointestinal and menstrual disorders. Based on this fact, ASHOUR et al. (2012) investigated influence of extract from aerial parts of *B. marginatum* on two cancer cell lines HepG2 (is a human liver cancer cell linea) nd HeLa. The research showed presence of two bioactive lactones-9--(ben-zo[d][1,3]dioxol-5-yl)-6,7,8-trimethoxy-3a,4,9,9a-tetrahydronaphtho[2,3--c]furan-1(3H)-one (marginatoxin) (**27**) and (3,4-Dimethoxybenzyl)-2-(3,4-0-methylene-dioxybenzyl)butyrolactone (**28**) – Figure 12. Using MTT assay, IC50 was determined (Table 4). These lignans are closely related to podophyllotoxin – compound, which prevents microtubule formation and blocks cell division in the late G2 phase of cell growth. This similarity might suggest the lignans course of action.



27 28

Fig. 12. Structures of marginatoxin (**27**) and (3,4-Dimethoxybenzyl)-2-(3,4-methylene- dioxybenzyl)-butyrolactone (**28**)

As shown in the Table 4, the most cytotoxic compounds are glaucolide (**23**), hirsutinolide (**24**) and marginatoxin with IC_{50} 2.1 μM, 3.3 μM and 16.90 μM respectively. Hirustinolide (**26**) and (3,4-Dimethoxybenzyl)-2-(3,4-methylenedioxybenzyl)butyrolactone also showed good level of anticancer activity. Hirustinolide (**25**) was the weakest against HeLa cells. Glaucolide (**23**) and hirsutinolide (**24**) have epoxy groups, which could be related to stronger cytotoxic effect.

Vernonia guineensis Benth, described earlier, also shows antitumor activity. TOYANG et al. (2013) have tested acetone extract from *V. guineensis* leaves against several human cancer cell lines, including human lung cancer A549 cells. The NMR analysis showed presence of two lactone compounds – vernopicrin (**14**) and vernomelitensin (**15**). The anti-proliferation activity was determined using WST-1 (*WSTs* – Water-soluble Tetrazolium salts – are a series of other water-soluble dyes for MTT Assays, developed to give different absorption spectra of the formed formazans) assay (Table 5).

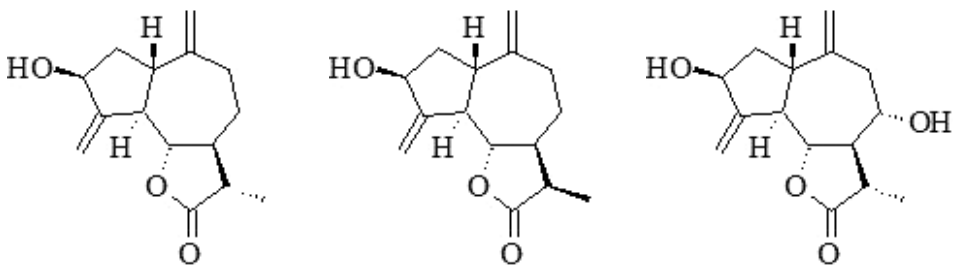
Table 5

Comparison of cytotoxic activity of sesquiterpenoid compounds against A549 cells

Compound	A549 IC_{50} [μM]
Vernopicrin (14)	2.04 ± 1.040
Vernomelitensin (15)	1.13 ± 0.380
Sausinlactone A (29)	0.01 ± 0.12
Sausinlactone B (30)	2.89 ± 0.11
Sausinlactone C (31)	na.
Millerenolide (32)	40
Thieleanin (33)	32

Source: own study based on XIAO et al. 2011, CHEN et al. 2010, TAYLOR et al. 2008

Sausinlactones A, B and C are three new compounds isolated from aerial parts of *Saussurea involucrate* by XIAO et al. (2011). *S. involucrate*, commonly known as snow lotus, is a rare Asian flowering plant. In traditional Chinese medicine it has been used to treat dysmenorrhea, rheumatoid arthritis, stomachache and cough (CHEN et al. 2010). Spectroscopic studies have revealed absolute structures of three new 3-hydroxyl-11, 13-dihydrodehydrocostuslactones (Figure 13). All compounds have different ring configuration than other guaiane-type sesquiterpene lactones – they possess *trans* junctions at C (**2**)-C (**6**). Anticancer activity of sausinlactones was tested on A549 lung cancer cell line using MTT method (Table 5).

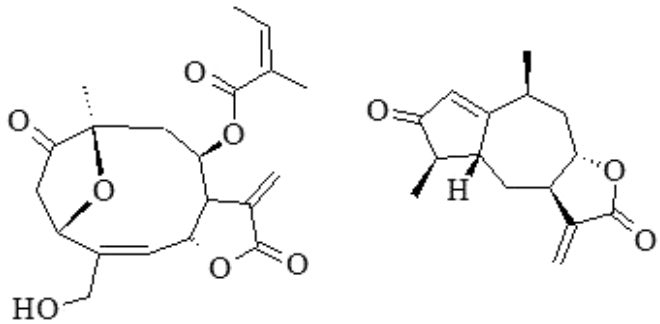


29 30 31

Fig. 13. Structures of sausinlactones A (**29**), B (**30**) and C (**31**)

TAYLOR et al. (2008) evaluated anticancer properties of two SLs extracted from *Viguiera sylvatica* and *Decachaeta thieleana* – millerenolide (**32**) and thieleanin (**33**) – Figure 14. *V. sylvatica* and *D. thieleana* are flowering plants from Asteraceae family. Both compounds were examined *in vitro* on A549 cells using Promega MTS/PMS chromogenic assay (Table 5) and *in vivo* on mice infected with melanoma cells. *In vivo* research revealed that millerenolide (**32**) caused significant, approximately 50%, depletion of tumor size, while thieleanin (**33**) did not affect melanoma at all. Further studies are important to find course of action of this compound and evaluate possible application in cancer treatment.

As shown in Table 5, all analyzed compounds showed similar level of antitumor activity. The strongest compound against A549 cells was sausinlactone A from *S. involucrate*, while the weakest was millerenolide (**32**) from *V. sylvatica,* with IC_{50} of 0.01 and 40 μM, respectively. The difference between tested compounds is not significant, hence, after further studies, application of these SLs could be possible in cancer treatment.



32 33

Fig 14. Structures of millerenolide (**32**) and thieleanin (**33**)

Conclusions

Lactones are an important group of natural products found in many herbs. Many of them contain the unique γ-lactone ring, which often comprises a α-methylene group.

Lactones demonstrate a diversified range of activities due to many fragments – alkyl, lipophilic, as well as the presence of sulfhydryl groups. These compounds have aroused widespread interest among scientists as potential drugs due to diverse biological activity e.g.: antibacterial, antimalarial, antiviral and anticancer.

Increasing the number of people diagnosed with cancer this class of compounds has become the cornerstone of continuous anticoagulation therapy due to the wide spectrum of biological properties.

Lactones also have the potential to be antileukemic drugs. Furthermore, recent studies have shown that lactones react with thiol groups, i.e. cysteine residues in proteins. The metabolism of lactones can contribute to the inhibition of tumor growth due to reduction of the enzymatic activity or the presence of abnormalities. This mechanism, initiated by lactones, leads to inhibition of a variety of cellular functions, which in turn leads to cellular apoptosis.

Nature is an inexhaustible source of biologically active substances. The chemistry of natural products is currently experiencing a renaissance due to the development of separation techniques, analytical methods and bioassays. Diversity of lactones enables studying mechanisms of action and structural-biological activity relationship. Examples presented in this article suggest that lactones play a crucial role in actions of other biomolecules (enzymes, receptors).

Translated by AUTHORS

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THE GENESIS AND CURRENT STATE OF *ACTINIDIA* COLLECTION IN M.M. GRISHKO NATIONAL BOTANICAL GARDEN IN UKRAINE

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K e y w o r d s: kiwiberry, kiwifruit, mini kiwi, selection, morphology, yield.

A b s t r a c t

The genus *Actinidia* was established by LINDLEY in 1836. The most recent systematic revision of *Actinidia* distinguish about 54 different species 52 of which occur naturally in China (44 of it are endemic). Recently *Actinidia* fruit has become famous for its nutritious, especially high content of vitamin C, and is evaluated as a healthy fruit. The results from the introduction of *Actinidia* species in M. M. Grishko National Botanical Garden of National Academy of Sciences of Ukraine (Kiev) are presented. These collections of *Actinidia* are the largest in Ukraine and includes 6 species and over 300 different cultivars and forms. This collection provides valuable material for further selective work with *Actinidia* in Forest-Steppe of Ukraine. The most promising for hybridization are *Actinidia arguta* and *A. arguta* var. *purpurea*. The result of the selection work are at least 15 cultivars established and registered in Ukraine. This paper describe the history and current status of kiwiberry selection work made in Botaical Garden in Kiev, Ukraine.

HISTORIA I STAN OBECNY KOLEKCJI AKTINIDII W NARODOWYM OGRODZIE BOTANICZNYM IM. M.M. GRISZKO NA UKRAINIE

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S ł o w a k l u c z o w e: kiwiberry, kiwifruit, mini kiwi, selekcja, morfologia, plon.

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Abstrakt

Rodzaj aktinidia utworzył LINDLEY w 1836 roku. Według najnowszej rewizji rodzaju wyróżnia się około 54 gatunki, z czego 52 naturalnie występują w Chinach (z tego 44 są endemitami). W ostatnim czasie owoce aktinidii stały się bardzo popularne z powodu swoich dużych wartości odżywczych, głównie wysokiej zawartości witaminy C i są uważane za owoce zdrowia.

W pracy przedstawiono wyniki introdukcji aktinidii do Narodowego Ogrodu Botanicznego im. M.M. Griszko w Kijowie na Ukrainie. Znajdująca się tu kolekcja aktinidii jest największą na Ukrainie i liczy 6 gatunków oraz ponad 300 odmian i form. Zbiór ten stanowi cenny materiał do dalszej pracy hodowlanej aktinidii na leśno-stepowym obszarze Ukrainy. Najbardziej obiecujące dla hybrydyzacji są *Actinidia arguta* i *A. arguta* var. *purpurea*. Efektem prac selekcyjnych jest ponad 15 odmian, które zarejestrowano na Ukrainie. W artykule przedstawiono historię i stan obecny prac hodowlanych prowadzonych w Narodowym Ogrodzie Botanicznym UAN w Kijowie na Ukrainie.

Introduction

The intensive introduction into the culture of new uncommon plants is related with the increasing demand for medical-dietary qualities in gardening products, because many of the uncommon fruit and berry plants contains an abundant amount of biologically active substances. Additionally, these plants possess a high level of resistance to pests and diseases; they practically needn;t chemical protection. One of these uncommon genus is *Actinidia* Lindl. It is a relatively new crop. Its commercial cultivation dates back to last century and is associated mainly with *A. deliciosa* and *A. chinensis*, which is more commonly known as kiwifruit. Nowadays, kiwiberry (*Actinidia arguta*) is a new product on the market with growing worldwide consumer acceptance. *A. arguta* in contrast to kiwifruit are small grape-sized fruit, with thin, edible predominantly green skin. The fruit have high sensory quality, is very aromatic with a sweet, intense flavour that has been compared to blackcurrant, pineapple, ripe strawberry, pear, banana, melon and other tropical flavours and is highly accepted by the consumers (LATOCHA and JANKOWSKI 2011, LATOCHA et al. 2011). Its great advantage is not only pleasant taste but also rich chemical composition having significant antioxidant activity (LATOCHA et al. 2010, BIENIEK 2012a, LATOCHA et al. 2013). Currently, kiwiberry is treated as one of the most nutrient dense fruit and are usually referred to as "healthy fruit". A great kiwiberry advantage is its delicate, edible skin containing up to 15 times more antioxidants than fruit pulp (KIM et al. 2009, LATOCHA et al. 2015). This paper describe the history and current status of kiwiberry selection work made in Botanical Garden in Kiev, Ukraine.

Systematics of the genus *Actinidia*

The genus *Actinidia* was established by LINDLEY (1836) based on the specimens WALLICH no. 6634 collected from Nepal in 1821. Taxonomy of the genus *Actinidia* was firstly proposed by GILG (1893). The author distinguished only eight species and based on inflorescences has divided it into two groups, *Monanthae* (with solitary flower) and *Pleianthae* (with cymes). The first comprehensive taxonomic revision of the genus was published in 1911 by DUNN (1911). He recognized 24 species and almost 40 varieties and forms worldwide and established four sections *Vestitae*, *Maculate*, *Ampulliferae* and *Leiocarpae* based mainly on the shape of the ovary and the degree of pubescence. The next major revision made by LI (1952) listed 36 species and 14 varieties. Based on the structure of leaf hairs he divided the section *Vestitatae* into two sections *Stellatae* (with stellate hairs) and *Strigosae* (with simple hairs). The section *Ampulliferae* was merged into section *Leiocarpae*. The revision by LIANG (1984) significantly increased the number of taxa to a total of 51 species and 35 varieties and six forms. Many of them were found in China. One of the most important taxonomical changes was including *A. purpurea* into *A. arguta* variability (as varieties of *A. arguta*) based on fruit colour and leaf serration. Finally, the last systematic revision made by LI et al. (2007) distinguish 54 species and 21 varieties (52 of which occur naturally in China and 44 are endemic). He concluded that fruit colour of *A. arguta* var. *purpurea* is not consistently correlated with leaf serration and is not useful as diagnostic feature. The variety was therefore merged with *A. arguta* var. *arguta*. Regardless of these systematic revisions some authors described different number of *Actinidia* species. SENETA (1991) mention 30–40 species, SENETA and DOLATOWSKI (2012) – about 40 species, HUANG et al. (2003) – 66 species and 118 taxa and FERGUSON and HUANG (2007) describe 76 species and 125 taxa. As genus *Actinidia* is very diverse and many natural hybrids occur, the list of species will probably change many times.

Some *Actinidia* species are successfully cultivated in M.M. Grishko National Botanical Garden (NBG) of National Academy of Sciences of Ukraine. The garden, located in Kyiv (N 50°27'; E 30°31'), has one of the largest scientific and practical Ukrainian centers for the introduction and acclimatization of uncommon fruit species, selection and spreading of new cultivars, which may be successfully cultivated on commercial plantations and private gardens as well. The most attention is paid to frost resistance species such as *A. arguta* and *A. kolomikta*.

Botanical garden environmental characteristics

The botanical garden is located on the border of two climatic zones – the wooded district and forest-steppe in the southeastern part of the city on the slopes of Pechersk hills near Dnipro river. The main type of soil is dark-gray podzolic. Due to the crossing of locality, the soil is rather washed-out and characterized by low humus content. The climate of the area is moderately continental with an average annual temperature of 7.6°C; the average temperature in January is -5.5°C and in June 20.4°C. The winter in Kyiv is softened by periodic changes of Atlantic air masses. The frost-free period in Kyiv is 165–180 days on average. According to long-term data the sum of active temperatures in Kiev is 2000–2500°C. The average annual amount of precipitation in Kyiv is 550–650 mm, relative humidity is 73–76%. The duration of frost free period, the temperature conditions, rainfall during the active growing season create suitable possibilities for successful cultivation of different fruit plants from regions with similar and sometimes more mild climate conditions. The whole collection of the Department of Fruit Plants Acclimatization of NBG has more than 150 species and 2000 cultivars. Among them an exclusive place belongs to the genus *Actinidia* (KLIMENKO and SKRYPCHENKO 2013).

Description of *Actinidia* species and cultivars from the collection

In Ukraine, the introductory work with *Actinidia* species was initiated by academician N. Kaschenko in Acclimatization Garden (Kyiv) in the 20s and 30s of the 20th century. His investigations with *A. kolomikta* and *A. arguta* demonstrated the possibility of its cultivation under climatic conditions of forest-steppe of Ukraine (SHAJTAN et al. 1983). In NBG the first seedlings of *A. kolomikta*, *A. arguta* and *A. polygama* were obtained by researcher Y. HOTSYK from seeds collected during an expedition to Far East of Russia in 1949 (HOTSYK 1955), which were later transferred to the geographical area 'Far East' in the Garden. Systematic creation of collection and selection work of *Actinidia* was carried out by well-known breeders I. SHAITAN and R. KLEYEVA since 1958 (SHAJTAN et al. 1983). Now the NBG collection of *Actinidia* is the largest in Ukraine – it includes 6 species and over 300 different forms and cultivars of *Actinidia* (SKRYPCHENKO and MOROZ 2002, KLIMENKO and SKRYPCHENKO 2013, SKRYPCHENKO 2016). The collection is constantly supplemented through exchanges with various botanical institutions and through breeding work focused on creating high-performance cultivars, which are adapted to the conditions of forest-steppe of Ukraine. The principal

Actinidia species with which we work with in Kyiv are described below (SKRYPCHENKO and MOROZ 2002, KLIMENKO and SKRYPCHENKO 2013).

Actinidia kolomikta (Rupr et Maxim.) Maxim. (Figure 1). The length of the vines in nature reaches (if supported) 15 m. Annual wooden shoots are smooth, reddish-chestnut in color with numerous white lenticels of elliptical oblong shape. Leaves have rounded-elliptical or ovoid form with linearly-lanceting tip and reddish hair on the veins. The upper leaf surface is green, matt, but the reverse side is lighter. During the growing season some of the leaves change colour several times – the whitening of the tip of the leaf or the whole leaf is observed during leaf formation; 8–15 days later (before flowering period) the leaves become partially crimson. After flowering leaf colour fades but is preserved till the end of vegetation. Some leaves remain green without colour changes. This phenomenon is most prevalent in male plants, which are located under direct sunlight.

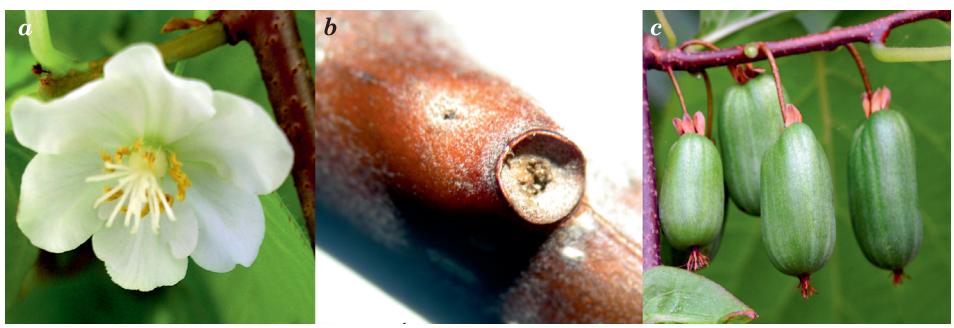

Fig. 1. *Actinidia kolomikta*: *a* – female flower; *b* – bud; *c* – fruit

A. kolomikta flowers are white with wide ovoid petals and have a strong odour. The male flowers mainly are in inflorescences (up to three) and have up to 30 stamens per one flower with a rather large yellow anthers. Female flowers are mainly alone, the diameter of flower is up to 2 cm. There is a large flower ovary in the center, which is surrounded by stamens. *A. kolomikta* fruits are oblong elliptical and have emerald green smooth surface. The beginning of fruit ripening season occurs in Kyiv in the second half of July. Ripe berries weigh about 3–5 g, are soft and sweet with pineapple flavour. The ripening is not simultaneous and ripe fruit fall off, but peduncles remain on the shoots for a long time.

Actinidia arguta (Siebold et Zucc.) Planch. ex Miq. (Figure 2). The seeds of *A. arguta* were obtained from China (Beijing) in 1958. In native location *A. arguta* is a liana, climbings up to 25 m. The shoots are mainly gray-brown with large number of longitudinal small lenticels. The buds are fully covered by

stem bark. Leaves have elongated-ovoid or elliptical form up to 15 cm in length and up to 10 cm in width. The leaf is dark green, leathery with light veins. The flowers are about 2–2.5 cm in diameter, white to light cream colored with gray anthers and a slight odour; the male flowers are gathered to 5–10 in an inflorescence, but the female ones are usually singlet or triplet (seldom up to 7). Petals are ovoid, sepals are light green.

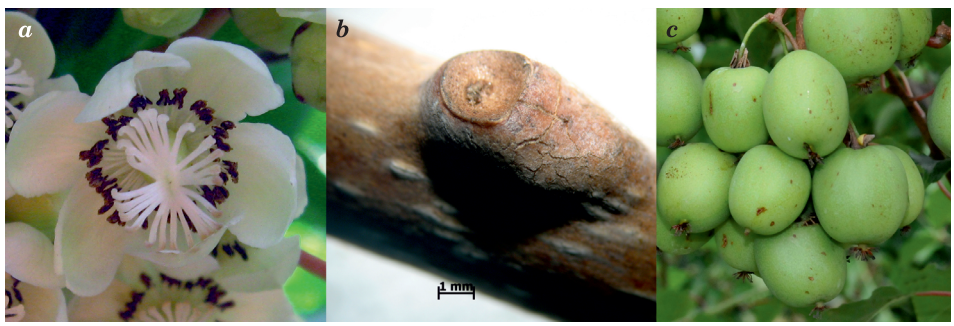

Fig. 2. *Actinidia arguta*: *a* – female flower; *b* – bud; *c* – fruit

Fruits of *A. arguta* are elliptical, almost spherical or elongated, with weight of 5–20 g. The berries are sweet and sour, with a specific delicate aroma. Their surface is smooth, dark or light green with or without blush. Berries begins to ripen in the Garden at mid-September. Ripe berries do not fall off and remain on stems until the first frost.

A. arguta var. *purpurea* (Rehder) C.F. Liang ex Q.Q. Chang (Figure 3). According to most recent taxonomy revision (Li et al. 2007) this variety is included in the variability of the *A. arguta* but for a long time it was considered a separate species. Seeds of this *Actinidia* were obtained from Beijing in 1958. This is a vigorous vine with shoots covered by numerous brown lenticels and leaves more elongated than in most *A. arguta* – with crimson petioles 3–5 cm in length. Flowers are white 2–2.5 cm in diameter, with five-six petals, on long thin peduncles, in female plants mostly two-three in a inflorescence. The male flowers are up to ten in inflorescence. The oblong-cylindrical, dark purple berries weigh 6–12 g. They ripen in Kyiv by the end of September or by the beginning of October and do not fall off after ripening. The last two species were used to produce many highly productive *Actinidia* hybrid cultivars, selected in NBG.

Actinidia polygama (Siebold et Zucc.) – Figure 4. As noted above, *A. polygama* was introduced to Ukraine in 1949 from seeds collected by an expedition to the Far East of Russia (Primorsky Krai). *A. polygama* is a liana growing up to 6 m. The bark on its stems is dark brown with furrow-like white

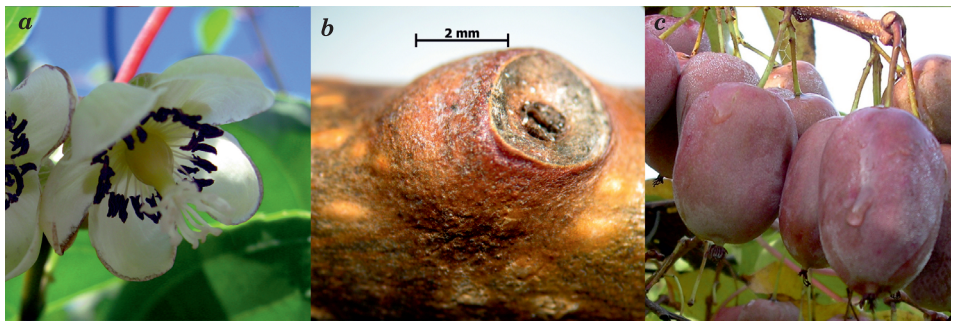

Fig. 3. *A. arguta* var. *purpurea*: *a* – female flower; *b* – bud; *c* – fruit

lenticels. The stem core is solid, unlike the previous species. The buds are not fully covered by stem bark, with apexes well visible at the bases of petioles. The leaves are alternate, broadly ovate to oblong-elliptical, with light green petioles. During leaf formation, some leaves change color to silvery white, sometimes remaining till the end of the summer. The flowers of *A. polygama* are white with a strong pleasant odour. Flowers are 2–3 cm in diameter. The male flowers are a bit larger than female. Fruits of *A. polygama* are orange, matte and smooth with oblong lighter streaks. The shape is cylindrical with thin tips; sepals remain until the full fruit maturation. Fruits ripen in the first decade of September and taste like hot or sweet peppers.

Fig. 4. *Actinidia polygama*: *a* – female flower; *b* – bud; *c* – fruit

A. macrosperma C.F. Liang (Figure 5). This species grows in the homeland in damp areas of mixed forests in eastern Chinese provinces. Seeds of this species were introduced to Ukraine in 2007; since 2013 the plants began fruiting. Leaves are small, 3–6 × 1.7–3.5 cm, with serrate margins and lightly pubescent petioles. Flowers are white with two or three ovate bared sepals and 5–12 ovoid petals. Fruits ripen in late September and are round, up to 4 cm in

diameter and mature with yellow-orange skin and pulp. Ripe fruit taste bitter. This species is promising for further hybridization because of beautiful fruit appearance (as mandarins).

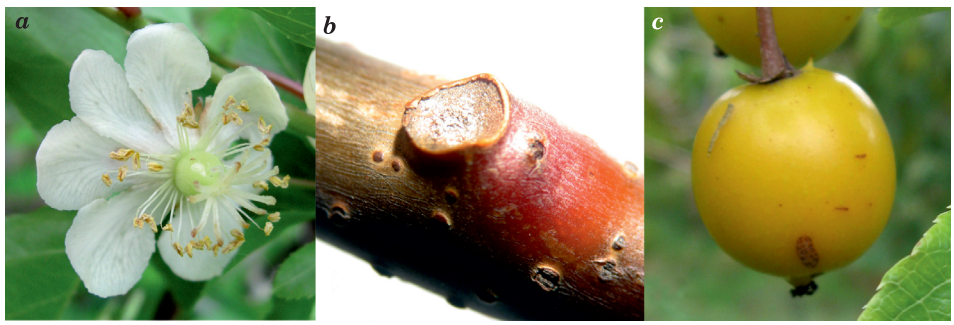

Fig. 5. *Actinidia macrosperma: a* – female flower; *b* – bud; *c* – fruit

Actinidia deliciosa (A. Chev) C.F. Liang et A.R. Ferguson (Figure 6). This species was firstly introduced in NBG in the 1970s (under the name *A. chinensis*) from Batumi and the first plants were male. In the 1990s, seedlings from kiwifruits from Italy were obtained but unfortunately most of these seedlings died due to the low winter temperatures. Only the most winter-hardy of our plants, which were able to withstand winter temperatures up to -19°C have survived. *A. deliciosa* is a strong woody vine, growing up to 8 m in height, with vigorous shoot growth. Young shoots are green-brown or red-brown and densely pubescent with relatively large hairs. The buds are covered with stem bark. The leaves are well-rounded to elliptical, with pubescent mostly red petioles. Leaves are dark green with smooth topside and a densely hairy underside (stellate hairs).

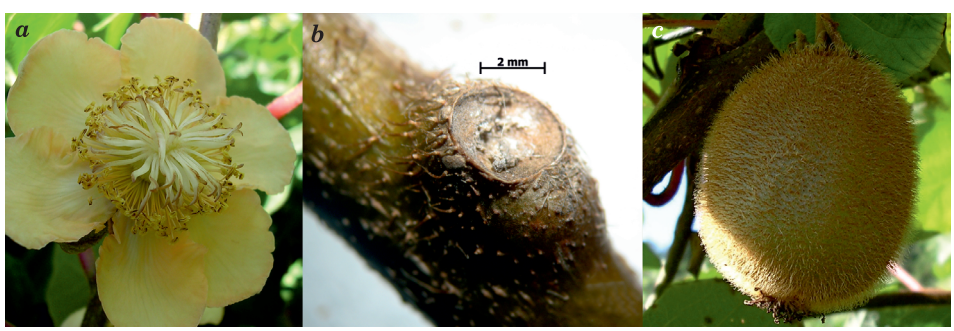

Fig. 6. *Actinidia deliciosa: a* – female flower; *b* – bud; *c* – fruit

Flowers of *A. deliciosa* are large, up to 5 cm in diameter, white; at the end of flowering they are yellow-orange with ovoid petals and three-four sepals on a long peduncle. At the center of female flowers there is a pistil with ray-like stigmas, surrounded by a lot of stamens with yellow anthers. The fruits are very fragrant, spherical to ellipsoid, weigh 45–60 g with sweet-sour taste. The fruit skin is tick, light brow and densely covered by hairs. In forest-steppe conditions, fruits of *A. deliciosa* do not ripen until the first frost, but are harvested before first frost occurs. They mature during storage and acquire the desired taste.

As the results of the selection work based on the above presented *Actinidia* collection new cultivars such as 'Sentiabrskaya'(*A. arguta*), 'Purpurnaya Sadovaya'(*A. arguta* var. *purpurea*) and 'Pomarancheva'(*A. polygama*) were obtained (Table 1). These cultivars were received as the results of interspecific and intervarietal hybridization. Despite of information given in the Table 1, they are characterized by a stable annual crop, abundant biochemical fruit composition and good frost resistance (SKRYPCHENKO 2016). Among these cultivars 'Kievskaya Krupnoplodnaya', 'Figurnaya', 'Karavaievskaya Urozhainaya', 'Nadiya', 'Zagadkova', 'Rubinovaya', 'Originalnaya' are most promising. Afterwards new winter-hardy cultivars 'Lasunka', 'Perlyna Sadu', 'Krasunia', 'Juvileyna', 'Smaragdova' were selected. All these cultivars were registered in Ukraine State Register. The male cultivar 'Don Juan' as a pollinator for all female cultivars was selected. But for further research work other species of *Actinidia* are of great interest: *A. polygama* and *A. kolomikta* as they are a source of high amount of biologically active substances in fruits, *A. macrosperma* due to its original fruit and *A. deliciosa* as a species with well-known fruits.

Long-term (10–15 years) phenological observations of *Actinidia* species showed that their vegetation in Kyiv begins on average in the third week of March, with intense weeping The growth of *Actinidia* shoots begins in the second week of April. There are significant differences in the biology of flowering and fruiting in the introduced species mostly due to their origin. Plants of *A. kolomikta* are the first to flower (middle-late May), whereas *A. arguta, A. arguta* var. *purpurea,* and *A. deliciosa* begin to flower several days later. The last to flower are *A. polygama* and *A. macrosperma* (first or the second week of June). The male plants usually start growing at Spring and flowers one-three days earlier than the female ones. Duration of the flowering of the studied *Actinidia* species is 6–19 days, depending on the weather conditions. As an example comparing differences in the phenological development of different *Actinidia* species, the results of the observation from 2014 are shown (Figure 7).

Table 1
Fruit characteristics and yield of actinidia cultivars from NBG selection (fruit weight for the year 2015 as average of 100 fruit ± standard deviation; yield as long-term average)

Cultivar	Origin	Fruit skin colour	Fruit weight [g]	Average yield [kg per plant]
'Purpurnaya Sadovaya'	*A. arguta* var. *purpurea*	purple	11.7 ± 1.4	20–35
'Sentiabrskaya'	*A. arguta*	green	10.3 ± 1.8	9–12
'Krasunia'	*A. arguta*	green	13.3 ± 1.2	9–12
'Kievskaya Gibridnaya'	*A. arguta* × *A. arguta* var. *purpurea*	green	10.6 ± 1.8	10–18
'Kievskaya Krupnoplodnaya'	*A. arguta* × *A. arguta* var. *purpurea*	green	18.5 ± 2.4	20–25
'Figurnaya'	*A. arguta* × *A. arguta* var. *purpurea*	green	8.6 ± 1.4	20–25
'Rima'	'Sentiabrskaya' × *A. arguta* var. *purpurea*	green	6.8 ± 1.5	15–20
'Zagadkova'	'Sentiabrskaya' × *A. arguta* var. *purpurea*	green with purple blush	9.3 ± 1.6	15–20
'Originalnaya'	'Sentiabrskaya' × *A. arguta* var. *purpurea*	green	10.4 ± 1.9	15–20
'Lasunka'	'Sentiabrskaya' × *A. arguta* var. *purpurea*	green with purple blush	9.3 ± 1.3	10–11
'Karavaievskaya Urozhainaya'	'Purpurnaya Sadovaya' × *A. arguta*	purple	6.6 ± 1.1	10–12
'Nadiya'	'Purpurnaya Sadovaya' × *A. arguta*	purple	9.0 ± 1.4	15–20
'Rubinovaya'	'Purpurnaya Sadovaya' × *A. arguta*	purple	6.1 ± 1.0	10–13
'Perlyna Sadu'	'Purpurnaya Sadovaya' × *A. arguta*	green	8.2 ± 1.1	12–15
'Juvileyna'	('Kievskaya Krupnoplodnaya' × 'Purpurnaya Sadovaya' × *A. arguta)*	brown–purple	14.8 ± 2.1	12–15
'Smaragdova'	'Sentiabrskaya' × 'Don Juan'*	dark green	10.2 ± 1.8	12–15
'Pomarancheva'	*A. polygama*	orange	6.5 ± 1.0	3–5

* – 'Don Juan' – male cultivar (pollinator)

The fruits of *A. kolomikta* begin to ripen in late July – early August (30–40 days after petal fall) and fall down just after ripening. The fruits of *A. arguta* begin to mature in the first week of September (ripe fruits do not fall off and can remain on stems for a long time). The harvest can be done in two-three steps or once after they fully ripen. Fruits of *A. arguta* var. *purpurea* ripen in late September – early October. Berries of *A. polygama* ripen simultaneously with fruits of *A. arguta* and do not fall down after ripening due to strong short

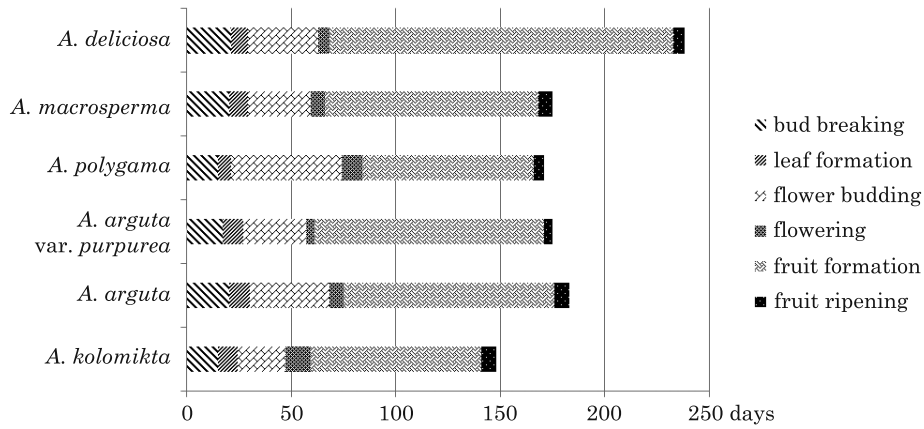

Fig. 7. Comparison of phenological spectrums of different *Actinidia* species in Kyiv. An example from the year 2014 (zero corresponds to 1th March)

peduncles. Ripe fruit can remain on a stems for up to a month. Fruits of *A. macrosperma* ripen in the middle of September. Fruits of *A. deliciosa* in our area are harvested when not fully mature before the first autumn frost occur, but they ripen during storage.

Leaves of *A. kolomikta*, *A. arguta* and *A. polygama* begin to acquire their autumn color in September, and defoliation happens in the first and the second week of October. Plants of *A. deliciosa* and *A. arguta* var. *purpurea* have green foliage at the first frost with leaves falling down after the air temperature drops below zero. Hence, vegetation periods of these species end with induced defoliation at the end of October or even the beginning of November.

With exception of *A. deliciosa* all other introduced species of *Actinidia* undergo their full circles of seasonal development hence the Forest-steppes of Ukraine climate conditions are favorable for widespread cultivation of these plants.

Some of these Ukrainian kiwiberry selections has already been introduced into North-Eastern Poland. Cultivars 'Kiyewskaya Gibridna', 'Purpurnaya Sadovaya', 'Figurnaya' and 'Kievskaya Krupnoplodnaya' were successfully cultivated in UWM Experimental Station in Olsztyn (BIENIEK 2012a,b, BIENIEK and DRAGAŃSKA 2013). Research made by Bieniek et al. (2016) shown that the sum of effective temperature (SET) during kiwiberry growing in years 2005–2014 in Olsztyn estimated between 1455 and 1839°C, on average. It was not much less than SET noted in Ukraine. Authors indicated late spring frost as the most important kiwiberry growing limitation in North--Eastern Poland.

Conclusions

The plant collection of *Actinidia* species of M. M. Grishko NBG of NAS of Ukraine is a basis for research of anatomical, morphological and biochemical properties of the new cultivars suitable for cultivation in Forest-steppe of Ukraine. The collection is an important source for hybridization work carried out in order to obtain new highly productive cultivars of *Actinidia*, with high contents of biologically active substances. The *A. kolomikta*, *A. arguta, A. polygama,* and *A. macrosperma* are characterized by high introduction potential, undergo all phases of development and produce high quality seeds.

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THE EFFECT OF STORAGE CONDITIONS ON SELECTED QUALITY MARKERS OF FROZEN VEGETABLES

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K e y w o r d s: frozen vegetables, broccoli, sensory evaluation, vitamin C, folate.

A b s t r a c t

At the end of frozen food distribution chain, there is home storage including storing at refrigerator. However, not much is known how it affects frozen vegetables quality. The aim of the study was to evaluate the effect of storage conditions (2°C and 8°C from 1 to 7 days) on frozen broccoli and green beans sensory properties and vitamin C and folate content. Sensory evaluation was conducted with the use of the 5-point category scale method, while vitamins were determined with the use of HPLC technique. The results showed constant tendency in sensory quality and vitamins content decrease, the rate of which was higher at 8°C than at 2°C. The study concluded, that folate content analysis can be good chemical indicator for storage period of frozen vegetables next to vitamin C and sensory quality. However, each product requires individual assessment of its shelf-life at refrigerating temperature.

WPŁYW WARUNKÓW PRZECHOWYWANIA NA WYBRANE WYRÓŻNIKI JAKOŚCI MROŻONYCH WARZYW

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S ł o w a k l u c z o w e: mrożonki warzywne, brokuły, ocena sensoryczna, witamina C, foliany.

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Abstrakt

Ostatnim etapem dystrybucji żywności mrożonej jest jej przechowywanie w domu, np. w lodówce. Jednak niewiele wiadomo, jaki ma ono wpływ na jakość mrożonych warzyw. Celem pracy była ocena wpływu warunków przechowywania (2°C i 8°C, od 1 do 7 dni) wybranych mrożonek warzywnych, brokułów i zielonej fasolki szparagowej, na ich właściwości sensoryczne, zawartości witaminy C i folianów. Ocenę sensoryczną przeprowadzono z wykorzystaniem 5-punktowej skali kategorii, a zawartość witamin oznaczono z wykorzystaniem techniki HPLC.

Wykazano stałą tendencję obniżania się jakości sensorycznej i zawartości witamin w mrożonkach. Była ona wyższa w temperaturze 8°C niż w 2°C. Wykazano, że analiza zawartości folianów może być dobrym wskaźnikiem chemicznym okresu przechowywania mrożonych warzyw obok zawartości witaminy C i ich jakości sensorycznej. Jednak każdy produkt wymaga indywidualnej oceny okresu przydatności do spożycia podczas przechowywania w temperaturze chłodniczej.

Introduction

Changes in the consumer preferences and lifestyle related to the lack of free time, make frozen vegetables more and more popular as convenient for quick and easy meal preparation (JOHANSSON et al. 2008). Nowadays, freezing is considered one of the best method for retaining not only sensory properties of the raw material, but also nutritive compound including vitamins. The quality of frozen product is highly dependent on the raw vegetable characteristics, environmental conditions, parameters of freezing processes, storage temperature and time. The lower the temperature is provided, the longer the shelf-life is (GONCALVES et al. 2011). According to official recommendations, the storage temperature must be stable and maintained below -18°C for a storage period between 6 and 24 months (EU Directive 89/108). Information on freezing storage conditions is written on the product packaging. However, according to some manufacturers, consumers are often interested in frozen vegetable shelf-life when it is stored in the refrigerator. Many deep-frozen products, available nowadays in retail, are marked with additional information about the storage time at temperatures above 0°C next to time of storage at -18°C.

However, at higher temperatures, the quality decrease might be observed very quickly. Numerous parameters of frozen food can be affected under such storage conditions. These are not only analytical parameters with nutritive and chemo-protective properties (e.g. vitamins, sugars, amino acid, fatty acid content) but also sensory attributes, which determine consumers acceptance (BERGER et al. 2007). Generally, to establish food products' shelf-life, microbiological criteria are considered. However, in case of frozen vegetables, sensory evaluation is used most of the times together with the use of some objective measurements, such as content of nutritional compounds, e.g. vitamin C (CORBO et al. 2006, GONCALVES et al. 2011). Although literature data on

vitamin C content in frozen vegetables under various storage conditions are available, many are old and the analysis were conducted with the use of antiquated photometric methods instead of the modern methods using high performance liquid chromatography, HPLC. Besides, knowing that chemical parameters can change in the same manner as important sensory attributes, there is a need to determine more of them, including another vitamins, as these can be considered as an important back-up to sensory methods (BERGER et al. 2007).

The aim of the study was to evaluate the effect of various storage conditions at the temperature above 0°C on frozen vegetables selected quality parameters, such as sensory properties, vitamins C and folate content.

Materials and Methods

Material

Test material, consisting of frozen green beans and broccoli, was purchased from company CHŁODNIA OLSZTYN in Olsztyn city which produces frozen fruit and vegetables. The company prepared 12 bags (450 g each) of both frozen vegetables which were stored in a manufactory cold room at -18°C until delivery to the Department's laboratory for analysis. The sensory evaluation together with determination of vitamin C and folate content were carried out at the vegetables samples after thawing and after 1, 3 and 7 days of storing in refrigerator at 2°C and 8°C.

Sensory evaluation

The sensory properties of vegetables samples were evaluated with the use of the 5-point category scale method by a panel of selected assessors. During the test, the following attributes were evaluated: shape, color, odor and firmness. All the selected properties were characterized, described in words and the appropriate number of points (5 – very good, 4 – good, 3 – satisfactory, 2 – poor, 1 – bad) were assigned to them using previously prepared standard cards (BARYŁKO-PIKIELNA and MATUSZEWSKA 2009). The definitions and evaluation techniques were agreed upon by the assessors during training before evaluation (*Sensory analysis...* ISO 8586-1:1993). All samples of each vegetable were presented to panelists in the single sessions during which they were provided with a response sheet with written instructions for the test. Sample were prepared and coded with three random numbers and the order of their

presentation on the plates was completely randomized among assessors (*Sensory analysis*... ISO 6658:2005). The sensory evaluation took place in the test room (*Sensory analysis*... ISO 8589:2007).

Folate analysis

Standards. 5-Methyltetrahydrofolate (5-CH_3-FH_4), tetrahydrofolate (FH_4), 5-formyltetrahydrofolate (5-HCO-H_4folate), 10-formyltetrahydrofolate (10-HCO-H_2folate) were purchased from Sigma-Aldrich (USA) and prepared as described by KONINGS (1999), and concentrations were calculated using molar absorption coefficients given by BLAKLEY (1969).

Enzymes. α-amylase (A-6211) was purchased from Sigma Chemical Co. and prepared as described by GUJSKA et al. (2014), by dissolving in 0.05 M potassium phosphate buffer, pH 6.1, at the concentration of 20 mg ml^{-1} 50 ml of rat plasma folate conjugase was prepared according to JASTREBOVA et al. (2003) by dialyzing for 24 h at 4°C in 1 l of 0.05 M phosphate buffer, pH 6.1, containing 2% sodium ascorbate and 0.1% 2-mercaptoethanol.

Extraction. Samples of vegetables were blended and the extraction was carried according to CZARNOWSKA and GUJSKA (2012), in a 0.1 M phosphate buffer (pH 6.1) containing 2% sodium ascorbate and 0.1% 2-mercaptoethanol. After homogenizing, samples were placed for 15 min in a boiling water bath, then cooled in ice. The homogenate was subjected to enzyme treatment: 0.25 ml rat plasma folate conjugase and 1 ml α-amylase (4 h at 37°C). Deconjugation was stopped by keeping tubes for 5 min in boiling water bath, then cooling in ice. Samples were centrifuged twice for 20 min at 12000 rpm, at 4°C. Supernatants were collected, filled to 50 mL with the extraction buffer and filtered, then stored at -80°C until HPLC analysis. All samples were prepared in triplicate.

Purification and HPLC analysis. Extracts were purified according to the JASTREBOVA et al. (2003) by solid-phase extraction with the use of Bakerbond SPE columns (500 mg 3 ml^{-1}, J.T. Baker 7091-03). HPLC folate analysis were conducted with the method described by CZARNOWSKA and GUJSKA (2012) with Shimadzu Series LC-10A instrument equipped with Phenomenex Synergi C18 column. The chromatographic condition for gradient elution were as described: flow rate: 1 ml min^{-1}, volume injection 100 µl, column temperature 25°C, fluorescence detector – 290 nm excitation/ 356 nm emission. The mobile phase was acetonitrile and 30 mM phosphoric acid buffer, pH = 2.3. Peak identification was based on the retention time of standards and samples peaks. Quantification was based on calibration curves of identified folate forms.

Vitamin C analysis

Materials. L-ascorbic acid and dithiotreitol were purchesed from Sigma Aldrich, while metaphosphoric acid (HPO_3) from P.P.H. "STANLAB" Sp.J.

Sample preparation and HPLC analysis. Vitamin C content in each vegetable sample was analyzed in triplicate according to GÖKMEN et al. (2000). After blending, 1 g of the sample was dissolved in water and HPO_3 (4:1) and shaken in a water bath, then filtered. To determine total vitamin C content, dehydroascorbic acid reduction to ascorbic acid was carried by adding to the filtrate (1 ml) dithiotreitol solution (2 ml) at the concentration of 2 mg ml^{-1}. After keeping in darkness for 2 h, samples were filtered and analyzed on Agilent 1200 Liquid Chromatograph equipped with Lichrospher RP 18 column (5.0 μm, 250 x 4.6 mm). The flow rate was 0.5 ml min^{-1}, volume injection – 50 μl, column temperature – 30°C, UV spectroscopic detector (λ = 245 nm) was used. Peak identification was based on the retention time of standards and samples peaks. Quantification was based on ascorbic acid calibration curve.

Statistical analysis

Data were analysed using the Analysis of Variance (Statistica version 10). Duncan multiple range test, with the significance level at $p < 0.05$, was applied to the results to test the effect of different storage conditions on folate and vitamin C content in tested vegetables.

Results and Discussion

Sensory evaluation

Sensory properties of the product are crucial for consumers. Meanwhile, one of the main change leading to reduction of frozen vegetables quality, is decrease in sensory properties due to degradation of natural colors, flavor changes and browning. The result of sensory evaluation of frozen vegetables is strongly effected by storage conditions, temperature and time (PUKSZTA 2013).

The sensory quality of broccoli immediately after thawing was assessed quite low at 3.7 (Figure 1). Similarly, as in the studies conducted by GONCALVES et al. (2011), the highest impact on that score had product color, which at the same time had the most influence on consumer choice. Color alternations in green vegetables during storage, including frozen storage, are attributed to the fade of the vivid green color of chlorophyll to an olive brown, characteristic

of pheophytin (MARTINS and SILVA 2002). In turn, the main features of lowering the total score of broccoli stored at 2 and 8°C for 1–7 days, were aroma and firmness. Also in MARTINS and SILVA (2004) model studies, the flavor retention has shown to be always the limiting factor of shelf-life at +5, -6 and -12°C. In our test samples after one day storing at 8°C, an unpleasant odor was noticed, firmness was also unsatisfactory. However, the product was assessed as poor no sooner than after 7 days of storage at 8°C with 50% decrease of total score (Figure 1).

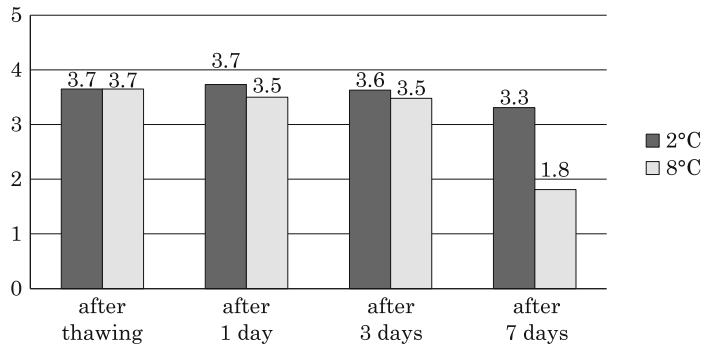

Fig. 1. Total score of broccoli sensory evaluation in the 5-point scale

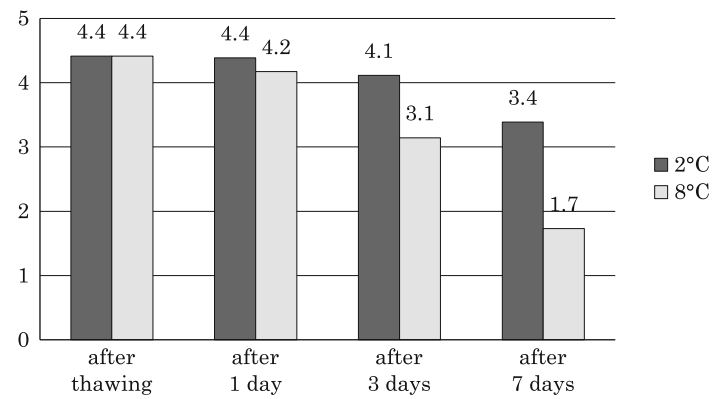

Fig. 2. Total score of green beans sensory evaluation in the 5-point scale

Sensory quality of green beans after thawing was assessed as good (4.4). The main feature of lowering the overall sensory score of this product throughout the storage period were color and firmness. The sensory quality decrease of almost 30%, was noticed no sooner than after 3 days of storing at 8°C and after 7 days at 2°C (Figure 2). Similarly, in the model studies conducted by MARTINS and SILVA (2004), only slow retention of flavor, texture and color was observed during 24 h storage at 5°C. After 7 days at 8°C, our test samples with quality decrease of 60%, received a "poor" note (Figure 2).

Folate

Folate, vitamin B, are reduced derivatives of folic acid, which naturally occur in foods, and are necessary for proper functioning of the body. The rich source of folate in the human diet are vegetables, especially green leafy ones (spinach, broccoli) but also legumes, cereals, dairy products and liver (RAMPERSAUD et al. 2003). Unfortunately, these vitamins are very unstable compounds and quickly lose their biologically activity. They undergo degradation at high temperature, sunlight, during storage time and food product preparation (JOHANSSON et al. 2008, XUE et al. 2011). The results available in literature indicate high folate lability in some food matrices at refrigeration temperatures. Researchers have called for further studies to explain the relationship between storage temperature and food matrix with which folate are bound in various foodstuffs. According to HAWKES and VILLOTA (1989), in vegetables due to the use of different technological treatments (soaking, cooking, blanching, steaming, freezing, thawing), folate reduction can vary from 22% in asparagus up to 84% in cauliflower. PUUPPONEN-PIMIÄ et al. (2003) and STEA et al. (2006), noticed that folate loss due to pea blanching (98°C/ 2 min) may range from 12 up to 35%. In another studies, CZARNOWSKA and GUJSKA (2012), observed folate reduction with the time of frozen storage (up to 12 months) at -18°C. For example, in frozen cauliflower, folate loss exceeded 95% just after three months of frozen storage while in green and yellow beans, significant loss (75% and 95%, respectively) was observed no earlier than after nine months.

In our study in all broccoli and green beans samples two folate forms were identified, tetrahydrofolate FH_4 and 5-metyltetrahydrofolate, 5-CH_3-FH_4. The conducted studies showed that frozen broccoli and green beans were good folate sources and its content after thawing was at the level of 129.3 and 91.1 μg $100g^{-1}$, respectively. In case of frozen broccoli stored at 2°C and 8°C up to 7 days, the significant folate loss of 20% was observed after 3 days at 2°C. The highest decrease of 30%, was noticed after 7 days at 8°C, while after the same time at 2°C, the loss didn't exceed 20%. In green beans significant folate loss of 10% was observed after 1 day at 2°C and almost 20% at 8°C. Folate decrease during storage for 3 and 7 days remained at the level of 20% and was not significantly different to the loss noticed after 1 day at 8°C (Table 1).

Vitamin C

Vitamin C is a water soluble vitamin that is an important antioxidant in the human body, which can prevent cancer (MARTINS and SILVA 2004). Due to its high sensitivity, it is widely considered as an appropriate marker for monitoring changes during processes including storage in the whole frozen chain.

Table 1
The effect of selected storage conditions on folate and vitamin C content in frozen broccoli and green beans*

Product/ storage conditions	$5CH_3FH_4$ μg 100 g^{-1}	FH_4 μg 100 g^{-1}	Total folate* μg 100 g^{-1}	Vitamin C mg 100 g^{-1}
Broccoli				
After thawing	121.7 ± 9.9	7.6 ± 0.5	129.3^{a}**	20.7 ± 0.9^{a}
After 1 day at 2°C	122.9 ± 2.9	7.9 ± 0.3	130.8^{a}	20.7 ± 0.3^{a}
After 1 day at 8°C	117.6 ± 7.0	7.2 ± 0.6	124.7^{a}	18.7 ± 1.5^{b}
After 3 days at 2°C	94.8 ± 1.2	10.6 ± 0.3	105.4^{b}	16.6 ± 0.5^{c}
After 3 days at 8°C	95.5 ± 5.7	11.1 ± 0.1	106.6^{b}	14.5 ± 0.7^{d}
After 7 days at 2°C	95.1 ± 3.7	10.9 ± 0.1	105.9^{b}	13.5 ± 0.3^{d}
After 7 days at 8°C	79.9 ± 1.5	8.7 ± 0.6	88.6^{c}	10.7 ± 0.1^{e}
Green beans				
After thawing	65.4 ± 2.7	25.8 ± 1.3	91.1^{a}	12.0 ± 0.6^{a}
After 1 day at 2°C	64.7 ± 6.3	18.1 ± 0.3	82.9^{b}	11.7 ± 0.1^{a}
After 1 day at 8°C	57.6 ± 1.8	17.5 ± 0.5	75.1^{c}	12.2 ± 0.9^{a}
After 3 days at 2°C	55.3 ± 2.3	20.7 ± 1.1	75.9^{bc}	11.5 ± 0.3^{a}
After 3 days at 8°C	53.4 ± 0.9	21.2 ± 0.8	74.6^{c}	11.5 ± 0.8^{a}
After 7 days at 2°C	55.1 ± 2.4	21.5 ± 1.3	76.6^{bc}	9.5 ± 0.5^{b}
After 7 days at 8°C	55.8 ± 4.1	16.7 ± 1.2	72.5^{c}	4.7 ± 0.1^{c}

* Measured as the sum of $5CH_3FH_4$ and FH_4, expressed as folic acid
** Means with the same letter are not significantly different ($p < 0.05$)

Vitamin C degradation is caused by two factors: L-ascorbic acid oxidation and by destructive effects of specific enzymes (SERPEN et al. 2007). According to PALICH and PUKSZTA (2001), the interaction of both factors is greatly enhanced under temperature fluctuations during storage. The authors observed that even the increase of storage temperature from -18°C to -8°C, resulted in a significant increase in the rate of enzymatic reactions causing vitamin C degradation and led to a reduction in the nutritional value of frozen vegetables and fruits. Also in GONCALVES et al. (2011) study, frozen storage of broccoli under isothermal conditions (from -30°C to -5°C within 57 days) caused 51% loss in vitamin C content.

In our study after freezing operations, frozen storage and thawing, broccoli presented vitamin C content of 20.7 mg 100 g^{-1}, which was lower than 34 mg 100 g^{-1} and 36.07 mg 100 g^{-1} reported by SIKORA et al. (2008) and GONCALVES et al. (2011), respectively. In green beans after thawing, vitamin C content was at the level of 12.0 mg 100 g^{-1}, which corresponds well with results obtained for fresh product (10–25 mg 100 g^{-1}) by MARTINS and SILVA (2002) and SERPEN et al. (2007). In our broccoli samples significant vitamin C reduction of almost 10% was observed after 1 day at 8°C. After 7 days at refrigerator, the loss was 35 and 49% at 2°C and 8°C, respectively. In green beans the significant reducing was observed after 7 days at 2°C and 8°C at the level of 21 and 61%, respectively. Significant loss in vitamin C content in raw

green beans after 4 days storage at 20°C was also noted by BERGER et al. (2007). The author observed the reduction of around 20% after one day of storage. FAVELL (1998) found that 40% of the original amount of the total ascorbic acid content in fresh green beans was lost after 3 days of storage at 4°C. These differences of his results to BERGER et al. (2007) study, can result from different experimental procedures applied for vitamin C determination. According to BERGER et al. (2007), the reducing trend for vitamin C is the same during deep-frozen green beans storage. When stored for 12 months at -18°C, 75% of the total ascorbic acid content was left.

Conclusions

Both time and temperature had a significant effect on vitamins content and sensory properties of frozen green beans and broccoli. The total ascorbic acid content is considered to be good chemical indicator for the storage period of frozen vegetables. Our study showed that also another vitamins, folate for instance, seemed to be an important quality parameter of frozen products during their shelf-life at refrigerating temperatures. In case of sensory evaluation it was noticed that total quality of analyzed products is determined by different quality attribute.

For all tested parameters it was observed, that lower temperature (2°C) at refrigerator, extended the frozen vegetable shelf-life comparing to 8°C. However, the dynamics of these changes, were different for each product. Because of this, the knowledge of the exact influence of storage for each frozen vegetable would help to provide the optimal conditions to enhance quality and prolong frozen vegetables shelf-life during home storage, which includes storing at refrigerator.

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EFFECT OF DIFFERENT CULTIVATION TECHNOLOGIES ON CORRELATIONS BETWEEN THE SELECTED WHEAT GRAIN QUALITY FEATURES AND THE CONTENT OF PHENOLIC COMPOUNDS

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K e y w o r d s: falling number, phenolic compounds, alkylresorcinols, correlations.

A b s t r a c t

The main objective of this study was to determine the effect of cultivation technologies (extensive vs. high-input) on correlations between wheat grain quality parameters, such as falling number value, wet gluten content and grain hardness, and the content of phenolic compounds, such as total free phenolic compounds and alkylresorcinols. ąIt was found high values of correlation coefficient between falling number value and wet gluten, and among analysed groups of polyphenols. Grain hardness was associated with content of wet gluten and falling number values. Additional significant correlations were observed inside each cultivar. Determined technological value indices (falling number value, wet gluten content and grain hardness) were primarily dependent on the cultivation technology, whereas the contents of the phenolic compounds and alkylresorcinols were mostly related to the wheat genotype.

WPŁYW TECHNOLOGII UPRAWY PSZENICY NA KORELACJE MIĘDZY WYBRANYMI WYRÓŻNIKAMI JAKOŚCI TECHNOLOGICZNEJ ZIARNA ORAZ ZAWARTOŚCIĄ POLIFENOLI

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S ł o w a k l u c z o w e: liczba opadania, związki fenolowe, alkilorezorcynole, korelacje.

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Abstrakt

Głównym celem pracy było określenie wpływu technologii uprawy (ekstensywna i wysokonakładowa) na korelacje między wskaźnikami jakości technologicznej ziarna (liczba opadania, zawartość glutenu mokrego, twardość ziarna) a zawartością związków fenolowych ogółem oraz alkilorezorcynoli w ziarnie pszenicy.

Wykazano wysokie wartości współczynnika korelacji między wartością liczby opadania i zawartością glutenu mokrego oraz między badanymi grupami związków fenolowych. Twardość ziarna była związana z wartością liczby opadania i zawartością glutenu mokrego. W obrębie poszczególnych odmian zaobserwowano inne istotne korelacje tych wyróżników. Wyróżniki wartości technologicznej ziarna były zależne głównie od technologii uprawy, podczas gdy na zawartość związków fenolowych i alkilorezorcynoli większy wpływ miał genotyp pszenicy.

Introduction

The Falling Number (FN) is a quick and easy test used to evaluate the degree of starch hydrolysis in wheat and rye grain samples. This index describes the degree of dextrinisation of starch caused by amylolytic enzymes, mainly by α-amylase. Increased activity of the enzyme is observed during the pre- or post-maturity sprouting of grain (LUNN et al. 1998). Even a small increase in the α-amylase activity results in a dramatic decrease in the viscosity of starch (JOHANSSON 2002, MARES and MRVA 2008). Low FNs values (60–90 s) are the indicator of sprouted grain, whereas numbers exceeding 300 s indicate that the activity of α-amylase is low and in consequence that the viscosity of gel made from the flour studied is high (LUNN et al. 2001). An increase in amylases activity is accompanied by an increase in the activity of other hydrolytic enzymes of cereal products (CORDER and HENRY 1989, KUBICKA et al. 2000). For example, cereal carbohydrates are broken down also by xylanase, β-glucanase and glycosidase (Corder and Henry 1989). However, endogenous hydrolytic enzymes are not only enzymes affecting the quality of grain. Grain condition and/or flour quality may be affected by the possible microbiological contamination, which can take place during either cultivation or grain postharvest storage. It has been shown that grain contaminated by the *Fusarium* spp. is more abundant in enzymes able to penetrate to external barriers of caryopsis. These include for example xylanases, which break down arabinoxylan structure of the cell wall (SCHMIDT et al. 2016). Generally, the activity of hydrolytic enzymes can cause degradation of biopolymers of the caryopsis (mainly starch and lignocelluloses) (FAULDS et al. 2006, SCHMIDT et al. 2016). The transformation of grain macropolymers leads to release of low molecular bioactive components such as phenolic compounds (KONOPKA et al. 2014).

Phenolic compounds are found almost exclusively in outer layer of grain. These compounds comprise a complex of diverse components, with dominating phenolic acids and phenolic lipids, called alkylresorcinols (ARs) or resorcinol

lipids. Ninety percent of phenolic acids (FARDET 2010) and 99% of all ARs (LANDBERG et al. 2008) in grain are found in the hyaline layer, testa and inner pericarp of caryopsis. Ferulic acid is the main phenolic acid found in wheat grain (VITAGLIONE et al. 2015). Its content can be as high as 500 μg g^{-1} of common wheat grain (*Triticum aestivum* L.) (OKARTER et al. 2010). Most of ferulic acid is present in the form of stable polymers with non-starch polysaccharides (ANSON et al. 2009, HEMERY et al. 2010). From the nutritional point of view, monomers and dimers of this acid are more desirable, since they are quickly and easily bioavailable from the alimentary tract. ARs are unique amphiphilic polyphenols of the cereal grain. In terms of their structure, these compounds consist of a benzene ring with two hydroxyl groups at the *meta* positions and an alkyl chain at no. 5 carbon atom (KOZUBEK and TYMAN 1995, KOZUBEK and TYMAN 1999). Cereal grain ARs occur as saturated, and mono- and bi-unsaturated homologues with an odd (from 13 to 27) number of carbon atoms in aliphatic chains (KOZUBEK 1984). The content of these compounds changes within the broad range of ca. 200–1500 mg per kg of wheat grain (HENGTRAKUL et al. 1990, ROSS et al. 2001, ROSS et al. 2003, CHEN et al. 2004). Wheat grain ARs exist primarily as free form, and are easily extractable by acetone (SAMPIETRO et al. 2009).

The kind of cultivation technology is one of "environmental" factors, which is often considered as the primary factor influencing grain quality (DENČIĆ et al. 2011). However, it is highly dependent on the cultivar sensitivity. The aim of this study was to determine the effect of cultivation technologies (extensive vs. high-input) on FN value, wet gluten content and grain hardness and the contents of total free phenolic compounds and alkylresorcinols in selected population and hybrid cultivars of common wheat (*Triticum aestivum* L.) grain.

Materials and Methods

Materials

Four cultivars of common wheat (*T. aestivum* L.) were used in this study, such as the common cultivars of Batuta and Bogatka and two hybrid cultivars of Hybred and Hymack. All of the cultivars are bread class B winter cultivars and are quite resistant to moulds and parasites (COBORU 2014). Grain samples were produced with different cultivation technology in 2014. Extensive and high-input cultivation technologies were applied (Table 1). Each cultivation technology was conducted on three independent plots for four cultivars. More details of the field experiment were presented by BUCZEK et al. (2016).

Table 1

Field experiment scheme

Sample number	Variety	Technology	Plot number
1	Batuta	extensive[1]	1
2			2
3			3
4		high-input[2]	1
5			2
6			3
7	Bogatka	extensive	1
8			2
9			3
10		high-input	1
11			2
12			3
13	Hybred	extensive	1
14			2
15			3
16		high-input	1
17			2
18			3
19	Hymack	extensive	1
20			2
21			3
22		high-input	1
23			2
24			3

[1] extensive technology – cultivation without the use of nitrogen fertilization and pesticides
[2] high-input – cultivation with the use of 120 kg N ha^{-1}, 2 x herbicide (Puma Uniwersal 069 EW, Sekator 125 OD), insecticide (Karate Zeon 050 CS), fungicide 2x (Juwell TT 483 SE, Swing Top 183 SE) and growth regulator treatments (Moddus 250 EC)

After harvesting, 500 g samples were manually cleaned from all foreign materials and stored at 8 ± 2°C. A fraction of small and damaged grains were separated from each wheat grain sample by sieving through a 2.2 mm sieve mesh. Before milling, the grain moisture was standardized to 14%. The grain samples were milled to wholemeal using IKA A11 mill (IKA-Werke, Germany) and roller-milled using a 300 μm sieve (FQC-2000, Inter-Labor, Hungary). Roller-milled flour was used to determine the content of wet gluten, whereas wholemeal flour was used for the other tests.

Methods

Moisture content. Moisture content of wheat samples was determined according to *Cereals and cereal...* ISO 712:2009.

Wheat grain qualities. FN was determined according to *Wheat, rye...* ISO 3093:2010 on a Falling Number 1600 device; the content of wet gluten was determined according to ISO *Wheat and wheat...* 21415-2:2015 on the gluten testing line type SZ-1 (ZBPP Sp. z o.o., Bydgoszcz, Poland). Grain hardness was determined by the NIR test on the NIR System Infratec™ 1241 Analyser (Foss, Hillerod, Denmark). Samples were scanned from 570 to 1,050 nm with 2 nm intervals.

Alkylresorcinols content. The content of ARs was determined according to the method of SAMPIETRO et al. (2009). Extraction was carried out with acetone by ultrasonic bath (InterSonic, Olsztyn, Poland) for 15 minutes. Subsequently, samples with the solvent were put aside to a dark place at room temperature for 24 hours. Then, the 15-minute ultrasonic treatment was repeated, after which the extract was separated in an Eppendorf centrifuge (Hamburg, Germany) at 1,000 rpm for 10 minutes. The extracts were evaporated to dryness on a vacuum evaporator (Büchi, Flawil, Switzerland). The sample was re-dissolved in 1 ml of methanol. Colour reaction was carried out using the Fast Blue RR reagent. After 20 minutes, the absorbance was measured at 480 nm using a ATI UNICAM UV/Vis UV2 (Cambrige, United Kingdom) spectrophotometer. The content of ARs was expressed as μg of olivetol per 1 g of grain based on the calibration curve.

Total phenolic compounds content. Phenolic compounds were determined according to the method described by KONOPKA et al. (2015) as later modified. The ground samples were extracted by the use of methanolic (80%, v/v) solution. The extracts were subjected to reaction with the Folin-Ciocalteau reagent. The absorbance of reaction mixture was measured after 60 minutes at 720 nm by using an Unicam UV/Vis UV2 spectrophotometer. The content of phenolic compounds was expressed as μg of D-catechin per 1 g of grain based on the calibration curve.

Statistical analysis. A statistical analysis was carried out with STATISTICA v. 12.5 software (StatSoft, Kraków, Poland). The calculations were made at the level of significance $p \leq 0.05$. The analysis was carried out with Duncan's test for homogeneous groups and with a two-way analysis of variance. Pearson's linear correlation coefficient was also calculated.

Results and Discussion

The results of analysed features are presented in Table 2. It has been shown that FNs varied from 100 to 296 s and were, on average, nearly 1.5 times larger for wheat grain samples grown under the high-input cultivation (224 s) than that for grain obtained by extensive cultivation (146 s). This was

Table 2
The amount of wheat grain soluble phenolic compounds and the results of selected technological quality parameters

Sample number	FN [s]		Phenolic compounds [μg g^{-1}]		ARs [μg g^{-1}]		Grain hardness (NIR)		Wet gluten [%]	
	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD
1	159	3	345	6	317	30	59.40	6.89	17.60	0.28
2	165	2	335	11	316	30	20.25	0.67	18.45	0.35
3	177	5	359	17	321	29	64.25	1.47	17.75	0.35
4	207	4	333	15	271	28	64.10	5.66	20.60	0.28
5	245	1	311	11	256	29	61.05	2.05	24.55	0.07
6	264	4	303	14	290	29	72.15	3.18	25.30	0.28
7	172	5	339	4	284	29	36.70	6.58	18.25	0.07
8	182	2	344	6	316	29	18.35	4.28	20.05	0.07
9	191	1	358	1	308	29	53.65	6.89	18.50	0.14
10	228	3	328	4	286	27	46.35	7.85	22.50	0.14
11	247	3	318	31	305	27	51.10	0.35	24.40	0.71
12	296	3	364	22	324	26	59.80	0.88	27.25	0.07
13	101	3	316	3	263	22	33.84	1.26	16.60	0.42
14	106	4	313	7	269	23	13.05	0.74	16.55	0.07
15	125	1	313	3	249	24	54.35	7.81	16.25	0.35
16	182	1	300	6	224	24	51.16	7.39	20.50	0.14
17	198	4	309	9	251	20	55.50	1.85	23.30	0.28
18	216	6	299	6	249	19	71.60	0.64	24.05	0.21
19	108	4	335	13	267	17	34.02	1.48	16.45	0.49
20	175	1	296	48	291	16	7.45	3.61	19.55	0.21
21	100	1	330	23	295	17	54.05	1.81	16.15	0.35
22	174	1	329	5	267	10	51.18	1.18	19.75	0.21
23	196	1	305	5	272	12	59.80	2.14	22.40	0.14
24	238	4	307	1	274	4	72.05	1.54	24.65	0.49
Cultivar										
Batuta	202 ± 42^{BC}		331 ± 22^{BC}		295 ± 29^{BC}		56.9 ± 18.5^{A}		20.71 ± 3.29^{A}	
Bogatka	219 ± 45^{C}		342 ± 20^{C}		304 ± 25^{C}		44.3 ± 14.9^{A}		21.83 ± 3.41^{A}	
Hybred	154 ± 48^{A}		309 ± 8^{A}		251 ± 17^{A}		46.6 ± 20.4^{A}		19.54 ± 3.41^{A}	
Hymack	165 ± 51^{AB}		317 ± 23^{AB}		278 ± 17^{B}		46.4 ± 22.8^{A}		19.83 ± 3.17^{A}	
Cultivation technology										
Extensive	146 ± 35^{A}		332 ± 22^{B}		291 ± 29^{B}		37.4 ± 19.5^{A}		17.68 ± 1.31^{A}	
High-input	224 ± 35^{B}		317 ± 21^{A}		272 ± 28^{A}		59.6 ± 9.0^{B}		23.27 ± 2.18^{B}	

$^{A–D}$ – denotes statistically significant differences separately between cultivars and cultivation technologies ($p \leq 0.05$), $n = 24$

similar to the results of the study carried out by KINDRED et al. (2005), who showed that the application of nitrogen fertilization increases the falling number in two ways. First, kernels are filled more completely, which reduces empty spaces inside, thereby decreasing the probability of a disruption between the endosperm and the testa. This reduces the amount of α-amylase produced at the places of structure disruptions. Moreover, nitrogen fertilisa-

tion delays crop maturity and extends its dormancy, which reduces the risk of the kernels achieving the incipient or actual post maturity sprouting (KINDRED et al. 2005). Considerable inter-cultivar differences ($p \leq 0.05$) were also observed, the greatest between Bogatka (219 s) and Hybred (154 s).

The values of wet gluten content varied from 16.15 to 27.25%, while the grain hardness values were from 7.45 to 72.15 NIR units (Table 2). Both features were significantly higher for grain cultivated using high-input technology: by ca. 60% and ca. 32%, respectively. According to the scale of relative wheat grain hardness presented by HRUSKOVA and SVEC (2009), grain cultivated using extensive cultivation was classified as medium soft (37–48), while that of high-input cultivation as medium hard (49–60). Variation of these both features was insignificant among used cultivars.

The content of compounds extracted by an alcohol solution (usually 70–80%) and simultaneously reacting with the Folin-Ciocalteu reagent is regarded as the equivalent of soluble phenolic compounds (PASQUALONE et al. 2014). However, it is worth remembering that it may be falsified by aromatic amines, sugars and ascorbic acid which can interfere with this reagent (AINSWORTH and GILLESPIE 2007). In present study content of these compounds changed from 296 to 359 μg g^{-1} of grain (Table 2). These levels were significantly lower (approx. 30%) than those found by KONOPKA et al. (2012) for wheat grown under organic and mineral fertilization regimes. Cited authors claimed that the fertilisation system can induce ca. 20% variation of phenolic compounds content. It was confirmed by present study, since grain from extensive and high-input technology significantly differed in accumulation of soluble phenolic compounds (more abundant was grain from extensive technology).

ARs ranged from 224 to 324 μg g^{-1} of grain (Table 2). Among cultivars, the highest average content was determined in grain of the Bogatka (304 μg g^{-1}) and the lowest in grain of the Hybred cultivar (251 μg g^{-1}). Determined contents were close to the lower limits showed by ANDERSSON et al. (2008) and SHEWRY et al. (2013), who found these compounds in range from 220 to 677 μg g^{-1}. The relatively low amount of ARs in our study may suggest favourable growing conditions, since ARs content is strongly affected by environment stresses, which stimulate a plant to produce compounds with defensive properties (ASAMI et al. 2003, WINTER and DAVIS 2006).

The results of present study indicated the significant differences ($p \leq 0.05$) in the content of the phenolic compounds and ARs between extensive and high-input cultivation technologies. Generally, their concentration was the highest when extensive technology was applied since the limited availability of nitrogen favours the biosynthesis of carbon-containing secondary metabolites (KONOPKA et al. 2012). The cultivation technology had a significant effect on

differentiation of phenolic compounds, however differences between extensive and high-input cultivation did not exceed 7% (ARs – 272 vs. 291 μg g^{-1}; phenolic compounds – 332 vs. 317 μg g^{-1}). Studies carried out by SHEWRY et al. (2013), ANDERSSON et al. (2010) and BELLATO et al. (2012) showed that both the genotype (cultivar) and cultivation conditions (especially the climate) during the phase of grain filling determines the content of ARs. According to ANDERSSON et al. (2010), a warm and dry climate favours the accumulation of ARs in grain. Cited study also demonstrated that there was a negative correlation between the content of ARs and the thousand kernel weight.

Table 3 shows the Pearson correlation coefficients between the parameters under study. Taking into account the whole batch of the samples, no statistically significant correlation was shown to be present between the technological indices (FN, wet gluten, grain hardness) and the content of phenolic compounds and ARs. In contrast, it has been noted high values of correlation coefficient between FN and wet gluten ($r = 0.94$), and among analysed group of polyphenols ($r = 0.59$). We also found that grain hardness was associated with content of wet gluten ($r = 0.50$) and FN ($r = 0.51$). It points that when biopolymers in a kernel are degraded (lower FN and gluten values), endosperm structure becomes looser, which is accompanied by physical changes (a decrease in hardness). Such process takes place, for example, during germination, which damages the endosperm and reduces its hardness (MIŚ and GRUNDAS 2002). Phenolic compounds, released during these processes, may modify grain starch and proteins through making a cross-links and physical complexes, which may affect wet gluten formation (SIVAM et al. 2010) and/or starch gelatinization (PALANI SWAMY and GOVINDASWAMY 2015).

Additional strong relationships were determined within individual cultivars; however, they were not representative for all of the tested samples. For example FN correlated strongly with polyphenols in Batuta ($r = -0.81$) and Hybred cultivars ($r = -0.76$), whereas in the case of other two cultivars such dependence was not observed. This inconsistency may be affected by two contradictory mechanisms (MILDNER-SZKUDLARZ et al. 2011). On the one hand, a higher activity of amylolytic enzymes (lower FNs) causes the release of bound phenolic compounds while, on the other hand, phenolic compounds are capable of inhibiting the activity of amylolytic enzymes. In a study by MILDNER-SZKUDLARZ et al. (2011), the authors showed that breads supplemented with phenols were harder and more gumminess. They suggested that phenols can restrict the activity of amylase, resulting in inadequate maltose for yeast activity during proofing. *In silico* studies suggested that polyphenols may inhibit active site of amylases through hydrogen bonds (WILLIAMSON 2013). This means that, at some point, the increased concentration of these compounds will inhibit further release, which will result in a lack of correlation between the two distinguishing features.

Table 3

Correlation (–) and variation [%] coefficients calculated for the studied parameters

Parameter	Variation coefficient	FN	ARs	Phenolic compounds	Wet gluten content
All samples					
FN	28.22	–	–	–	–
ARs	10.65	0.16	–	–	–
Phenolic compounds	6.99	-0.01	0.59*	–	–
Wet gluten content	16.31	0.94*	-0.01	-0.21	–
Grain hardness	38.13	0.51*	-0.13	-0.01	0.50*
Batuta cv.					
FN	20.58	–	–	–	–
ARs	9.73	-0.70*	–	–	–
Phenolic compounds	6.66	-0.81*	0.68*	–	–
Wet gluten content	15.89	0.98*	-0.71*	-0.88*	–
Grain hardness	30.97	0.55	-0.33	-0.19	0.43
Bogatka cv.					
FN	20.65	–	–	–	–
ARs	8.37	0.27	–	–	–
Phenolic compounds	5.92	0.1	0.37	–	–
Wet gluten content	15.62	0.97*	0.28	-0.01	–
Grain hardness	32.03	0.70*	0.08	0.17	0.55
Hybred cv.					
FN	30.87	–	–	–	–
ARs	6.88	-0.52	–	–	–
Phenolic compounds	2.59	-0.76*	0.55	–	–
Wet gluten content	17.45	0.97*	-0.37	-0.66*	–
Grain hardness	41.69	0.81*	-0.52	-0.57	0.72*
Hymack cv.					
FN	30.67	–	–	–	–
ARs	6.03	-0.19	–	–	–
Phenolic compounds	7.23	-0.48	-0.21	–	–
Wet gluten content	15.99	0.98*	-0.23	-0.46	–
Grain hardness	46.74	0.36	-0.24	0.12	0.48
Extensive cultivation technology					
FN	23.84	–	–	–	–
ARs	10.13	0.57*	–	–	–
Phenolic compounds	6.78	0.4	0.45*	–	–
Wet gluten content	7.38	0.86*	0.49*	0.15	–
Grain hardness	51.06	0.01	0.13	0.48*	-0.4
High-input cultivation technology					
FN	15.62	–	–	–	–
ARs	10.28	0.71*	–	–	–
Phenolic compounds	6.56	0.36	0.64*	–	–
Wet gluten content	9.36	0.90*	0.55*	0.14	–
Grain hardness	14.71	0.33	0.01	-0.25	0.44*

* - statistically significant at $p \leq 0.05$, $n = 24$

It has been found a significant effect of a cultivar on the results of ARs, FNs and the content of phenolic compounds (Table 4), with the greatest impact on content of ARs (46.78% of explained variation). In contrast, the technology of cultivation had the greatest effect on content of wet gluten (71.70% of explained variation), the FNs (56.27% of explained variation), and grain hardness (36.76% of explained variation). Generally, environmental variation is often considered as the primary factor influencing grain end-use quality, but it depends on the genetic variability of the tested genotypes (DENČIĆ et al. 2011). Determined in our study relatively low content of phenolic compounds, accompanied by the low impact of cultivation technology imply that other growth conditions (like climate) were suitable for plants and did not favour accumulation of these compounds (KONOPKA et al. 2012).

Table 4

Results of two way analysis of variance

Specification	Cultivar	Cultivation technology	Cultivar x Cultivation technology	Others
ARs	46.78*	10.15*	8.65*	34.41
Phenolic compounds	32.50*	10.70*	4.33	52.47
FN	26.28*	56.27*	0.35	17.16
Wet gluten content	7.30*	71.70*	0.50	20.60
Grain hardness	7.11	36.76*	2.17	53.97

* – statistically significant at $p \leq 0.05$, $n = 24$

In conclusion it was find that wheat grain produced using high-input cultivation technology is characterized by higher FNs, wet gluten content and hardness what suggest its better baking quality. On the other hand such grain is less abundant in pro-health phenolic compounds compared to grain from extensive cultivation technology.

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DISTRIBUTION AND PHYSIOLOGICAL ACTIVITY OF HETEROTROPHIC BENTHIC BACTERIA IN LAKES WITH DIFFERENT TROPHIC CONDITIONS LOCATED IN THE BORY TUCHOLSKIE NATIONAL PARK (POLAND)*

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K e y w o r d s: benthic bacteria, microbiological activity, bottom sediments, microbial ecology, lakes.

A b s t r a c t

Research on ecophysiology of benthic bacteria indicates that microbiological processes which occur in bottom sediments have a major influence on the development of water bodies. Organic matter accumulated on the bottom makes lakes shallower and accelerates their aging. Microbiological processes are particularly important in small lakes, which are a common element of the environment. This article presents the results of the investigation of the distribution of heterotrophic bacteria in the bottom sediments of four lakes with different trophic conditions in the Bory Tucholskie National Park and the relationship between the number of benthic bacteria and their physiological activity. The total number of benthic bacteria and the ability of isolated strains to carry out selected physiological processes were assessed by cultivating bacteria on appropriate substrates. We also determined the coefficient of physiological activity, which was then used for the correlation analysis and verification of the research hypothesis. The highest number of benthic bacteria was found in the eutrophic and dystrophic lakes. The lowest number was recorded in lake sediments of the mesotrophic lake, although at this site the studied strains were the most active. As a result, the correlation analysis confirmed the hypothesis that there is no relationship between the metabolic potential of benthic bacteria and their number in a sample.

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WYSTĘPOWANIE I AKTYWNOŚĆ FIZJOLOGICZNA HETEROTROFICZNYCH BAKTERII BENTOSOWYCH W ZRÓŻNICOWANYCH TROFICZNIE JEZIORACH PARKU NARODOWEGO „BORY TUCHOLSKIE"

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S ł o w a k l u c z o w e: bakterie bentosowe, aktywność mikrobiologiczna, osady denne, ekologia mikroorganizmów, jeziora.

A b s t r a k t

W badaniach nad ekofizjologią bakterii bentosowych prowadzonych na przestrzeni lat potwierdzono, że procesy mikrobiologiczne zachodzące w osadach dennych mają fundamentalny wpływ na kierunek kształtowania się zbiorników wodnych. Zdeponowane na ich dnie substancje organiczne przyczyniają się do wypłycania i starzenia się zbiorników jeziornych. Procesy te mają szczególne znaczenie w kontekście niewielkich jezior, które stanowią bardzo liczny i ważny komponent środowiska naturalnego.

W artykule zaprezentowano wyniki badań podjętych w celu ilościowej oceny występowania bakterii heterotroficznych w osadach zróżnicowanych troficznie jezior położonych w Parku Narodowym „Bory Tucholskie" oraz wpływu liczebności populacji bakteriobentosu na ich aktywność fizjologiczną. Ogólną liczbę bakterii bentosowych oraz zdolność wyizolowanych szczepów do przeprowadzenia wybranych procesów fizjologicznych oznaczono metodami hodowlanymi na podłożach testowych. Wyznaczono ponadto wskaźnik aktywności fizjologicznej, który umożliwił wykonanie analizy korelacji i weryfikację hipotezy badawczej. Na podstawie analiz największą liczebność bakterii bentosowych stwierdzono w jeziorze eutroficznym oraz dystroficznym. Najmniejszą liczbę bakterii odnotowano w osadach jeziora mezotroficznego, jednakże na stanowisku tym analizowane szczepy najintensywniej przeprowadzały procesy fizjologiczne. W konsekwencji w analizie korelacji potwierdzono hipotezę o braku zależności potencjału metabolicznego szczepów bakterii bentosowych od ich liczebności w badanej próbie.

Introduction

Heterotrophic bacteria constitute one of the largest and most important groups of microorganisms in aquatic environments. Due to their different physiological properties and ability to degrade organic compounds, they play a key role in self-purification of lakes. Their number, growth and changes in population size and structure reflect the current condition of water bodies (DONDERSKI and KALWASIŃSKA 2003). Bottom sediments make an integral part of these ecosystems, offering habitat for many aquatic organisms (MADEYSKI and TARNAWSKI 2006). Being an important source of nutrients, they participate in regulating the biogeochemical cycle in the environment (KALINOWSKI and ZAŁĘSKA-RADZIWIŁŁ 2009). Researchers around the world have attempted to define the role of benthic bacteria in degradation and transformation of organic matter (MUDRYK et al. 2000, KREVS et al. 2006, SUSLOVA et al. 2009,

KIERSZTYN et al. 2012). On the one hand, there is a widely held belief that the content and availability of organic nutrients have a major impact on the distribution of bacterial populations in aquatic environment (KALINOWSKA et al. 2013). CHRÓST and SIUDA (2006) proved that higher numbers of aquatic bacteria are found in hypertrophic and eutrophic lakes, and lower, in oligotrophic. On the other hand, it has been argued that the trophy of a particular water body has little influence on the number of bacteria in bottom sediments. Since the exoenzymes of bacteria display different activity, the rate and type of degradation in sediments are lake-specific (WOBUS et al. 2003, MIELNIK 2005).

The existing information gap and little scientific concern for the problem inspired the authors to undertake a study whose main objective was to determine the relationship between the number and physiological activity of benthic bacteria in lakes of different trophic conditions. Taking into account current scientific reports and our own observations we put forward a hypothesis that the relationship of this kind does not exist.

Materials snd Methods

Object of the study

The study was conducted in four lakes with different trophic conditions, located in the Bory Tucholskie National Park. The trophic status of each lake was established by park authorities. Three of these lakes are legally protected. The morphometric parameters and the location of research sites are presented in Table 1.

Table 1

Selected parameters of the research sites

Lake name	Number of site	Trophic level	Area [ha]	Depth [m]	Location
Mielnica	I	Eutrophic	11.3	1.0	N 53°48.396' E 017°31.171'
Olbrachta*	II	Mesotrohic	2.46	3.5	N 53°48.289' E 017°31.717'
Głuche*	III	Oligotrophic	3.3	8.0	N 53°49.265' E 017°32.635'
Rybie Oko*	IV	Dystrophic	0.2	3.5	N 53°48.828' E 017°32.315'

* protected lakes

Sampling

Samples were collected from a surface layer of bottom sediments (up to 10 cm deep), using a tube water sampler, from the deepest part of the studied lakes. All samples were transported to the laboratory in sterile glass containers at ± 6°C, and examined immediately. The research was conducted in a seasonal cycle from summer 2014 to spring 2015. Triplicate sediment samples were collected at each research site. In situ physico-chemical parameters of water collected from above the bottom sediments were also measured at all research sites (Table 2). There were measured parameters such as temperature, pH, electrolytic conductivity and oxygen concentration.

Table 2

Correlations between physico-chemical parameters of water collected from above the bottom sediments and the number of heterotrophic benthic bacteria

Parameter	Correlation (r)	*p*
Temperature [°C]	-0.73	> 0.05
pH	0.2	
Electrolytic conductivity [μS cm^{-1}]	0.44	
Oxygen concentration [mg dm^{-3}]	0.63	

Determining the number of heterotrophic bacteria

We determined the total number of heterotrophic bacteria (CFU) in the samples of sediment using the spread plate method according to *Jakość wody*... PN-EN ISO 8199:2010. Before inoculation we diluted the samples in sterile peptone saline (0.85% aqueous NaCl solution with 1 g of peptone/l). Next, we prepared inoculations of 100 fl of each sample on sterile nutrient agar (three parallel repetitions). The plates were incubated at +20°C for 72 hours. After that we counted grown colonies using the formula from the above ISO standard, and calculated the results per gram of dry mass. Some colonies (pure cultures) were preserved for further analysis. We isolated of 25 strains.

Physiological properties

To assess the ability of benthic bacteria to degrade selected organic compounds we inoculated them on media enriched with suitable organic polymers. We used of 25 strains from each research site. To examine the degradation of each compound, we supplemented the media accordingly with tributyrin (fats), casein (proteins), and starch, colloidal chitin, and

carboxymethylcellulose (sugars). After the incubation, some plates were covered with suitable reagents in order to read the results. The result was considered positive when there was a hydrolysis zone around the colony. The following were also tested: the ability of the studied strains to ammonify, to reduce nitrats to nitrites, and to produce hydrogen sulphide (LALKE-PORCZYK and DONDERSKI 2005).

Coefficient of physiological activity

Based on the results of physiological processes we determined the coefficient of physiological activity of heterotrophic bacteria (FA_{HB}) according to the formula proposed by DAHLBÄCK et al. (1982):

$$FA_{HB} = \frac{\sum_{i=1}^{n} a_i}{n}$$

where:
a_i – the percentage of strains exhibiting specific physiological properties
n – number of physiological tests.

Statistical

STATISTICA 12 software was used for statistical analysis of the results. The analysis of the differences between the sets of data and the comparison of independent factors was prepared using ANOVA analysis of variance or a non-parametric test (Kruskal-Wallis). Tukey test (post-hoc) was used to determine the differences within the sets of data. Pearson correlation coefficient was determined in order to analyze the relationship between the obtained values. All statistical tests were carried out at $p < 0.05$.

Results

The number of heterotrophic bacteria in the bottom sediments of the investigated lakes is presented in Figure 1. The highest average number of benthic bacteria was recorded at research site I, i.e. in the eutrophic lake ($1.61 \cdot 10^6$ CFU g^{-1} dry weight) and site IV, i.e. in the dystrophic lake ($1.51 \cdot 10^6$ CFU g^{-1} dry weight). The number of benthic bacteria was consider-

ably lower at the sites located in the meso- and the oligotrophic lake. Benthic bacteria were the most numerous in summer ($2.45 \cdot 10^6$ CFU g^{-1} dry weight on average). Taking into account an average of all investigated lakes, the lowest number of these bacteria was noted in winter ($0.35 \cdot 10^6$ CFU g^{-1} dry weight on

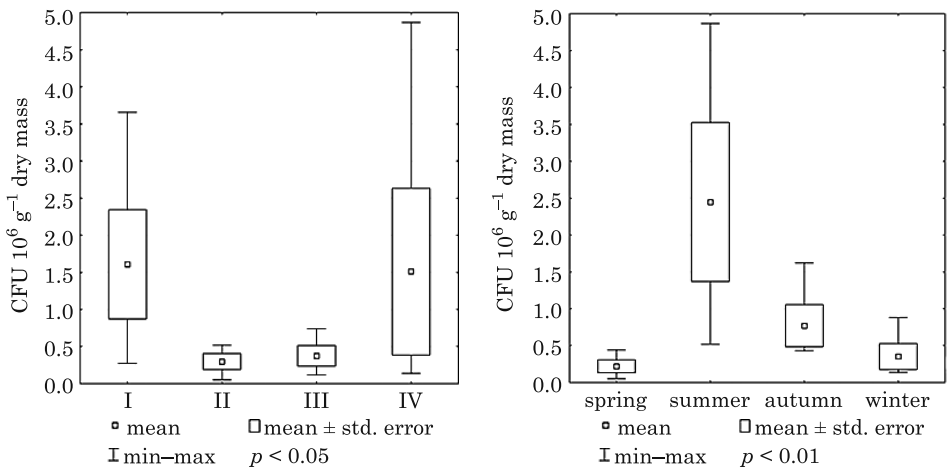

Fig. 1. Number of heterotrophic benthic bacteria at the 4 research sites in all seasons and in the research seasons at all sites: I – eutrophic lake, II – mesotrohic lake, III – oligotrophic lake, IV – dystrophic lake

average). The analysis of variance revealed statistically significant differences in the number of benthic bacteria between the research sites and seasons ($p < 0.05$). Table 3 show that differences in the number of benthic bacteria between lakes was mainly statistically insignificant. In an annual cycle the temperature and the number of benthic bacteria had the strongest negative correlation ($r = -0.7$), compared to the remaining physico-chemical parameters of water from above the sediment) However, this result was not statistically significant (Table 2). Table 4 presents the percentage of benthic strains involved in particular physiological processes. Some repeatibility of the results was observed for ammonification: all studied strains had this ability. Regardless of the season and research site, only a small percentage of the strains had the ability to depolimerize chitin. The other physiological processes had different intensity. On the basis of the presented results we determined the coefficient of physiological activity of heterotrophic bacteria. Figure 2 shows that the highest activity of benthic strains was recorded at research site II, i.e. in the mesotrophic lake, the lowest, in the lake with a low content of organic substances (site III). Moreover, the only statistically significant differences ($p < 0.05$) were noted between these two sites. The physiological activity of the

bacteria was not significantly correlated with the season. The highest was observed in autumn, and slightly lower, in spring and summer. In winter physiological activity of the analyzed strains decreased considerably. On the basis of the above results we determined the correlation between the number and activity of heterotrophic benthic bacteria. The low correlation coefficient together with the lack of statistical significance suggests that the metabolic potential of the studied bacteria does not depend on their number in the sample (Figure 3).

Table 3
Statistical differences in the number of heterotrophic benthic bacteria between the groups of data

	I	II	III	IV	Spring	Summer	Autumn	Winter
I								
II	*							
III	ns	ns						
IV	ns	ns	ns					
Spring								
Summer					**			
Autumn					*	ns		
Winter					ns	**	ns	

Explanation: I – eutrophic lake, II – mesotrohic lake, III – oligotrophic lake, IV – dystrophic lake
ns – not significant; * $p < 0.05$; ** $p < 0.01$

Table 4
Physiological properties of benthic bacteria: *P* – proteolytic; *L* – lipolytic; *A* – amylolytic; *C* – cellulolytic; Ch – chitinolytic; Am – ammonification; Ni – nitrates reduction; H_2S – hydrogen sulfide production

Season	Sites	Physiological group of bacterial strains [%]							
		P	*L*	*A*	*C*	Ch	Am	Ni	H_2S
Spring	I	36	32	40	44	8	100	44	68
	II	76	80	72	64	4	100	20	16
	III	36	44	32	36	16	100	24	64
	IV	44	60	52	28	0	100	88	56
Summer	I	36	40	40	44	8	100	48	64
	II	72	76	76	68	4	100	16	20
	III	32	40	36	36	12	100	24	68
	IV	52	84	52	32	4	100	56	40
Autumn	I	32	72	44	44	8	100	56	68
	II	88	88	80	72	8	100	12	36
	III	32	40	36	36	8	100	24	68
	IV	52	100	56	32	4	100	28	24
Winter	I	16	20	36	24	0	100	16	40
	II	72	64	68	52	4	100	8	28
	III	24	32	32	20	4	100	0	20
	IV	48	56	36	20	0	100	0	64

Explanation: I – eutrophic lake, II – mesotrohic lake, III – oligotrophic lake, IV – dystrophic lake

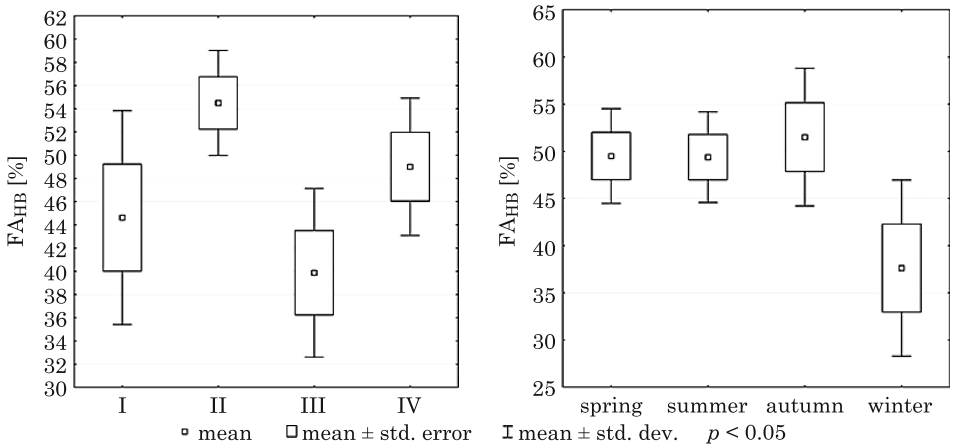

Fig. 2. Physiological activity of heterotrophic benthic bacteria: I – eutrophic lake, II – mesotrohic lake, III – oligotrophic lake, IV – dystrophic lake

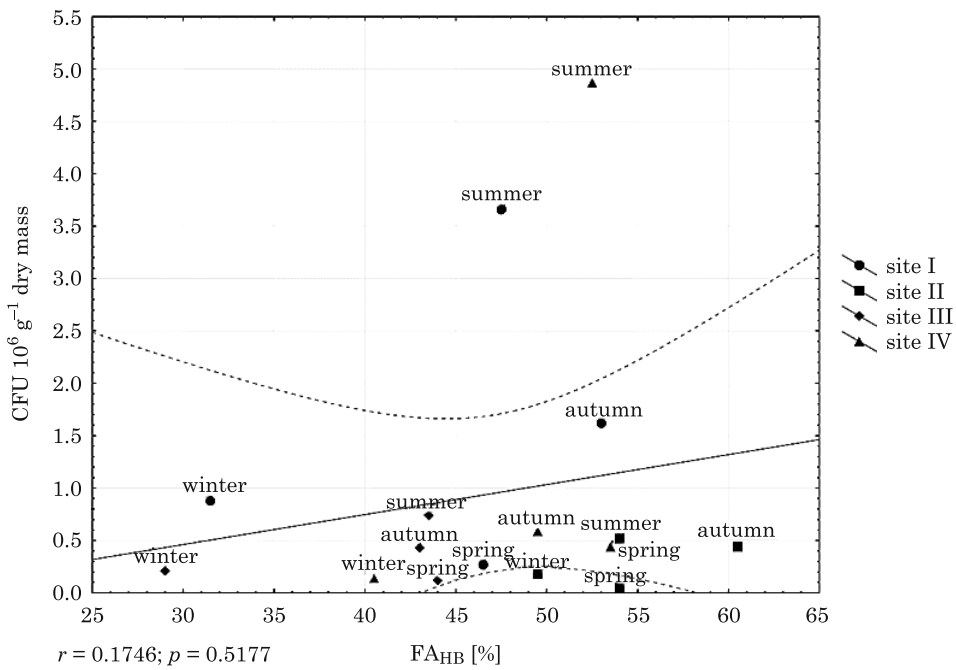

Fig. 3. Correlation between physiological activity and the number of heterotrophic benthic bacteria: site I – eutrophic lake, site II – mesotrohic lake, site III – oligotrophic lake, site IV – dystrophic lake

Discussion

Due to physico-chemical and biological factors water bodies undergo dynamic changes. Microbiological processes observed in bottom sediments play a key role in shaping these aquatic ecosystems (KALWASIŃSKA and DONDERSKI 2005). Abundance is one of the basic parameters characterizing the microbial population in the natural environment. It may determine the fertility of the given ecosystem (i.e its trophic status) to a large extent. This observation is reflected in the results of this study, as we recorded the highest number of heterotrophic bacteria in the sediments of the eutrophic lake. Much lower numbers were recorded in the meso- and the oligotrophic lake. PORTER et al. (2004) also confirmed a relationship between the total number of planktonic bacteria and the trophic status of lakes. A high number of benthic bacteria was noted in the bottom sediments of a dystrophic lake with low pH owed to the presence of humic acids. Low water pH normally limits bacterial growth in aquatic environments. This observation was confirmed by LINDSTRÖM (2000) investigating five lakes with different trophic conditions in Sweden. He recorded higher bacterioplankton biomass in lakes with higher pH values. On the other hand, humic acids are susceptible to microbiological degradation, and can stimulate the activity of heterotrophic bacteria (MUNSTER et al. 1992). Organic polymers of high molecular weight such as proteins, fats and carbohydrates are common in aquatic systems. These compounds cannot be directly assimilated by microbial cells. In order to depolymerize macromolecular compounds and to release monomers ready for assimilation, bacteria regulate the synthesis and activity of specific hydrolytic exoenzymes (UNANUE 1999). Our research into the microbial decomposition of organic compounds indicates that lipolytic bacteria were dominant in the sediments of the investigated lakes. On average 58% of strains had the ability to hydrolyze tributyrin. Lipid compounds constitute one of the dominant fractions of organic matter in aquatic environments. They offer an important energy substrate for bacteriocenosis. According to many authors, dead and alive zooplankton and phytoplankton are the main source of lipid compounds in both saltwater and freshwater ecosystems (ARTS et al. 1992, GAŠPAROVIĆ et al. 2007). Chitinolytic bacteria were the least numerous among bacteria hydrolyzing organic compounds in the bottom sediments of the studied lakes. Their average share in the studied isolates was only 5.75%. POULICEK and JEUNIAUX (1991) noted that chitin level is lower in the sediments than in water of lakes. This may account for a small percentage of strains capable of degrading this polysaccharide in the studied sediments. Similar results were also obtained by SWIONTEK-BRZEZINSKA et al. (2008), who analyzed the activity of chitynolitic strains in lake Chełmżyńskie. Among the bacteria isolated from this water body they

noted 11–19% of chitinase-producing strains. Only 3–8% of benthic strains had this ability. Many capacities of bacteria to carry out physiological processes may determine the ecological success of water bodies. The highest physiological activity was observed among strains isolated from the sediments of the mesotrophic lake (54.5%), the lowest, in the oligotrophic lake (39.9%). The latter result can be caused by the low content of nutrients which could be transformed microbiologically. Our results correspond with those obtained by LALKE-PORCZYK et al. (2004), who recorded 40–49% physiological activity of epiphytic bacteria in Lake Chełmżyńskie. The highest FAHB cofficient was noted in spring. The studied benthic strains were more active in autumn (51.5%) than in spring (49.7%).

Conclusions

As has been already mentioned, the availability of substrates determines to a large extent the development of aquatic microbiomes. However, bacterial activity depends on other factors as well. Our results indicate that throphic conditions influence the physiological activity of bacteria: statistically significant differences were recorded between the mesotrophic and the oligotrophic lake. There was no correlation between physiological activity of benthic bacteria and their number in a sample.

Due to the fact that a range of physico-chemical and biological factors make water bodies one of the most dynamically changing natural environments, our results may not be valid for all lake ecosystems. However, they led to positive verification of the initial hypothesis.

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IMPACT OF MICROWAVE HEATING ON THE EFFICIENCY OF METHANE FERMENTATION OF ALGAE BIOMASS*

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K e y w o r d s: microalgae, microwave radiation, biomass, methane fermentation, biogas.

A b s t r a c t

Providing the optimal temperature is a means of increasing the effectiveness of methane fermentation processes. The use of an electromagnetic microwave field enables energy to be directed to a mixture of anaerobic sludge and processed biomass this reduces energy losses.

The aim of this study was to determine the effect of electromagnetic microwave radiation in stimulating thermal conditions in anaerobic reactors, on the effectiveness of methane fermentation process of microalgae biomass and on the qualitative composition of biogas produced. The quantity of gaseous metabolites of anaerobic bacteria produced in both experimental variants (convective and microwave heating) averaged approximat's 450 cm^3 g^{-1} VS. The electromagnetic microwave radiation proved to have an immediate impact on the improvement in the qualitative composition of biogas produced. The stimulation of thermal conditions using electric heaters resulted in a methane contetnt of 65% in biogas, whereas the use of microwaves assured ca. 69% in sewage gas.

WPŁYW OGRZEWANIA MIKROFALOWEGO NA EFEKTYWNOŚĆ FERMENTACJI METANOWEJ BIOMASY GLONÓW

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S ł o w a k l u c z o w e: mikroalgi, promieniowanie mikrofalowe, biomasa, fermentacja metanowa, biogaz.

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Abstrakt

Zapewnienie optymalnej temperatury jest sposobem na zwiększenie efektywności procesów fermentacji metanowej. Użycie promieniowania mikrofalowego umożliwia bezpośrednie skierowanie energii do mieszaniny osadu beztlenowego i przetwarzanej biomasy, co znacznie ogranicza straty energii.

Celem badań było określenie wpływu zastosowania elektromagnetycznego promieniowania mikrofalowego jako czynnika stymulującego warunki termiczne w reaktorach beztlenowych na efektywność procesu fermentacji metanowej biomasy mikroglonów i skład jakościowy wytwarzanego biogazu. Ilość produkowanych gazowych produktów metabolizmu bakterii beztlenowych w obu wariantach eksperymentalnych wynosiła średnio (konwekcyjne i mikrofalowe ogrzewanie) 450 cm^3 g s.m.o.$^{-1}$. Udowodniono, że elektromagnetyczne promieniowanie mikrofalowe wpłynęło bezpośrednio na poprawę składu jakościowego uzyskiwanego biogazu. Stymulowanie warunków termicznych za pomocą grzałek elektrycznych pozwoliło na uzyskanie prawie 65% metanu w biogazie, natomiast wykorzystanie mikrofal zapewniło około 69% zawartość tego komponentu gazu fermentacyjnego.

Introduction

The rate of biochemical degradation of organic matter depends on the activity of microorganisms or – to be more specific – on the rate of enzymatic reactions. In turn, the activity of enzymes is determined by environmental factors, including process temperature. For this reason, providing the optimal temperature is a means of increasing the effectiveness of methane fermentation processes (LANGE and AHRING 2001, TAKASHIMA et al. 2006).

The thermal conditions inside anaerobic reactors may be effectively controlled via microwave radiation. The use of an electromagnetic microwave field enables energy to be directed to a mixture of anaerobic sludge and processed biomass (the body absorbing the radiation). This reduces energy losses as a result of absorption by structured elements of the reactor (HA et al. 2011). Our earlier study demonstrated a significant increase in the effectiveness of organic matter degradation and methanogenesis in a microwave-modified system (CYDZIK-KWIATKOWSKA et al. 2012). Genetic studies carried out in systems with conventional heating and with electromagnetic microwave radiation have shown significant changes in the species structure of microorganisms that are affected by the microwave radiation (ZIELIŃSKI and ZIELIŃSKA 2010). Results achieved so far seem to confirm theories of non-thermal effects of microwaves, as changes were observed not only in the activity of particular enzymes but also in entire bacterial populations. These findings are the grounds for investigations aimed at determining the effects of thermal and athermal properties of microwaves on the effectiveness of methane fermentation of typical energetic plants used in agricultural biogasworks (ZIELIŃSKI et al. 2007, ZIELIŃSKI et al. 2009, ZIELINSKI and KRZEMIENIEWSKI 2007).

The aim of this study was to analyze the effect of an electromagnetic microwave radiation, as a factor stimulating thermal conditions in anaerobic reactors, on the effectiveness of methane fermentation process of microalgae biomass and on the qualitative composition of biogas produced.

Experimental procedures

Study design

The study was divided into two experimental stages with different methods of assuring appropriate thermal conditions in the reaction tanks. In stage I, the reactors were placed in a thermostating cabinet in which thermal conditions were provided by electric heaters. In stage II the cabinet was replaced by an electromagnetic microwave field.

Materials

The microalgae that constituted the substrate for the fermentation process originated from the waters of the Vistula Lagoon. The biomass was separated and concentrated with the use of a fractional-technical scale instalation. Before the algae biomass was fed into the anaerobic reactors, the material was concentrated in a laboratory centrifuge. The physicochemical characteristics of the algae biomass used in the study is presented in Table 1.

Table 1

Characteristics and properties of microalgae biomass used in the study

Characteristic	Unit	Mean value	Standard deviation
Dry matter	[%]	10.40	1.49
Organic dry matter	[% TS]	87.69	1.06
Dry mineral matter	[% TS]	12.31	1.06
Total nitrogen	[mg g^{-1} TS]	45.97	3.92
Total phosphorus	[mg g^{-1} TS]	4.36	0.94
TC	[mg g^{-1} TS]	463.82	25.31
TOC	[mg g^{-1} TS]	437.26	19.77
C:N ratio	–	9.51	0.43
Protein	[% TS]	28.73	2.45
Lipids	[% TS]	19.96	1.39
Saccharides	[% TS]	15.84	2.55
pH	8.82–7.03		

The microalgae biomass analyzed in the study was harvested from June till August. The species composition of obtained microalgae was determined with optical microscope equipped with camera MF346 Optech 3MP.

Qualitative analyses of samples of phytoplankton biomass collected in this period demonstrated that the *Cyanoprokaryota* was the predominant taxonomic group (82.6 ± 7.4% of all algae). Taxonomic diversity in the Vistula Lagoon was also affected by algae of the *Chlorophyta* group (11.1 ± 4.7%) and *Bacillariophyceae* (4.7 ± 2.3%). The most abundant species of blue-green algae included *Aphanizomenon flos-aqae* and *Microcystis sp*. Species of other planktonic algae (*Pyrrophyta*, *Euglenophyta*, *Chrysophyta*: *Chrysophyceae*) were in the minority, and their combined contribution did not exceed 1.6 ± 0.4% of all algae.

Anaerobic sludge used as the inoculum of anaerobic reactors in the experiments originated from the closed fermentation tanks of the Municipal Wastewater Treatment Plant "Łyna" in Olsztyn. Its characteristics are presented in Table 2.

Table 2

Characteristics of anaerobic sludge used in the study

Characteristic	Unit	Mean value	Standard deviation
Dry matter	[%]	3.81	0.21
Organic dry matter	[% TS]	68.46	2.53
Dry mineral matter	[% TS]	31.54	2.53
Total nitrogen	[mg g^{-1} TS]	33.08	3.35
Total phosphorus	[mg g^{-1} TS]	1.66	0.23
TC	[mg g^{-1} TS]	309.05	28.37
TOC	[mg g^{-1} TS]	199.42	34.29
C:N ratio	–	9.34	0.08
Protein	[% TS]	20.67	2.77
Lipids	[% TS]	3.12	0.51
Saccharides	[% TS]	1.57	0.36
pH	7.52–6.89		

Experimental equipment

Experiments were conducted using anaerobic reactors with full mixing and an active volume of 4.0 dm^3 (total volume – 5.0 dm^3). The initial concentration of anaerobic sludge in the tanks was kept at ca. 4.0 g TS dm^{-3}. Different heating systems were used depending on the stage of the study, i.e., stage I, electric heaters, and stage II, electromagnetic microwave radiation. A magnet-

ron served as the source of microwave radiation. Radiation was transmitted from the magnetron via a wave-guide to a cabinet with fermentation tanks. The microwave generator (Plazmatronika) applied in the study has a fluent power control that ranges from 0 to 600 W. The frequency of the radiation was 2.45 GHz. The heating systems were controlled via a thermal controller that responded immediately to indications of temperature sensors located inside the reactors. Methane fermentation was conducted at 35°C, with a hysteresis of ± 1°C. The process of anaerobic degradation of organic substrates was run under conditions of mesophilic fermentation, with a load of 2.0 kg VS dm^{-3} d and a hydraulic retention time of 20 days. A scheme of the experimental set-up is presented in Figure 1.

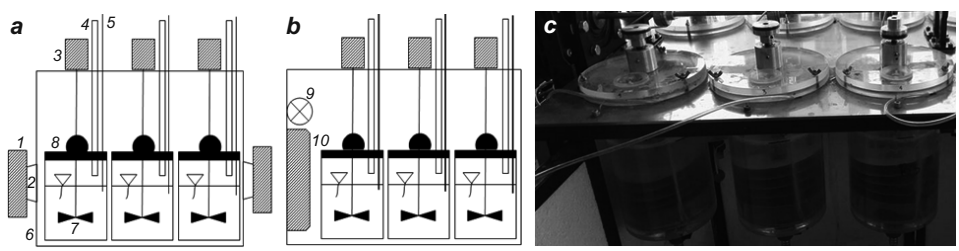

Fig. 1. Design of experimental equipment: *a* – microwave heating; *b* – convective heating (*1* – magnetron, *2* – wave-guide, *3* – stirrer drive, *4* – pressure measurement, biogas collection, *5* – temperature sensor, *6* – steel cabinet, *7* – stirrer, *8* – model fermentation tanks, *9* – ventilator, *10* – heater)

Analytical methods

The substrat composition was determined before and after the anaerobic digestion. The content of dry matter, organic dry matter and mineral dry matter was determined gravimetrically. In samples of biomass dried at 105°C, the content of total carbon (TC) was determined as well as, total organic carbon (TOC) and total nitrogen (N_{tot}). Analyses were carried out with a Flash 2000 elementary particle analyzer (Thermo company). Total phosphorus (P_{tot}) was assayed with the colorimetric method with ammonium(V) metavanadate and ammonium molybdate after sample mineralization in a mixture of sulfuric(VI) acid and chloric(VII) acid at a wavelength of 390 nm using a DR 2800 HACH Lange spectrophotometer. The content of total protein was determined by multiplying the content of N_{tot} by a protein conversion factor (x 6.25). The content of reducing sugars was measured with the colorimetric method with an anthrone reagent at a wavelength of 600 nm using the DR 2800 HACH Lange spectrophotometer. The concentration of lipids was determined with the

Soxhlet method, using Buchi extraction apparatus, whereas the pH of water was measured with a potentiometer.

Temporary and total flow of biogas from the continuous reactors was measured using a flow meter (Allborg SS-Body company). The composition and percentage contents of particular components of biogas were analyzed daily with the use of a GC Agillent 7890 A gas chromatograph.

Statistical analysis

The statistical analysis of experimental results was conducted using a STATISTICA 10.0 PL package. The hypothesis normality of distribution of each analyzed variable was verified using the W Shapiro-Wilk test. One-way analysis of variance (ANOVA) was conducted in order to determine differences between variables. Homogeneity of variance in groups was determined using a Levene test. The Tukey (HSD) test was applied to determine the significance of differences between the analyzed variables. In all tests, results were considered significant at $\alpha = 0.05$.

Results

The algae biomass used in the study was characterized by a organic matter content of 87.69 ± 1.06%, and TC and TOC of 463.82 ± 25.31 mg g^{-1} TS and 437.26 ± 19.77 mg g^{-1} TS, respectively. The mean content of N_{tot} was 45.97 ± 3.92 mg g^{-1} TS, whereas that of P_{tot} was around 4.36 ± 0.94 mg g^{-1} TS. In the analyzed biomass, the C:N ratio – being of great significance to the methane fermentation process – was very low, at 9.51 ± 0.43 on average (Table 1). The content of total protein, lipid substances and carbohydrates in the mixed culture of microalge was 28.73 ± 2.45% TS, 19.96 ± 1.39% TS and 15.84 ± 2.55% TS, respectively.

In stage I, during the period of stable operation, biogas production was 440.41 ± 8.45 cm^3 g^{-1} VS and methane content averaged 65.20 ± 2.04%, (Figure 2, Figure 3). Total production of biogas was 3523.28 ± 67.6 cm^3 d^{-1}, wherein methane was 2297.18 ± 71.87 cm^3 d^{-1}.

In stage II, during the period of stable operation biogas production was 453.11 ± 9.09 cm^3 g^{-1} VS (Figure 2). Total biogas production was higher than in stage I and amounted to 3624.88 ± 72.72 cm^3 d^{-1}. The use of microwave radiation gave a significantly higher concentration of methane, it was on average 68.78 ± 2.03% (Figure 3). Biogas production rate increased from 9.16 cm^3 g^{-1} VS d^{-1} to 21.2 cm^3 g^{-1} VS d^{-1} during the use of electromagnetic microwave radiation in stage II.

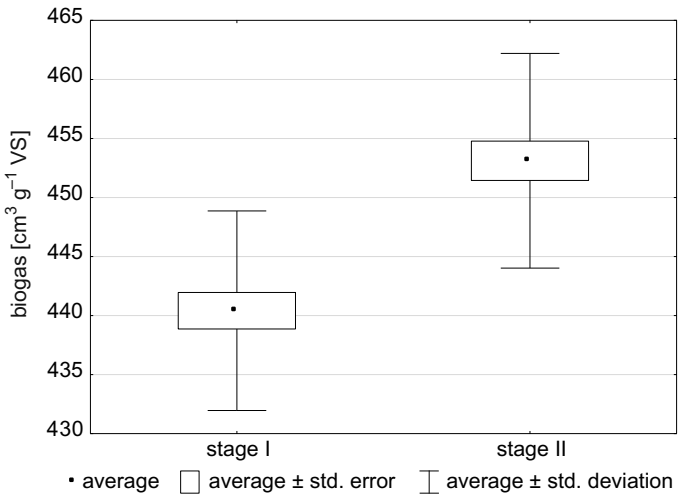

Fig. 2. The percentage content of biogas production after process of anaerobic sludge adaptation

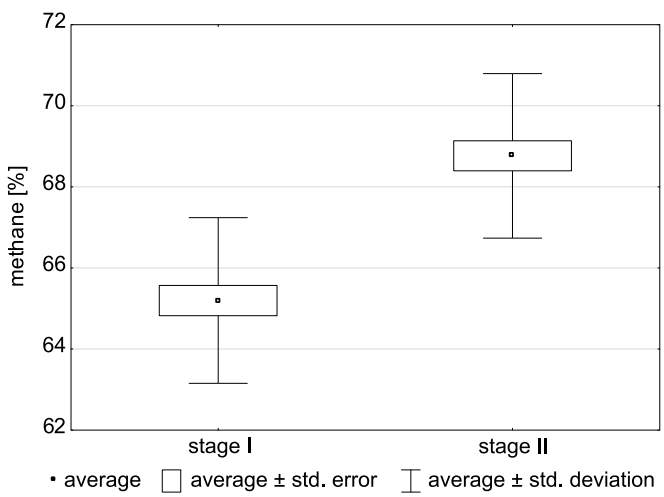

Fig. 3.The percentage content of methane production after process of anaerobic sludge adaptation

The study demonstrated that the use of electromagnetic microwave radiation (stage II of the study) had a direct impact on faster adaptation of the technological system and achieved greater stability of the methane fermentation process. The anaerobic sludge used as the inoculum originated from closed fermentation tanks of a municipal wastewater treatment plant, hence its metabolism was adjusted for sewage sludge degradation. Efficient algae biomass conversion to biogas required a period of sludge adaptation and its metabolism adjustment to a new substrate – in this case: the microalgae

biomass. The heating of the technological system with microwave radiation produced stable effects of biogas production as early as 30 days after exploitation (Figure 4). During stage I with convective heating, the process of anaerobic sludge adaptation lasted twice as long and stable effects of biogas production were achieved after nearly 60 days (Figure 4). The study also demonstrated that the impact of the physical factor was reflected more in a equalized concentration of methane in biogas (Figure 5, Figure 3).

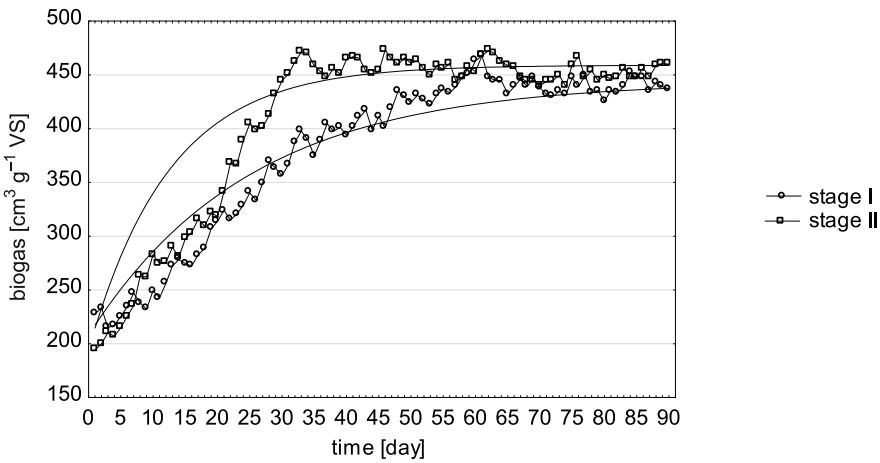

Fig. 4. The biogas production depending on the applied method of heating model fermentation tanks

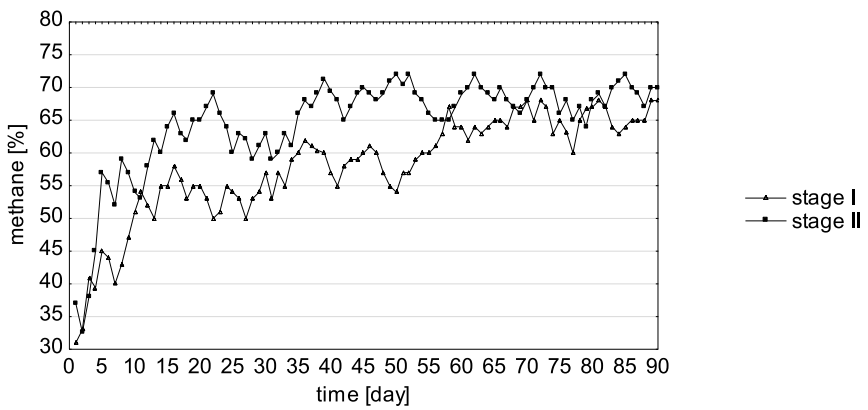

Fig. 5. The percentage content of methane in biogas depending on the applied method of heating model fermentation tanks

Discussion

The literature provides extensive descriptions of the use of microwave radiation in chemical syntheses processes (KAPPE 2002, LIDSTRÖM et al. 2001, OLSSON and JUSLIN 2000, VASS et al. 1999). Microwave heating increased production and quality of biogas from algae biomass. The impact of electromagnetic energy always involves energy transfer and usually leads to an increase of temperature. The term "athermal" refers to effects of microwaves specific to electromagnetic energy and not observed in the case of conventional heating (MUSSGNUG et al. 2010). For instance, PARKER et al. (1996) investigated the effect of microwaves on the activity of the hydrated enzyme – lipase. The rate of the enzymatic reaction heated with microwaves was seen to increase maximally several times more compared to conventional heating.

BANIK et al. (2006) analyzing biological effects of microwave radiation, point to, among other things, a positive impact of the radiation on the process of methane fermentation. The exposure of *Methanosarcina barkei* – DSM 804 bacteria to high frequency radiation between 13.5 GHz to 36.5 GHz produced an increased volume of biogas and increased methane concentration in biogas.

In the presented experiment, a significantly faster adaptation of the technological system was observed, which resulted in stable effects of the fermentation process, and an equalized concentration of methane. It confirms findings of ZIELIŃSKI et al. (2007) regarding the impact of microwave radiation on the activity of a biofilm in reactors with immobilized biomass. In those cases significant enhancement was observed in the activity of microorganisms exposed to microwaving. Significantly increased process efficiency was achieved under identical technological conditions especially in the case of nitrifying activity. At a temperature difference between the control reactor and the irradiated reactor not exceeding 3°C, the yield of the process increased by ca. 30%.

The mean level of biogas production in the present study was at 453,11 $cm^3\ g^{-1}$ VS. Comparable values were reported by De Schamphelaire and Verstraete (DE SCHAMPHELAIRE and VERSTRAETE 2009) in their research on the effectiveness of biogas production from mixed cultures of freshwater microalgae. The initial loading of periodical fermentation tanks at 0.6 g VS $dm^{-3}\ d^{-1}$ enabled methane production of 310 $cm^3\ CH_4\ g^{-1}$ VS after 45 days of biomass retention in the technological system. Samson and LeDuy (SAMSON and LEDUYT 1986) fermented biomass of blue-green algae (*Spirulina maxima)* in a reactor at a loading of 1.0 g VS $dm^{-3}\ d^{-1}$ and retention time of 33 days. They noted that methane production in the mesophilic process was 240 $cm^3\ CH_4\ g^{-1}$ VS

on average, and they observed 68.0% – 72.0% effectiveness of organic biomass degradation. RAS et al. (2011) obtained a biogas production yield of 150 cm^3 CH_4 g^{-1} VS and 240 cm^3 CH_4 g^{-1} VS at 16 days and 28 days hydraulic retention time, respectively. This experiment was conducted with biomass of *Chlorella vulgaris*, and fermentation tanks loading at a level of 1.0 g COD dm^{-3} d^{-1}. The effectiveness of biomass conversion to gaseous metabolites of anaerobic bacteria ranged from 29.0% to 49.0%.

In the presented experiment, the COD:N ratio reached 9.51 ± 0.43, however the content of total protein in the case of the mixed culture of microalgae reached ca. 28%. Similar observations were made by ZAMALLOA et al. (2012). In the case of testing biomass of *Spirulina platensis*, the COD:N ratio was higher and reached 10.3 ± 0.6. It was due to a very high contribution of proteins in biomass – reaching up to 60%. For two other analyzed species of algae the ratios of carbonic substances expressed by the COD value to nitrogen were comparable, i.e. 15.6 ± 7.4 for *Scenedesmus obliquus* and 13.4 ± 3.4 for *Phaeodactylum tricornutum*.

The potential of biogas production was proved to be directly determined by species, but no correlation was found between the taxonomic groups and process effectiveness. The fermentation of *Chlamydomonas reinhardtii* algae from the phylum *Chlorophyta* resulted in biogas production at a level of 587 ± 8.8 cm^3 g^{-1} VS , whereas in the case of *Dunaliella salina* – at a level of 505 ± 24.8 cm^3 g^{-1} VS. Anaerobic processes applied to blue-green algae *Arthrospira platensis* and *Euglena gracilis* resulted in production effectiveness of gaseous metabolites of fermentation bacteria reaching 481 ± 13.8 cm^3 g^{-1} VS. and 485 ± 3.0 cm^3 g^{-1} VS, respectively. Finally, biogas production from *Chlorella kessleri* and *Scenedesmus obliquus* algae was the lowest and accounted for 335 ± 7.8 cm^3 g^{-1} VS and 287 ± 10.1 cm^3 g^{-1} VS, respectively (MUSSGNUG et al. 2010).

Conclusions

The conducted experiments demonstrated that the quantities of produced biogas per load of organic substrate fed to the technological systems were similar irrespective of the applied method of heating fermentation tanks. At both experimental stages, the quantity of gaseous metabolites of anaerobic bacteria was at an average level of ca. 450 cm^3 g^{-1} VS. It was proved that the electromagnetic microwave radiation had a direct impact on the improved qualitative composition of biogas produced. The stimulation of thermal conditions using electric heaters produced nearly 65% of methane in biogas, whereas the use of microwaves assured methane content of ca. 69%. In addition, the

study demonstrated that the use of electromagnetic microwave radiation had a significant impact on faster adaptation of the technological system and on obtaining stable effects of methane fermentation.

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CONCEPTION OF THE "UNIQUE FERDYNANDOVIAN FLORA" GEOSITE IN THE ŁUKÓW PLAIN

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K e y w o r d s: geotourist site/geosite, Ferdynandovian interglacial, nature tourism, Łuków Plain.

A b s t r a c t

Geosites are places of particular importance for understanding history of the Earth. An example of such a place may be the Łuków site with fossil Ferdynandovian flora. The development of vegetation covers over 140 000 years of Pleistocene in this reference profile. The Łuków profile is one of only a few European complete profiles, in which two Ferdynandovian interglacial climatic optima are recorded.

The scientific uniqueness of the Łuków profile was evidenced by numerous papers. However, the site was not analysed in terms of its usefulness for tourism and educational purposes. In order to fill this gap, the method of geosite evaluation has been used to evaluate geotourism potential. The following features of the site were evaluated: scientific value, educational value, state of the object (including threats), and accessibility. The evaluation was qualitative in a scale 1–5. We propose to establish the geosite named "Unique Ferdynandovian flora". As a result, it will be possible to improve the accessibility and protect this site, as well as promote it as an object of nature tourism.

KONCEPCJA STANOWISKA GEOTURYSTYCZNEGO "UNIKALNA FLORA FERDYNANDOWSKA" NA RÓWNINIE ŁUKOWSKIEJ

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Abstrakt

Stanowiska geoturystyczne (geostanowiska) są to miejsca o szczególnym znaczeniu dla poznania dziejów Ziemi. Takim przykładem może być stanowisko kopalnej flory ferdynandowskiej na Równinie Łukowskiej. Przedstawia ono wzorcowy profil rozwoju szaty roślinnej w ciągu 140 tys. lat trwania plejstocenu. W skali europejskiej profil ten dołącza do zaledwie kilku pełnych tzw. dwuoptymalnych profili ferdynandowskich.

Naukowa wyjątkowość profilu Łuków podkreślona jest licznymi publikacjami. Nie była ona jednak analizowana pod kątem udostępnienia dla turystyki i edukacji. W celu uzupełnienia tej luki stanowisko kopalnej flory ferdynandowskiej poddano waloryzacji metodą stosowaną dla geostanowisk. Zakres jej ewaluacji obejmuje następujące cechy stanowisk: wartość naukową, wartość edukacyjną, stan obiektu i jego zagrożenia oraz dostępność. Waloryzacja była jakościowa w skali 1–5. Wykazano wysokie walory edukacyjne analizowanego obiektu dające podstawę do utworzenia stanowiska geoturystycznego pt. „Unikalna flora ferdynandowska". Zaprezentowana koncepcja geostanowiska przyczyni się do poprawy dostępności i ochrony obiektu wyjątkowego w skali europejskiej oraz jego promocji w zakresie szeroko rozumianej turystyki przyrodniczej.

Introduction

Geotourist sites (geosites) are valuable geological objects, which are of particular importance for understanding the history of the Earth (REYNARD 2004). Geosites are established in order to expose and protect the natural landscape elements, and to present the knowledge of their geological history. They are element of widely-understood geoheritage, focused on geology and landscape (cf. DOWLING and NEWSOME 2010, DOWLING 2011). These objects of inanimate nature (abiotic elements) are usually a record of geological-geomorphological processes forming landforms of different origin (e.g. GARAVAGLIA and PELFINI 2010, cf. MIGOŃ 2012). Fossil vegetation successions (biotic elements) are much less often exposed in geosites, though they provide equally valuable information on the geological past, including the history of life and climate changes. Reconstruction of these changes is mainly based on the analysis of plant remains (pollen, spores and macrofossils), which are very well preserved in the deposits of fossil lakes and mires.

From among numerous geological sites the stratotype profiles are especially noteworthy as they serve as the standards of reference in the reconstruction of the history of individual geological periods. This is the case of the sites with fossil Ferdynandovian flora occurring in the Łuków Plain – the mesoregion in eastern Poland (Figure 1). Their scientific uniqueness was evidenced by numerous papers published in leading Polish and international journals in the field of palaeobotany, palaeoecology and palaeoclimate (among others PIDEK 2013, PIDEK and POSKA 2013). However, these sites were not analysed in terms of their usefulness for tourism and educational purposes. In order to fill this gap, the method of geosite evaluation (cf. MIGOŃ 2012) has been used to evaluate geotourism potential of the Łuków site, which is the most representa-

tive, unique profile with the fossil Ferdynandovian flora. The purpose of our study is to present this evaluation and conception of the geosite together with the model of information board.

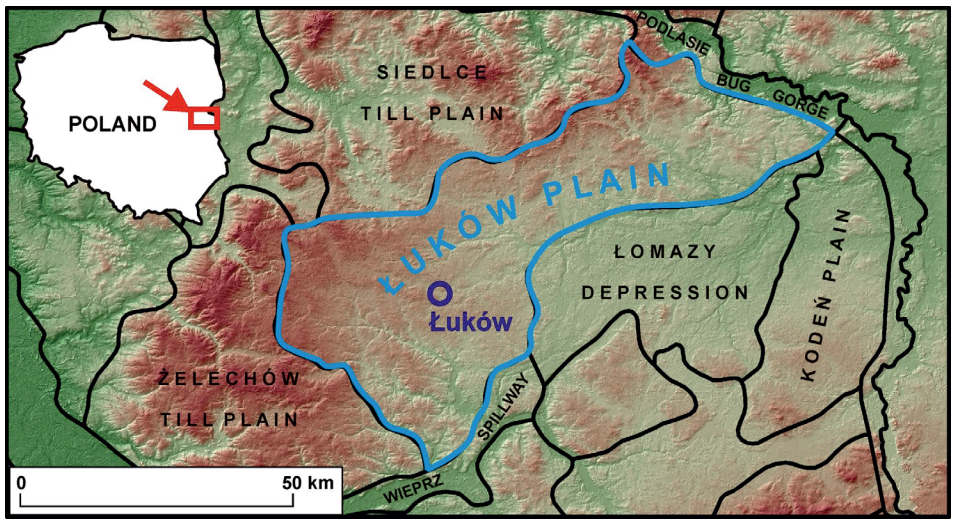

Fig. 1. Location of the Łuków geological site in the Łuków Plain mesoregion in relation to physico-geographical regionalization of Poland (authors' own study based on regions determined by Kondracki 2009)

Research area

The Łuków site with the fossil Ferdynandowian flora is located in the Łuków Plain. This physico-geographical mesoregion is a part of the South Podlasie Lowland macroregion (KONDRACKI 2009, Figure 1). It is flat area gently descending towards the SE from 170 to 140 m a.s.l. It is mainly composed of fluvioglacial sands and gravels, which were deposited on flat alluvial plains by braided rivers supplied with meltwater that flowed from the Wartanian ice-sheet front about 210 000–130 000 years ago.

Surface deposits of the Łuków Plain are underlain by fossil deposits of cyclically recurring cold (glacial) and warm (interglacial) periods, i.e. glacial sands, gravels and tills, and interglacial lacustrine and mire deposits. Within these deposits there were found the profiles with the so-called Ferdynandovian flora correlated with the Cromerian interglacial in Western Europe (Figure 2). Among them, the most unique is the Łuków site with the lacustrine-mire deposits filling the fossil basin of a lake formed 620 000 years ago. The site is located within the city of Łuków, in its eastern part, in the Zapowiednik quarter. A characteristic feature of the site location is a small distance (up to

100 m) from the forest complex (State Forests National Forest Holding, Łuków Forestry) surrounding the site from the west, north, and east, and from the Łuków – Lublin railway line in the south.

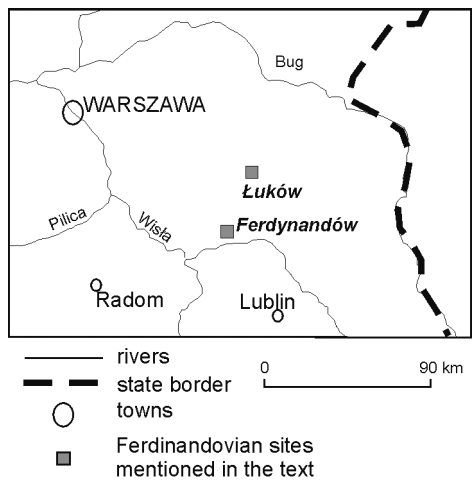

Fig. 2. Sites of the Ferdynandovian flora in the Łuków Plain

The site of Ferdynandovian flora in Łuków has been known since the 1960s due to the investigations conducted by SOBOLEWSKA (1969), but only the detailed research in 2010 indicated that the exceptionally complete vegetation succession (representing the Ferdynandovian s.l. period that lasted from 620 000 to 480 000 years ago) was recorded the lacustrine-mire deposits (PIDEK and MAŁEK 2010). This succession represents two interglacial periods together with separating them glacial cooling. Two warm climate units are named Ferdynandovian 1 and 2, and separating them cooling – Ferdynandovian 1 / 2 (LINDNER et al. 2004). The Ferdynandovian deposits are 13.6 m thick, and are covered by till deposited during the next glacial (Sanian 2).

Methods

The evaluation of geotourism potential of the Ferdynandovian flora site in Łuków was carried out in several stages, using the method developed by KNAPIK et al. (2009) and KNAPIK and MIGOŃ (2010).

In the first stage the sites with Ferdynandovian flora in the Łuków Plain were inventoried, based on the analysis of primary source materials, i.e. the

authors' own scientific publications. Then the most representative site (Łuków-3a) was selected.

The next stage of the work was the evaluation of the site for geotourism purposes. The following features of the site were evaluated: scientific value, educational value, state of the object (including threats), and accessibility. The evaluation was qualitative; each feature was evaluated in a scale 1–5 (*vide* Table 1).

Table 1
Elements of geosite evaluation according to KNAPIK et al. (2009) and KNAPIK and MIGOŃ (2010) method

Subject of evaluation	Description	Point value
Scientific value	the only site in the region; unique in over-regional scale; presented in international scientific journals	5
	object very important for regional studies; presented in national and international scientific journals	4
	object important for regional studies; presented in national and international scientific journals	3
	typical site of mediocre scientific value, presented in popular scientific magazines	2
	no special distinguishing features; the lack of publications	1
Educational value	number of presented problems: 5 and more	5
	number of presented problems: 4	4
	number of presented problems: 3	3
	number of presented problems: 2	2
	number of presented problems: 1	1
State of the object	well preserved, with no visible signs of degradation	5
	slightly disturbed structure of the site	4
	partially destroyed	3
	heavily changed by man	2
	destroyed, loss of geosite nature	1
Accessibility	site situated directly on the tourist trail or nature trail	5
	site situated off the trail but well visible from the trail, near a road or path	4
	site not visible from the tourist trail and poorly visible from other points, situated more than 500 m away from a road or path	3
	site difficult to access, e.g. overgrown or accessible by a route difficult to walk	2
	site inaccessible for tourists	1

Evaluation results

Scientific value

The results of geosite evaluation indicate that the Łuków site has the greatest geotourism potential in terms of scientific value (5 points). Scientific problems studied in the site are exceptional in regional scale, unique in over-regional scale, and the results are published in international scientific journals. The most important, unique feature of the site is a complete record of two interglacial warmings, which were characterized by climate warmer than nowadays, and exceptionally rich composition of vegetation communities, including also exotic species that do not occur in Poland nowadays. The history of vegetation near Łuków during 140 000 years is presented in the pollen diagram (Figure 3).

The phase of pioneer birch forests (Ł-1), following the retreat of the Sanian 1 ice sheet, represents the beginning of the Ferdynandovian 1 interglacial. The analysis of plant macrofossils has provided important information about the development of palaeolake and its trophy. The lake was very deep from the beginning of the interglacial. On the edge of the lake, in the peat-covered places, the communities of sedge rush developed, in which the plants typical of transitional mires occurred (among others Cyperaceae, *Menyanthes trifoliata, Comarum palustre*). *Typha latifolia* and *Schoenoplectus lacustris* occurred in the belt of typical rush (STACHOWICZ-RYBKA 2015). The complete succession of the Ferdynandovian 1 interglacial is represented by eight pollen zones (Ł-1–Ł-8), illustrating the development of rich, multi-species forests, at first dominated by elm and oak, and then with a high proportion of hazel and lime, and an admixture of exotic thermophilous tree (*Celtis*) and shrubs (*Buxus, Ligustrum*, and several other Mediterranean species). Vegetation composition indicates that climate was warm and humid, and the mean temperature of July was higher by at least 2°C from the present-day temperature, and annual precipitation total reached 800–900 mm (PIDEK and POSKA 2013). The occurrence of numerous seeds of *Najas marina* and *N. minor* (two species of the Hydrocharitaceae family) among macrofossils indicates that water in the lake was very eutrophic. The presence of *Zannichellia palustris* in the rush belt suggests the occurrence of habitats with varying water level and thereby with a high content of mineral salts (STACHOWICZ-RYBKA 2015).

The subsequent zones of the pollen diagram (Ł-9–Ł-11) reflect the stadial-interstadial changes occurring during a cold period of glacial nature (Ferdynandovian 1/2). The beginning of this period was characterized by the spread of boreal pine communities with spruce admixture. Then they were replaced by open steppe-tundra communities of herbs (grasses, *Artemisia*, sedges), and again by boreal birch and pine-birch forests.

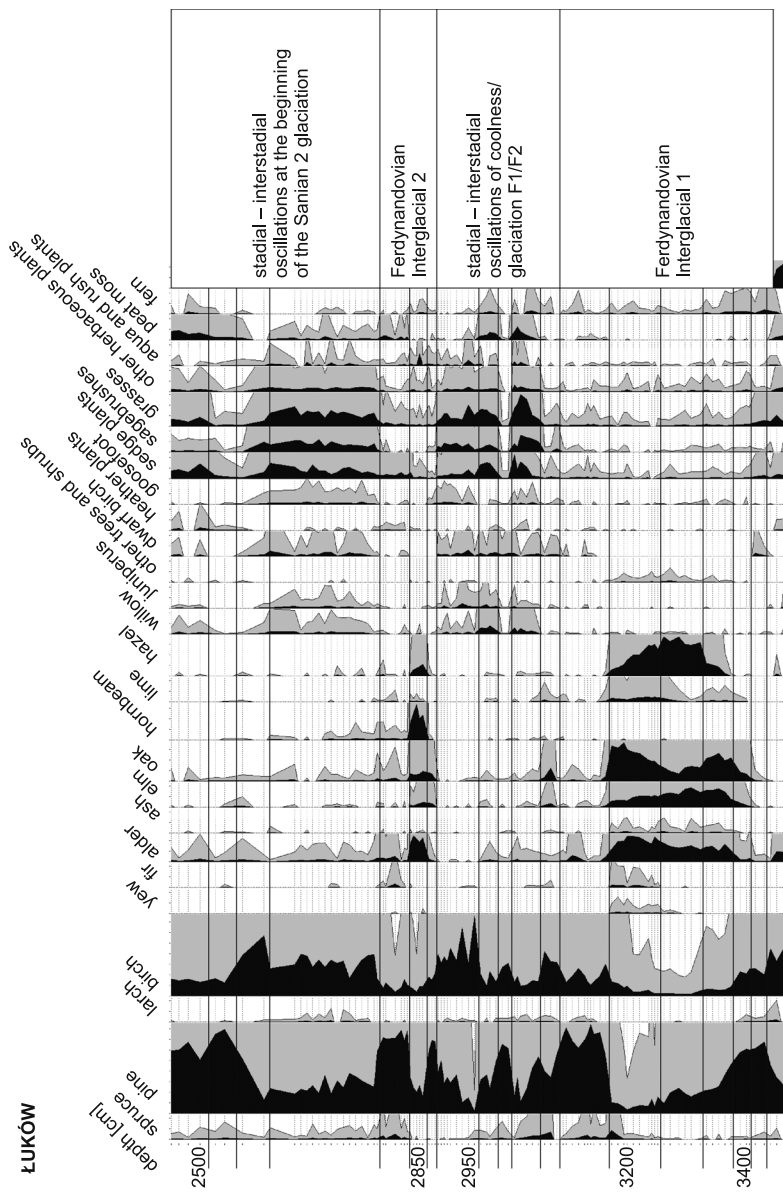

Fig. 3. Schematic pollen diagram of fossil lacustrine-mire deposits in the Łuków profile

The next interglacial succession (Ferdynandovian 2), including pollen zones Ł-12–Ł-15, is very different from the older one. This period was primarily characterized by the occurrence of vast hornbeam forests with an admixture of oak, lime, and hazel. Riverine communities, with elm, oak, and ash, were also present but the proportion of exotic species was lower than in the older interglacial. The estimated temperature and humidity seem to indicate that the climate was somewhat more continental though also warm, with the July mean temperature reaching 19°C (PIDEK and POSKA 2013). *Brasenia borysthenica* and *Aldrovanda borysthenica*, which were found among macrofossils, are very exotic taxa (STACHOWICZ-RYBKA 2015).

The next zones (from Ł-16) represent glacial conditions. The vegetation succession was characterized by the alternating occurrence of boreal forests of taiga type and steppe-tundra herbaceous communities.

Educational value

The analysis of educational value of the Łuków site indicates its high usefulness (5 points) for the explanation of phenomena and processes occurring in natural environment over 140 000 years of the early stage of Pleistocene. It was the period of several ice-sheet advances, which were separated by warmings when rich, multispecies, often exotic vegetation reappeared. Such changes are recorded in deposits due to the unique properties of exine (external membrane) of pollen and spore grains, which may be preserved under low-oxygen conditions for millions of years. The whole South Podlasie Lowland is unique in Europe because in this area there are preserved the fragments of fossil lakelands of different ages, which developed during different interglacials (ŻARSKI et al. 2005). These facts are not known to the broader public, but only discussed among scientists. In comparison with other sites located in the South Podlasie Lowland the Ferdynandovian flora site in Łuków is exceptional. In the Łuków profile we find a complete and continuous record of vegetation and climate changes during two warm interglacial periods separated by cold glacial period, i.e. the Ferdynandovian s.l. The overlying deposits represent the Sanian 2 glacial, and their vegetation succession is characterized by the alternating occurrence of boreal birch and pine-birch forests and replacing them herb-dwarf-shrub communities of steppe-tundra type. The West European authors also stress the exceptional value of the Ferdynandovian deposit sequences in eastern Poland (TURNER 1996, ZAGWIJN 1996), and the Łuków site is one of three profiles most suitable for reconstruction of the Cromerian climate and vegetation changes. Taking into account the fact that in the West European profiles the record of these changes is

incomplete and fragmentary, we think that the Łuków sequence should be exposed to the public for educational purposes.

Based on these new palaeobotanical data, it is possible not only to reconstruct the changes of climate and vegetation cover during the Ferdynandovian s.l. climatostratigraphic unit (LINDNER et al. 2004) but also to undertake broader palaeogeographical interpretations concerning the Southern Podlasie region. This region is of essential importance for the identification of palaeogeographical and palaeoclimate conditions in the periods of functioning of fossil lakelands of different ages (ŻARSKI et al. 2005).

State of the object

Location of the Łuków site, which is surrounded by forest and far from housing estates, has been favourable to its state of preservation. Therefore, the site should be assessed as well preserved, without visible signs of degradation (5 points). Due to such a location, it will be also possible to preserve the geological site for future generations.

However, good access to the area where the site is located may be a threat to it. The proximity of the access road may facilitate destructive actions or acts of vandalism. In addition, the proximity of the railway line with heavy traffic (domestic and international transit) may cause noise disturbing sightseeing or pose a threat to the visiting tourists.

Accessibility

As the Łuków-3a site is located within the limits of the city of Łuków, it is easy accessible from other cities of the region and the whole country due to well-developed road and railway infrastructure. Provincial roads from Radzyń Podlaski, Stoczek Łukowski and Siedlce intersect in Łuków making possible easy access to the site also from more distant cities: Warsaw, Lublin and Białystok. The Warszawa – Terespol railway line runs through the city, so it is also possible to easily reach the site also from further regions of the country (Figure 4).

According to the method of geosite evaluation for geotourism purposes the Łuków site is easily accessible (4 points). The site is situated off the tourist trails but in the short distance from them. The bike red trail named "Trail of the Łuków Land" runs about 7 km from the site, and the bike blue trial – about 3 km (Figure 4), so in the future it will be possible to establish trails running to the site itself. This solution is favoured by the location of the site on an access road, so that the access to it is easy.

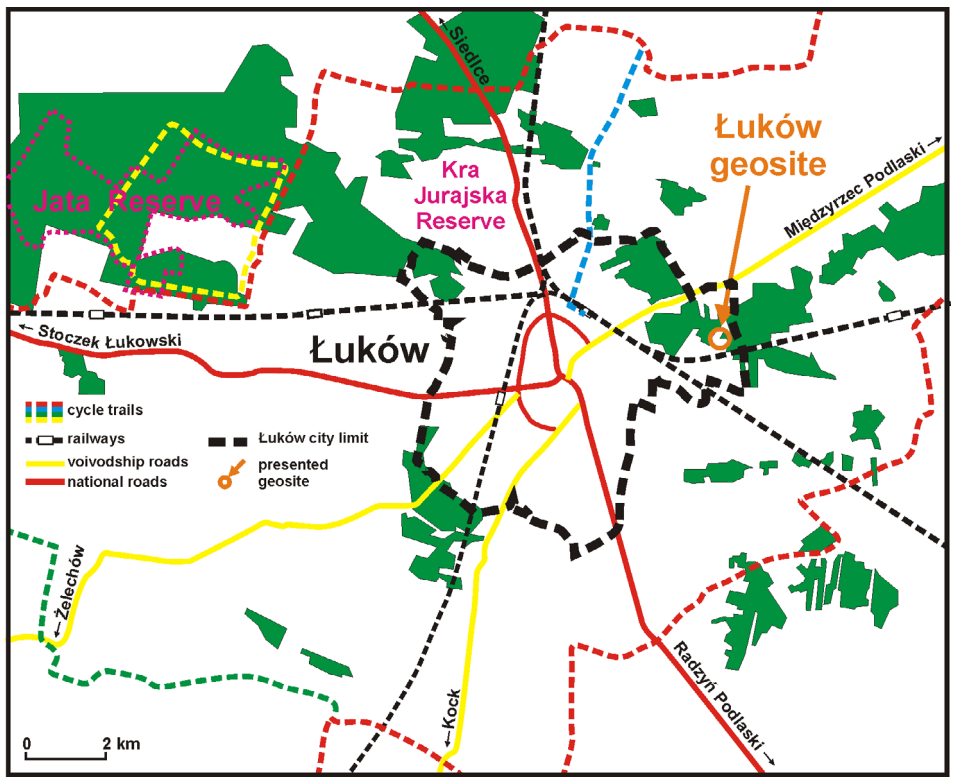

Fig. 4. Location of the Łuków-3a site against the background of the sketch of communication accessibility and tourist trails in the surroundings of the city of Łuków (author's own study based on the fragment of Sketch of communication accessibility and tourist trails in the surroundings of the city of Łuków by the Community Council of Łuków)

It is worth noting that in the city there are also other forms of nature protection, which present issues of geology, botany, etc. (Figure 4):

1) Jata Reserve – located in the Łuków Forests, protects the present vegetation of multispecies forest with fir reaching here the its north-western limit; springs of the Northern Krzna and Southern Krzna rivers occur in the reserve; three tourist trails run across the reserve: nature educational trail, historic walking trail, and historic bike trail. The fir forest in lowlands is never to be found in Europe. Its occurrence here is of special interest (*Centralny Rejestr*... 2017, Community Council of Łuków, own data).

2) Kra Jurajska Reserve – protects a rare geological object in the European scale, i.e. raft, which has been transported and left by the Scandinavian ice sheet; this very large slab of Jurassic clay is rich in fossils, especially ammonites (*Centralny Rejestr*... 2017, Community Council of Łuków, own data).

"Unique Ferdynandovian flora" geosite

The evaluation of geotourism potential of the Łuków-3a geological site indicates its high usefulness (19 points) for geotourism purposes. Therefore, we think that the site is suitable to establish geosite, i.e. the place where valuable geological information, recorded in the fossil deposits of the Łuków Plain, will be presented. In the case of such a geosite type the most common form of making knowledge about it available to the tourists is information board (MIGOŃ 2012). An example of such a board has been designed for the analysed Łuków-3a site (Figure 5).

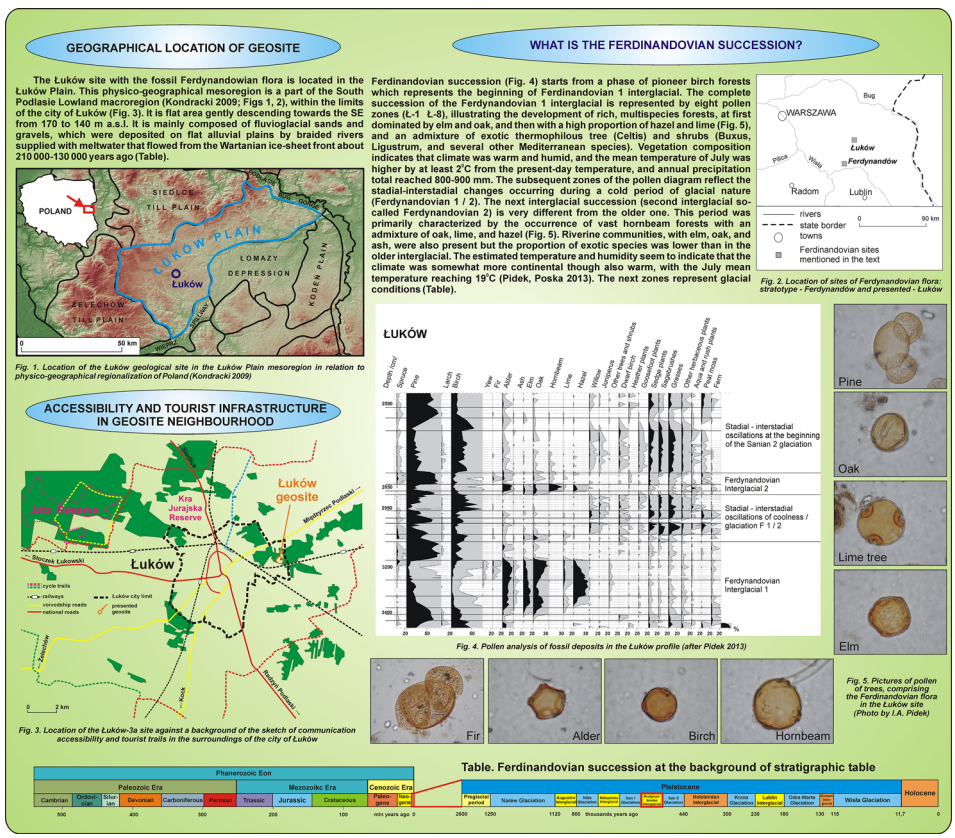

Fig. 5. Information board of Łuków-3a geotourist sites/geosite (authors; own study, pollen diagram plotted in the POLPAL software Nalepka and Walanus 2003)

Information board for a geosite should be prepared based on information resulting from the evaluation of scientific and educational values of a site, its state of preservation and accessibility. Information board should present all the knowledge about a geosite in a way accessible to potential unprofessional

tourist. The clear layout is a necessary form facilitating the assimilation of presented information. In addition to text information the proposed information board contains clear drawings (including the location map), tables and photos explaining and illustrating the processes, which have formed the fossil deposits with the Ferdynandovian succession in the analysed site.

Conclusions

The evaluation of geotourism potential of the Łuków-3a geological site leads to the following conclusions:

1. The abundance of information on the Pleistocene glacial – interglacial environment, which is recorded in the deposits of the Łuków Plain, is an excellent contribution to the education in the field of palaeoenvironmental sciences.

2. Based on the palynological data, it is possible not only to reconstruct the changes of climate and vegetation cover in the period of 140 000 years of Pleistocene (i.e. during the Ferdynandovian s.l. climatostratigraphic unit) but also to undertake broader palaeogeographical interpretations concerning the Southern Podlasie region, which is of essential importance for the identification of palaeogeographical and palaeoclimate conditions in the periods of functioning of fossil lakelands of different ages.

3. The analysis of plant macrofossils has provided important information about the development of palaeolake and its trophy.

4. Natural values of the geological site with the Ferdynandovian flora in Łuków, confirmed by scientific research, as well as the rarity of such sites (recording two interglacials in stratigraphic superposition) in Poland and in Europe indicate that the site meets the criteria for the establishment of another nature/geological reserve within the city of Łuków.

Translated by Maria Wilgat

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