UNIVERSITY OF WARMIA AND MAZURY IN OLSZTYN

Polish Journal of Natural Sciences

(2/2008) 23

PUBLISHER UWM
OLSZTYN 2008

The Polish Journal of Natural Sciences is indexed and abstracted
in Biological Abstracts and Biosis Previews

The Journal is also available (from volume 22) in electronic form. The online edition
is hosted by MetaPress (www.metapress.com) in partnership with Versita
(www.versita.com)

PL ISSN 1643-9953

PUBLISHER UWM OLSZTYN

Address
ul. Jana Heweliusza 14
10-718 Olsztyn-Kortowo, Poland
tel.: (48) (089) 523-36-61
fax: (48) (089) 523-34-38
e-mail: wydawca@uwm.edu.pl

Ark. wyd. 21,4 ark. druk. 17,5
Druk – Zakład Poligraficzny UWM w Olsztynie
zam. nr 345

TABLE OF CONTENTS

Agriculture

Biology

Environmental Protection

Fishery

Food and Nutrition Sciences

Veterinary Medicine

SPIS TREŚCI

Rolnictwo

Biologia

Ochrona Środowiska

Rybactwo

Nauka o Żywności i Żywieniu

Medycyna Weterynaryjna

POLISH JOURNAL OF NATURAL SCIENCES
Abbrev.: Pol. J. Natur. Sc., Vol 23(2): 269-283, Y. 2008

DOI 10.2478/v10020-008-0021-3

EFFECTS OF MINERAL FERTILIZATION ON SOIL FUNGAL COMMUNITIES OF ORIENTAL GOAT'S RUE *GALEGA ORIENTALIS* LAM.

***Bożena Cwalina-Ambroziak*[1], *Stanisław Sienkiewicz*[2]**

[1] Chair of Phytopathology and Entomology
[2] Chair of Agriculture Chemistry and Environment Protection
University of Warmia and Mazury in Olsztyn

K e y w o r d s: oriental goat's rue, fungi, rhizosphere, rhizoplane, roots, mineral fertilization.

A b s t r a c t

The objective of a study conducted in the years 2001-2003 was to determine the quantitative and qualitative composition of fungal populations colonizing the roots and radical zones – rhizosphere and rhizoplane – of oriental goat's rue cultivated with and without the application of fertilization. Root samples were collected from particular experimental objects before flowering to isolate fungi according to the methodology described by STRZELCZYK (1968). At the same time the influence of fungi of genus *Trichoderma* on potentially pathogenic fungi of *Botrytis cinerea* and genus *Fusarium* was investigated.

The largest fungal colony was found in the rhizosphere, where yeast-like fungi accounted for 80% of all isolates. Potentially pathogenic fungi of genus *Fusarium* constituted 2% only. They were isolated during all years of the study. Fungi of genus *Fusarium* were isolated more frequently from the rhizoplane and roots than from the rhizosphere (13.0 and 17.4% respectively). Among the other pathogens there were a few species of: *Botrytis cinerea*, *Sclerotinia sclerotiorum* and of genus *Ascochyta*. Fungi of the order *Mucorales* were quite common in the rhizoplane (43.3% of all isolates), whereas species of the genera *Gliocladium* and *Trichoderma*, antagonistic towards pathogens, accounted for 16.6%. Species belonging to the genera *Gliocladium* and *Trichoderma* were isolated more frequently from roots than from rhizoplane (25%), whereas fungi of the order *Mucorales* were less frequent (15.6%).

Mineral fertilization modified the quantitative composition, and to some degree also the qualitative composition, of fungi colonizing the radical zones of oriental goat's rue. The most isolates were obtained from the combination without fertilization (35.5% of all isolates). Potentially pathogenic fungi were isolated more frequently from the rhizoplane than from the rhizosphere in combination without fertilization and from the roots in the combination without fertilization as well as with fertilization at 80 kg P_2O_5 – and 160 K_2O kg x ha^{-1}. In the test of antagonism the species of genus *Trichoderma* inhibited growth of the tested pathogens of genus *Fusarium* and *Botrytis cinerea*.

Address: Bożena Cwalina-Ambroziak, University of Warmia and Mazury, ul. Prawocheńskiego 17, 10-720 Olsztyn, Poland, phone: (089) 523 41 47, e-mail: bambr@uwm.edu.pl

WPŁYW NAWOŻENIA MINERALNEGO NA ZBIOROWISKO GRZYBÓW ŚRODOWISKA GLEBOWEGO RUTWICY WSCHODNIEJ (*GALEGA ORIENTALIS* LAM.)

***Bożena Cwalina-Ambroziak*[1], *Stanisław Sienkiewicz*[2]**

[1] Katedra Fitopatologii i Entomologii
[2] Katedra Chemii Rolnej i Ochrony Środowiska

Słowa kluczowe: rutwica wschodnia, grzyby, ryzosfera, ryzoplana, korzenie, nawożenie mineralne.

Abstrakt

W przeprowadzonych w latach 2001-2003 badaniach podjęto próbę określenia liczebności i składu jakościowego zbiorowiska grzybów zasiedlających korzenie oraz strefy korzeniowe (ryzosferę i ryzoplanę) rutwicy wschodniej, uprawianej bez nawożenia i z zastosowanym nawożeniem mineralnym. W okresie przed kwitnieniem z poszczególnych obiektów składających się na kombinacje pobrano próby korzeni roślin w celu izolacji grzybów według metody STRZELCZYK (1968). Jednocześnie sprawdzono oddziaływanie grzybów z rodzaju *Trichoderma* na potencjalnie patogeniczne grzyby *Botrytis cinerea* i z rodzaju *Fusarium*.

Najbogatsze pod względem ilościowym zbiorowisko grzybów stwierdzono w ryzosferze. Dominowały grzyby drożdżopodobne (80% ogółu izolatów). Potencjalnie patogeniczne grzyby z rodzaju *Fusarium* stanowiły zaledwie 2%. Wyosobniano je we wszystkich latach badań. Częściej niż ryzosferę zasiedlały ryzoplanę (13%) i korzenie rutwicy (17,4%). Wśród pozostałych patogenów zidentyfikowano nieliczne gatunki: *Botrytis cinerea*, *Sclerotinia sclerotiorum* i z rodzaju *Ascochyta*. W ryzoplanie często występowały grzyby z rzędu *Mucorales* (43,3% ogółu izolatów), rzadziej antagonistyczne względem patogenów gatunki z rodzaju *Gliocladium* i *Trichoderma* (16,6%). Z korzeni wyosobniono więcej niż z ryzoplany antagonistów z rodzaju *Gliocladium* i *Trichoderma* (25% ogółu wyosobnień w tej strefie), a mniej grzybów z rzędu *Mucorales* (15,6%).

Nawożenie mineralne modyfikowało skład ilościowy i jakościowy zbiorowiska grzybów zasiedlających strefy korzeniowe rutwicy wschodniej. Najwięcej izolatów otrzymano spod uprawy rutwicy w kombinacji bez nawożenia (35,5% ogółu wyosobnień). Grzyby potencjalnie patogeniczne częściej niż z ryzosfery izolowano z ryzoplany w kombinacji bez nawożenia i z korzeni w kombinacji bez nawożenia i z nawożeniem (80 kg P_2O_5 i 160 K_2O kg ha^{-1}). W teście antagonistyczności gatunki z rodzaju *Trichoderma* hamowały wzrost badanych patogenów z rodzaju *Fusarium* i *Botrytis cinerea*.

Introduction

Soil biological diversity determines both soil fertility and the heath status of crops. The structure of fungal colonies is affected, among others, by root exudates, especially important in perennial crops, weather conditions and chemical protection (DEB, BORA 1996, FUNCK-JENSEN, HOCKENHULL 1984). Free amino-acid, metal ions, organic acid and enzymes exuded by roots of a plant species can inhibit or stimulate the development of pathogenic fungi as well as saprotrophic ones (ANGUS et al. 1994). Mineral fertilization also influences the biological activity of the environment in which plants are

cultivated and mutual relations between organisms inhibiting it (PATIL, VARADE 1998, SEHGAL et al. 1992). Numerous authors reported in their works the stimulating influence of fertilization on microorganic population limiting growth of pathogenic fungi (BOWEN, ROVIRA 1999, CWALINA-AMBROZIAK, MAJCHRZAK 2000b). The share of antagonistic species of genera *Gliocladium* and *Trichoderma* in the structure of fungal community populating the rhizosphere, rhizoplane and roots of plants is of particularly large importance in protection of plants against pathogens' that is why their presence in the soil is welcome (PASTUCHA 1999).

The experiment conducted aimed at determining the composition of fungal populations colonizing the rhizosphere, rhizoplane and roots of oriental goat's rue (*Galega orientalis* Lam.) – a perennial papilionaceous plant grown under conditions of differentiated mineral fertilization. At the same time influence of genus *Trichoderma* fungi on pathogenic fungi of genus *Fusarium* and *Botrytis cinerea* was investigated.

Material and Methods

The experiment was performed during the years 2001-2003 on an experimental field located in Bałcyny near Ostróda on wheat good soil complex. Oriental goat's rue was grown on three plots, each covering an area of 1 ha. The treatments were as follows: 1. no fertilization, 2. with fertilization at 40 kg P_2O_5 ha^{-1} – superphosphate and 80 kg K_2O ha^{-1} – potassium salt (17.46 kg P ha^{-1} and 66.45 kg K ha^{-1}), 3. with fertilization at 80 kg P_2O_5 ha^{-1} and 160 kg K_2O ha^{-1} (34.92 kg P ha^{-1} and 132.90 kg K ha^{-1}). P_2O_5 and K_2O fertilizers were applied pre-sowing. Plant samples representative of particular treatments were collected before flowering. Fungi were isolated from the rhizosphere, rhizoplane and roots of oriental goat's rue as described by STRZELCZYK (1968) on MARTIN'S (1950) nutrient medium. Samples of roots with soil were collected from 10 places of each experimental plot. 10 g portions were shaken for 30 min in 90 ml of sterile water obtaining the dilution of 10^{-1}. From that suspension further dilutions were obtained (each time shaking the next dilution for 2 min). After obtaining the final dilution of 10^{-3} and 10^{-4}, 1 ml samples of the suspension was placed on Petri dishes and inundated with Martin medium. Fungal colonies obtained after five days of incubation at 22°C were transferred to agar slopes and identified according to the key and monographic work. Aiming at isolation of fungi from the rhizoplane, the rots from the first dilution were cut into 5 cm long pieces, transferred to a fresh 90 ml portion of water and shaken for 10 min. The washed fragments of roots were divided into 5 mm sections and placed on Martin medium. Next the fungi that developed were

transferred on agar slopes for later identification. The experiment concerning isolation of fungi from the roots was as follows: roots washed in water were cut into 2 cm long fragments that were disinfected for 30 sec in 50% ethanol and hypochlorite and washed three times in sterile water. Disinfected roots were cut into 5 mm pieces and placed on PDA. The further procedure was as described earlier.

At the same time at the laboratory the linear growth of seven pathogenic species: *Botrytis cinerea, Fusarium avenaceum, F. culmorum, F. equiseti, F. fusarioides, F. oxysporum* and *F. poae* in presence of 3 fungal species of genus *Trichoderma: T. hamatum, T. harzianum* and *T. viride* was observed. In that experiment species of fungi obtained from soil on which oriental goat's rue was cultivated were used. In the test of antagonism, agar discs overgrown with 4-day mycelium were placed at a distance of 2 cm from each other in the center of Petri dishes on PDA medium. The controls consisted of the dishes with discs of one species of fungi. After 10 days of growing together the diameters of pathogen colonies were measured and the mycelium growth inhibition percentage index was calculated. Assessment of biotic relations between the tested species of pathogens and the fungi of genus *Trichoderma* was made using the method of biotic series by MAŃKA (1990).

Results and Discussion

1821 isolates of fungi representing 51 species, 3940 isolates of yeast-like fungi and 154 asporogenous cultures were obtained from the rhizosphere, rhizoplane and roots of oriental goat's rue (Table 1, Table 2, Table 3). The most fungi were isolated in 2003 (44.1% of all isolates Figure 1a). Analyzing the influence of mineral fertilization the richest community of fungi was found in the oriental goat's rue cultivation environment in combination without fertilization (35,5% of fungi in that combination – Figure 1b).

Table 1

Fungi colonizing the rhizosphere of oriental goat's rue during investigation period

Species	2001			Σ	2002			Σ	2003			Σ	Total
	K	40	80		K	40	80		K	40	80		
1	2	3	4	5	6	7	8	9	10	11	12	13	14
Acremonium strictum W. Gams	17	5	17	39		2	9	11	5	7	6	18	68
Alternaria alternata (Fr.) Keissler	1			1	7			7	8	4	2	14	22
Arthrinium sphaerospermum Fuckel			2	2	9		5	14	4			4	20
Ascochyta sp.									1			1	1

cont. table 1

1	2	3	4	5	6	7	8	9	10	11	12	13	14
Aspergillus sp.										1		1	1
Aureobasidium pullulans (Bary) Arnaud					9	4	2	15	1	2	1	4	19
Bipolaris sorokiniana (Sacc. in Sarok.) Shoem.	3			3						6		6	9
Botrytis cinerea Pers.			4	4									4
Chaetomium spp.									6			6	6
Cladosporium cladosporioides (Fres) Vries	24	7		31	14	6	8	28	17	16	9	42	101
Cladosporium herbarum Link:Vries		3	2	5									5
Coniothyrium spp.										4		4	4
Endothia spp.					1		3	4					4
Epicoccum sp.											1	1	1
Fusarium avenaceum (Fr.) Sacc.					6	4	20	30	1		1	2	32
Fusarium culmorum (W.G.Sm.) Sacc.	1	3		4		2		2	4			4	10
Fusarium equiseti (Corda) Sacc.		11	10	21		8		8			1	1	30
Fusarium oxysporum Schlecht.	7			7	1	4		5					12
Fusarium solani (Mart.) Sacc.										6		6	6
Gilmaniella humicola Barron							4	4		5		5	9
Gliocladium catenulatum Gilman & Abbott	2	6	2	10			1	1					11
Gliocladium fimbriatum Gilman & Abbott											3	3	3
Gliocladium penicillioides Corda	3			3	4	6		10					13
Gliomastix murorum (Corda) Gueguen		1		1	3			3					4
Humicola brevis Gilman et Abbott	6		9	15	6		2	8	18	1	5	24	47
Humicola grisea Traaen	2	2		4	1		7	8	12		11	23	35
Microdochium nivale (Fries) Samuels et Hallett	1			1									1
Monodictis laevis (Wilttshire) Hughes									1			1	1
Mortierella alpina Peyronel	10	11		21			3	3	2	4	4	10	34
Mortierella isabelina Qudemans	1	4	1	6						1	1	2	8
Mortierella zonata Linn.											2	2	2
Mucor circinelloides van Tieghem			2	2						4		4	6
Mucor hiemalis Wehmer	4	3	1	8			4	4	4	4	1	9	21
Paecilomyces roseum (Thom) Samson	4	8		12	4	5		9			3	3	24
Phoma medicaginis Malbr. Et Roum.		1		1									1

cont. table 1

1	2	3	4	5	6	7	8	9	10	11	12	13	14
Penicillium spp.	14	4	19	37	27	26	41	94	29	11	2	42	173
Rhizopus spp.	4	5	23	32	6	1		7	1	2		3	42
Sporotrichum carnis Books et Hansford									7	6		13	13
Sporotrichum olivaceum Fries		9	1	10	1			1					11
Trichoderma hamatum (Bon.) Bain		4	6	10		3		3					13
Trichoderma harzianum Rifai	3			3									3
Trichoderma polysporum (Link:Pers.) Rifai													
Trichoderma viride Rifai									8			8	8
Ulocladium spp.						4		4					4
Zygorhynchus spp.		3		3									3
Non sporulating fungi	7	8	9	24	16	15	37	68	16	16	11	43	135
Yeast-like fungi	484	459	403	1346	151	267	140	558	731	541	716	1988	3892
Total	598	557	511	1666	266	358	286	910	876	640	780	2296	4872

K – control (withous fertilization), 40 – 40 kg P_2O_5 and 80 K_2O ha^{-1}, 80 – 80 kg P_2O_5 and 160 K_2O ha^{-1}

Table 2

Fungi colonizing the rhizoplane of oriental goat's rue during investigation period

Species	2001			Σ	2002			Σ	2003			Σ	Total
	K	40	80		K	40	80		K	40	80		
1	2	3	4	5	6	7	8	9	10	11	12	13	14
Acremonium strictum W. Gams										2	2	4	4
Alternaria alternata (Fr.) Keissler									1	2	4	7	7
Ascochyta sp.							1	1					1
Aureobasidium pullulans (de Bary) Arnaud						1		1		1		1	2
Cladosporium cladosporioides (Fres) Vries		1		1	2			2		1	1	2	5
Fusarium avenaceum (Fr.) Sacc.	1			1	2	2		4	4	4	2	10	15
Fusarium culmorum (W.G.Sm.) Sacc.	2			2	5	1	4	10	2	4		6	18
Fusarium equiseti (Corda) Sacc.					4		5	9					9
Fusarium fusarioides (Frag. & Cif.) Booth					1		1	2					2
Fusarium oxysporum Schlecht.	1	2	2	5	9	1		10	1			1	16
Fusarium poae (Peck) Woll.					4	2		6					
Fusarium solani (Mart.) Sacc.										4	3	7	7
Gliocladium catenulatum Gilman & Abbott		2		2									2

cont. table 2

1	2	3	4	5	6	7	8	9	10	11	12	13	14
Gliocladium penicillioides Corda			1	1		2		2					3
Gliomastix murorum (Corda) Gueguen							3	3					3
Humicola brevis Gilman et Abbott		4	1	5		5	18	23					28
Microdochium nivale (Fries) Samuels et Hallett	1		1	2									2
Monodictis laevis (Wilttshire) Hughes											1	1	1
Mortierella alpina Peyronel	17	7	6	30	1	3	2	6	7		10	17	53
Mortierella isabelina Qudemans	5		7	12					1	4	3	8	20
Mucor circinelloides van Tieghem		4	3	7						2		2	9
Mucor hiemalis Wehmer	2	7	5	14	13	2	6	21	6	12	3	21	56
Paecilomyces roseum (Thom) Samson									1			1	1
Penicillium spp.		3		3	1		1	2	9	10	5	24	29
Rhizoctonia solani Kühn									2			2	2
Rhizopus spp.	16	24	19	59		8	4	12	6	4	11	21	92
Sclerotinia sclerotiorum (W.G.Sm.) Sacc.									1	3	1	5	5
Sporotrichum olivaceum Fries						3		3	3		2	5	8
Trichoderma hamatum (Bon.) Bain	13	3	4	20	14	16	3	33					53
Trichoderma harzianum Rifai		4	4	8	8	6		14	2			2	24
Trichoderma polysporum (Link:Pers) Rifai										1		1	1
Trichoderma viride Rifai							4	4	1			1	5
Zygorhynchus spp.					9		1	10					10
Non sporulating fungi		3	4	7			2	2		2		2	11
Yeast-like fungi	7			7	5	1		6	3	4	1	8	21
Total	65	64	57	186	78	53	55	186	50	60	49	159	531

Explanations as in Table 1

Table 3

Fungi colonizing the roots of oriental goat's rue during investigation period

Species	2001			Σ	2002			Σ	2003			Σ	Total
	K	40	80		K	40	80		K	40	80		
1	2	3	4	5	6	7	8	9	10	11	12	13	14
Acremonium strictum W. Gams		1	1	2			1	1					3
Alternaria alternata (Fr.) Keissler					3		2	5	6	2	1	9	14
Arthrinium sphaerospermum Fuckel						3		3					3
Ascochyta sp.					1			1					1
Aspergillus sp.									1			1	1

cont. table 3

1	2	3	4	5	6	7	8	9	10	11	12	13	14
Bipolaris sorokiniana (Sacc. in Sarok.) Shoem.									1			1	1
Botrytis cinerea Pers.			1	1		3	3	6					7
Cladosporium cladosporioides (Fres)Vries					2	4	3	9	3	2		5	14
Cylindrocarpon destructans Zins.Scholten					2	4	3	9					9
Endothia spp.					1	6		7					7
Epicoccum spp.							2	2					2
Fusarium avenaceum (Fr.) Sacc.	1			1		3	6	9	5	2	5	12	22
Fusarium culmorum (W.G.Sm.) Sacc.	5		7	12	3		4	7	1	4	1	6	25
Fusarium equiseti (Corda) Sacc.			1	1	2	2	1	5					6
Fusarium fusarioides (Frag. Ex Cif.) Booth			4	4									4
Fusarium oxysporum Schlecht.	4	4		8	7	1		8	3	2	2	7	23
Fusarium poae (Peck) Woll.						2		2					2
Fusarium solani (Mart.) Sacc.										4	3	7	7
Gilmaniella humicola Barron					1			1					1
Gliocladium catenulatum Gilman & Abbott	2		2	4		1		1					5
Gliocladium fimbriatum Gilman & Abbott											1	1	1
Gliocladium penicillioides Corda		2		2			1	1					3
Gliomastix murorum (Corda) Gueguen							2	2					2
Humicola brevis Gilman et Abbott										3		3	3
Humicola grisea Traaen									2		1	3	3
Monodictis laevis (Wilttshire) Hughes										1		1	1
Mortierella alpina Peyronel	4	2		6		1		1	1	1	2	4	11
Mortierella isabelina Qudemans	1			1		4		4	1	2		3	8
Mucor circinelloides van Tieghem	1			1	1		3	4		2		2	7
Mucor hiemalis Wehmer	2	3	9	14	2	1	1	4	3	7	4	14	32
Paecilomyces roseum (Thom) Samson									1			1	1
Penicillium spp.	8	11	3	22	8	8	8	24	12	12	10	34	80
Rhizopus spp.	5	4	3	12		4	2	6			4	4	22
Sclerotinia sclerotiorum (W.G.Sm.) Sacc.									2	4		6	6
Sporotrichum olivaceum Fries		2		2		1	2	3	5		3	8	13
Trichoderma hamatum (Bon.) Bain	17	15	12	44	20	14	16	50					94
Trichoderma harzianum Rifai		4	3	7	2	5		7	2			2	16
Trichoderma koningi Qudemans	1			1									1

cont. table 3

1	2	3	4	5	6	7	8	9	10	11	12	13	14
Trichoderma polysporum (Link:Pers) Rifai										1		1	1
Trichoderma viride Rifai						1		1		2	3	5	6
Zygorhynchus spp.		1	2	3	2			2		4		4	9
Non sporulating fungi	3		1	4		1	1	2		2		2	8
Yeast-like fungi		4	16	20	1	1		2	3	2		5	27
Total	54	53	65	172	58	70	61	189	52	59	40	151	512

Explanations as in Table 1

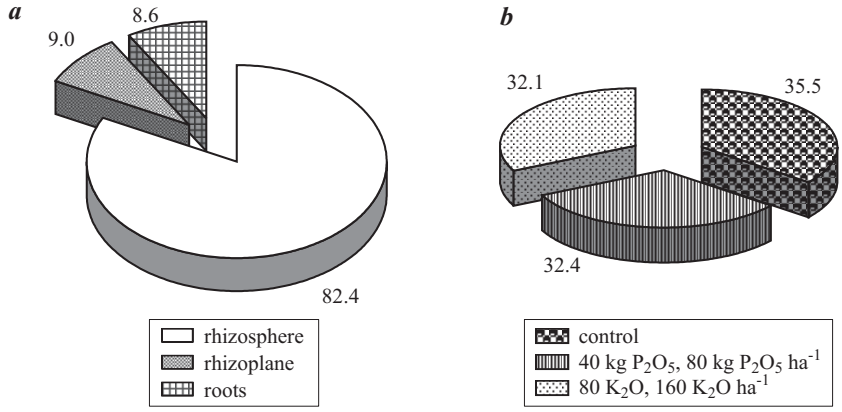


Fig 1. Percentage of fungi isolated from root zone of oriental goat's rue depending on: *a* – root zone *b* – kind of cultivation

During the conducted study, the largest fungal colony was fund in the rhizosphere, which is consistent with the results obtained by MAŃKA (1990 – Figure 1a). Yeast-like fungi (80% of all isolates – Figure 2) proved to be dominating. Potentially pathogenic fungi of genus *Fusarium* were represented by 6 species and constituted about 1.9% of the total population. The strongest represented among them were *F. avenaceum* and *F. equiseti*. Individual isolates of *B. cinerea* and fungi of genus *Ascochyta* were identified. During the analysis of individual experimental combinations (without and with fertilization) the share of pathogens in the rhizosphere soil without fertilization was 1.3%, while in the soil with fertilization it was 2.3 and 2.5% of the total number of isolates respectively (Figure 3a). Few saprotrophic fungi of the genera *Acremonium, Cladosporium, Gliocladium, Penicillium* and *Trichoderma*, and of the order *Mucorales* (*Mortierella* spp., *Mucor* spp., *Rhizopus* spp. and

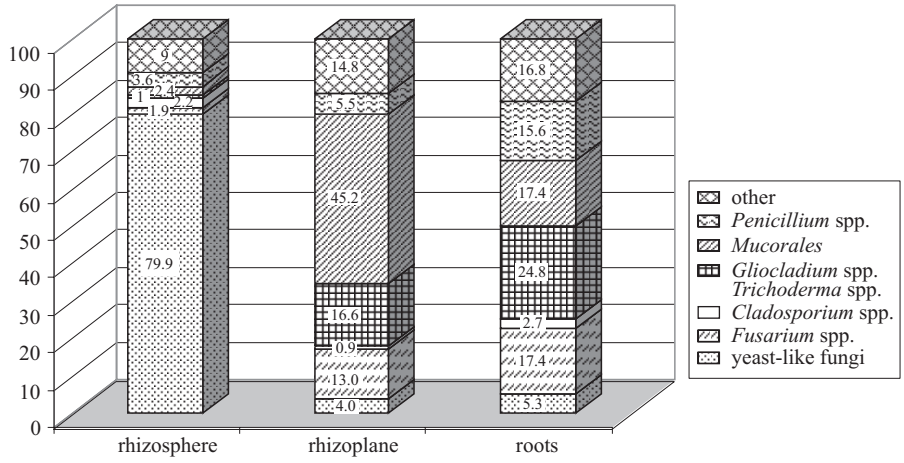


Fig. 2. Percentage of fungi most isolated from cultivated of oriental goat's rue depending on roots zone

Zygorhynchus spp.) were isolated. The quantities of those fungi in individual combinations showed low diversification and it ranged from 6.4 to 7.4%. The most numerously isolated fungi were those of genus *Penicillium*. Presence of those fungi in the environment is common, as they are known for their high adaptation potential to various environmental conditions and obtaining various sources of nutrients. DEB and BORA (1996) in their studies on the rhizosphere of peas fertilized with NPK obtained mainly species such as: *Aspergillus flavus, Curvularia lunata, Rhizopus nigricans, Fusarium oxysporum, Trichoderma lignorum, Penicillium lilacinum* and *Chaetomium* spp. Results of other experiments (PATIL, VARADE 1998) show that mineral fertilization stimulated development of bacteria and fungi, including those of genus *Aspergillus*, in the rizosphere of sorghum. We also learn from other studies that addition of sulfur to soil (HILAL et al. 1992) or nitrogen at adequately high doses (SEHGAL et al. 1992), caused an increase in the number of bacteria and fungi in the rhizosphere of various plant species.

In these studies fungi isolated from the rhizoplane and roots of oriental goat's rue represented 9% of the total number of isolates each (Figure 1a). In those cases pathogenic fungi of genus *Fusarium* were isolated more frequently than from the rhizosphere, 13.0 and 17.4% of total colonies respectively – Figure 2). They were isolated during every year of studies and they were represented by 7 species among which *F. avenaceum, F. culmorum* and *F. oxysporum* were isolated most frequently. The other pathogens from the rhizoplane of oriental goat's rue (*Ascochyta* sp. and *S. sclerotiorum*) represen-

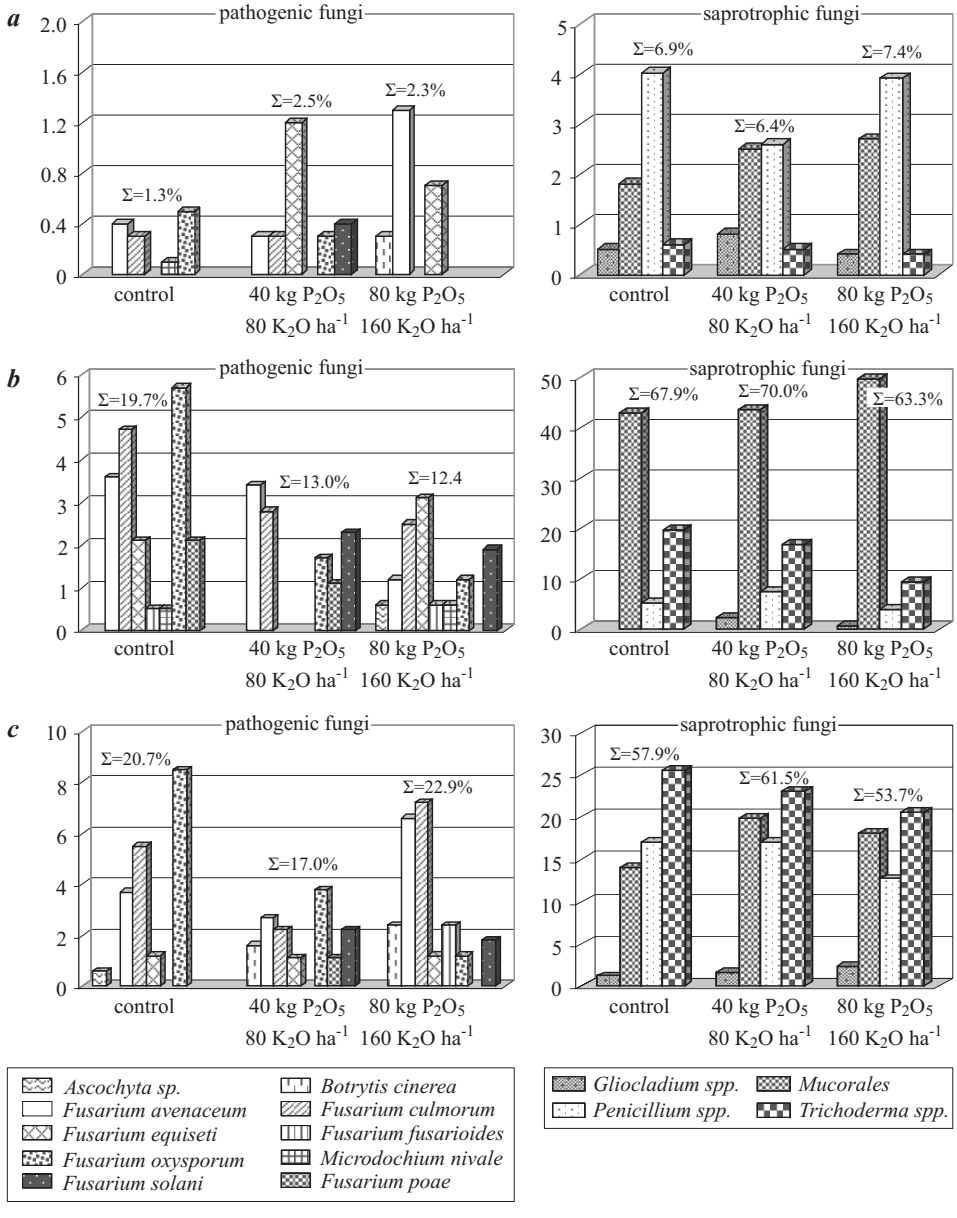


Fig. 3. Percentage of fungi isolated from roots zone of galega fodder: *a* – Rhizosphere, *b* – Rhizoplane, *c* – roots

ted just 1.2% of all isolates. DORENDA (1986) demonstrated that the fungi of genus *Fusarium* are common components of fungal populations found in the soil environment of papilionaceous plants. Those are polyphagous fungi,

which, similar to *Botrytis cinerea* and *Sclerotinia sclerotiorum*, commonly populate also seeds of papilionaceous plants including oriental goat's rue (NOWICKI 1995, CWALINA-AMBROZIAK, MAJCHRZAK 2000a).

In our studies the most pathogens were found in the rhizoplane of oriental goat's rue in combination without fertilization (19.7% – Figure 3b.). In the combinations with fertilization their share among the fungi isolated there did not exceed 14%. The largest presence of saprotrophic fungi possessing antagonistic influence in relation with pathogens was recorded in the rhizoplane of oriental goat's rue in combination with fertilization at 40 kg P_2O_5 ha^{-1} and 80 kg K_2O ha^{-1} (70% of the total presence of fungi in that combination). Fungi of the order *Mucorales* were numerously present in that zone (43 and 50% in the combination without fertilization and with the highest fertilization applied respectively). Species of genera *Gliocladium* and *Trichoderma* antagonistic in relation to pathogens were less numerous (16.6%). Fungi of genus *Gliocladium* were not recorded in the control combination. PASTUCHA (1999) as well as RODRIQUEZ, COTES (1999) also demonstrated that the above microorganisms contribute to reducing the size of pathogens' population. Strong antagonistic abilities of genus *Trichoderma* fungi in relation to pathogens are caused by production of metabolites and hydrolytic enzymes by those microorganisms (LEDERER et al. 1992). Also fungi of genus *Mucorales* (*Mucor* spp. and *Rhizopus* spp), thanks to high proteolytic abilities and chitin metabolism, limit growth of many pathogenic organisms. Fungi of genus *Penicillium* represented 5.5% of all isolates obtained from the rhizoplane. They are frequently present in the cultivated crops' environment because of a wide range of temperatures under which they can develop and the ability of using diverse sources of nutrients.

The share of pathogens in the total number of isolates obtained from the roots of oriental goat's rue was the highest as compared to the rhizosphere and rhizoplane. The largest number of isolates of those fungi was obtained from the roots of oriental goat's rue cultivated in combination with the highest fertilization level (22.9% of all isolates in that combination) and in the control combination without fertilization (20.7%). Fungi of genus *Fusarium* were the most frequent while in case of *B. cinerea* and *S. sclerotiorum* species and species of genus *Ascochyta* individual isolates only were found. Species *F. poae* and *F. fusarioides* were isolated the least frequently among fungi of genus *Fusarium* while *F. oxysporum* was represented by the largest number of isolates obtained from the roots of oriental goat's rue cultivated in the combination without fertilization (8.5% – Figure 3c). Antagonists belonging to genera *Gliocladium* and *Trichoderma* (jointly), and fungi of genus *Penicillium* were isolated more frequently from roots than from the rhizoplane, and their share in the total number of isolates from the roots was 25.0 and 15.6%

respectively. Similar to the rhizoplane, also in case of the roots the largest numbers of isolates of the above mentioned saprotrophic fungi were received from the combination with the highest fertilization at 40 kg P_2O_5 ha^{-1} and 80 kg K_2O ha^{-1} (61.5%). Fungi of genus *Gliocladium* and order *Mucorales* most frequently colonized the roots of fertilized plants and their shares were 2.4 and around 20% respectively.

Recapitulating the results of these studies we should point out the limitation in numbers of fungi in the oriental goat's rue cultivation environment in combination with mineral fertilization as compared to the experimental combination without fertilization. Similar regularities were presented by DEB and DUTTA (1992) in their studies on soy as well as by CWALINA-AMBROZIAK, MAJCHRZAK (2000b) in the earlier studies on the oriental goat's rue also. At the same time those studies revealed an increase in the numbers of fungi of genus *Penicillium*.

The results of antagonism test showed that fungi of genus *Trichoderma* limited growth of colonies of the tested pathogens: *Botrytis cinerea* and genus *Fusarium*. The smallest colony sizes and, as a consequence, the highest effectiveness in inhibiting growth of the mycelium of *F. culmorum* were recorded in case of *T. hamatum* presence (62.5%) as well as of the mycelium of *F. fusarioides* in case of *T. viride* (61.9%) presence were recorded as compared to the controls (Figure 4). The lowest competitiveness characterized all three species of genus *Trichoderma* during simultaneous growth with *B. cinerea* (in average 27.1% inhibition of mycelium growth, significantly lower

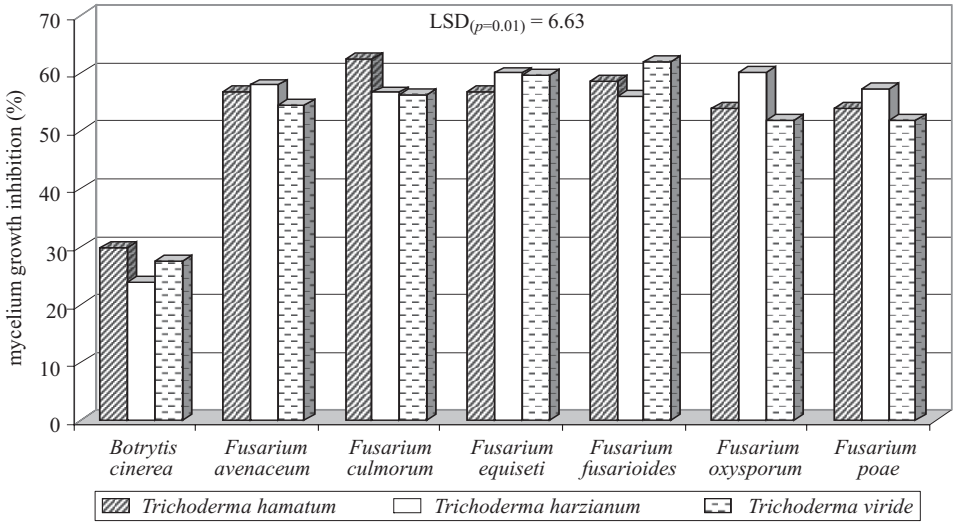


Fig. 4. Percentage of pathogenic fungi mycelium growth inhibition in PDA medium in the presence of fungi with genera *Trichoderma*

as compared to other pathogens tested). The recorded positive individual biotic effects confirm the limiting influence of antagonistic species of genus *Trichoderma* on the pathogens tested (Table 4).

Table 4

Biotic effects of fungi with *Trichoderma* genera against pathogenic fungi

Pathogens	*Trichoderma hamatum*	*Trichoderma harzianum*	*Trichoderma viride*
Botrytis cinerea	5*	5	5
Fusarium avenaceum	7	6	7
Fusarium culmorum	7	6	6
Fusarium equiseti	7	7	7
Fusarium fusarioides	7	7	7
Fusarium oxysporum	6	7	6
Fusarium poae	6	7	6

* positive values

Conclusions

1. Mineral fertilization influenced the number and composition of species in the population of fungi populating the root zones of oriental goat's rue. The least numerous population of potentially pathogenic fungi was obtained from the rhizoplane of oriental goat's rue in both combinations with fertilization and from the roots of plants in combination with the lower level of fertilization.

2. Fungi of genus *Fusarium* were present in the analyzed fungal populations in all the years of study. They were isolated more frequently from the rhizoplane and roots of oriental goat's rue than from the rhizosphere.

3. Species of genus *Trichoderma* tested in the laboratory showed strong antagonistic influence in relation to the pathogens tested.

Translated by Aleksandra Poprawska

Accepted for print 19.10.2007

References

Angus J.F., Hervaarden A.F. van , Howe G.N. 1994. *Productivity and break crop effects of winter-growing oilseeds.* Aust. J. Exp. Agric., 31: 669-677.

Bowen G.D., Rovira A.D. 1999. *The rhizosphere and its management to improve plant growth.* Adv. Agronomy, 66: 1-102.

Cwalina-Ambroziak B., Majchrzak B. 2000a. *Grzyby występujące na nasionach rutwicy wschodniej (Galega orientalis* Lam.). Acta Agrobot., 53(2): 15-23.

Cwalina-Ambroziak B., Majchrzak B. 2000b. *The structure of fungal population from Galega orientalis root system formed as the result of fertilization.* Acta Mycologica, 35(2): 311-321.

DEB P.R., BORA K.N. 1996. *Effect of chemical fertilizer on the rhizosphere mycoflora and nodulation of pea plant.* Environment Ecology, 14(4): 747-751.

DEB B., DUTTA B.K. 1992. *Effect of inorganic fertilizers on the development of foot rot diseases of soybean caused by Sclerotinia rolfsii.* Indian Phytopathol., 45(1): 89-97.

DORENDA M. 1986. *Badania mikoflory środowiska uprawnego koniczyny czerwonej i kupkówki pospolitej w aspekcie fitopatologicznym.* Acta Mycol., 22(1): 15-34.

FUNCK-JENSEN D., HOCKENHULL J. 1984. *Root exudation, rhizosphere microorganisms and disease control.* Vaxtskyddsnotier, 48: 49-54.

HILAL M.H., EL-LAKKANY H., EL-SHEEMY H. 1992. *Effect of sulphur and long term fertlizer application program on rhizosphere activity and yield of peanuts in a samdy soil.* Proceedings Middle East Sulphur Symposium 12-16 February 1990, Cairo, Egypt., pp. 217-227.

LEDERER W., LORENZ K. H, SEEMULLER E. 1992. *Studies on antagonistic effect of Trichoderma isolates against Phytophthora cactorum.* J. Phytopathol., 136(2): 154-164.

MAŃKA K. 1990. *Saprofityczna mikoflora środowiska glebowego a zdrowotność roślin.* Phytopathol. Pol., XI: 122-134.

NOWICKI B. 1995. *Patogeniczne grzyby zasiedlające nasiona łubinu wąskolistnego.* Acta Agrobot., 48: 59-64.

PASTUCHA A. 1999. *Ochronne działanie mikroorganizmów antagonistycznych w ograniczeniu występowania chorób korzeni soi (Glycine max (L.) Merrill).* Ann. Univ. Mariae Curie-Skłodowska, E, 7: 119-135.

PATIL R.B., VARADE P.A. 1998. *Microbial population in rhizosphere as influenced by high input rates of fertilizer application to sorghum an a Vertisol.* J. Indian Soc. Soil Sc., 2: 223-227.

RODRIQUEZ R.F., COTES M. 1999. *Biological control of the bean root rot (Rhizoctonia solani Kuhn) using pregermination control in the presence of Trichoderma koningii Qudemans.* ASCOLFI Informa, 25(1): 3-4.

SEHGAL J.S., SHANKER V., SEHGAL H.S., POKHRIYAL T.C. 1992. *Effect of nitrogen fertilizers on the nitrogenase activity and rhizosphere microflora in Enterolobium timbouva Mart.* Indian Forest. 118(3): 218-226.

STRZELCZYK A. 1968. *Metody badania grzybów glebowych*. Roczniki Gleboznawcze, 19(2): 405-424.

POLISH JOURNAL OF NATURAL SCIENCES
Abbrev.: Pol. J. Natur. Sc., Vol 23(2): 284-298, Y. 2008

DOI 10.2478/v10020-008-0022-2

EXCESSES AND DEFICIENCIES OF PRECIPITATION IN NORTH-EASTERN POLAND

***Jan Grabowski*[1], *Irena Brzozowska*[2], *Stanisław Suchecki*[1], *Ewelina Olba-Zięty*[1]**

[1] Chair of Meteorology and Climatology
[2] Chair of Agricultural Systems
University of Warmia and Mazury in Olsztyn

K e y w o r d s: mesoregion, precipitations, trends of precipitations, extreme precipitations.

A b s t r a c t

This paper aims at presenting the trends in sums of precipitations for years and year quarters and assessment of extreme precipitations appearance in three selected locations of north-eastern Poland. Those locations belong to different mesoregions of Mazury Lake District. Bałcyny is located in Iława Lake District, Łężany in Mrągowo Lake District and the study for those locations covered the years 1959-1995. Pozorty-Tomaszkowo is situated in Olsztyn Lake District and the study for that location covered the years 1953-1995. The characteristics of precipitations during the above periods are presented on the basis of criteria elaborated by KACZOROWSKA (1962). The variability of precipitations during the periods covered was as follows: Pozorty-Tomaszkowo 21%, Łężany 22% and Bałcyny 23%. The average volume of precipitations during the periods covered was from 593 mm in Pozorty-Tomaszkowo to 605 mm in Bałcyny and 596 mm in Łężany while the trends analysis showed decreasing trends that were statistically insignificant.

As concerns the volumes of precipitations during individual years of the periods covered compared to long-term standards, the largest number of average years (90-110% of the standard) was recorded for Pozorty-Tomaszkowo – 44%, for Łężany – 40%, and for Bałcyny – 32%.

NADMIARY I NIEDOBORY OPADÓW W POLSCE PÓŁNOCNO-WSCHODNIEJ

***Jan Grabowski*[1], *Irena Brzozowska*[2], *Stanisław Suchecki*[1], *Ewelina Olba-Zięty*[1]**

[1] Katedra Meteorologii i Klimatologii
[2] Katedra Systemów Rolniczych
Uniwersytet Warmińsko-Mazurski w Olsztynie

S ł o w a k l u c z o w e: mezoregion, opady atmosferyczne, tendencje opadów atmosferycznych, opady ekstremalne.

Address: Jan Grabowski, University of Warmia and Mazury, pl. Łódzki 1, 10-727 Olsztyn, phone: (089) 523 49 39, e-mail: jan.grab@uwm.edu.pl

A b s t r a k t

Celem pracy jest przedstawienie tendencji sum opadów atmosferycznych dla lat i kwartałów oraz ocena występowania opadów ekstremalnych w trzech wybranych miejscowościach Polski północno-wschodniej (Bałcyny, Łężany, Pozorty-Tomaszkowo). Miejscowości te należą do różnych mezoregionów Pojezierza Mazurskiego. Bałcyny znajdują się na Pojezierzu Iławskim, Łężany na Pojezierzu Mrągowskim, a Pozorty-Tomaszkowo należą do Pojezierza Olsztyńskiego. Okres badań w dwóch pierwszych miejscowościach obejmował lata 1959-1995, a w trzeciej – 1953-1995. Charakterystykę opadów w powyższych okresach przedstawiono na podstawie kryteriów opracowanych przez KACZOROWSKĄ (1962), a ich zmienność wynosiła: w Pozortach-Tomaszkowie – 21%, w Łężanach – 22% i Bałcynach – 23%. Średnia wielkość opadów kształtowała się od 593 mm w Pozortach-Tomaszkowie do 605 mm w Bałcynach, w Łężanach wynosiła zaś 596 mm, miała tendencję spadkową i była nieistotna statystycznie.

Pod względem wielkości opadów w poszczególnych latach badań okresów, w stosunku do przyjętych norm wieloletnich, najwięcej lat przeciętnych (90-110% normy) wystąpiło w Pozortach-Tomaszkowie – 44%, w Łężanach – 40%, a w Bałcynach – 32%.

Introduction

Scientific and technological developments result in an increasing dependence of man on climatic conditions. In Poland, the appearance of extreme weather conditions is characterized by high irregularity over both the time and space. That results from the geographic position as well as characteristics of the climate (BORYCZKA et al. 1992, TAMULEWICZ, STACH 2005). Determination of frequency and predictability of extreme weather conditions allows preventing negative consequences of their appearance. Depending on the type of unfavourable weather components, their intensity, level of agricultural technique, soil quality and plants development stage, the losses in yields of crops can reach as much as several tens percent (*Atlas*... 2001). During the last 50 years, the frequency of appearance of extreme weather phenomena such as strong winds, droughts and floods that could be the consequence of, among others, anthropogenic causes of climate changes, increased (SADOWSKI 2001, STANKIEWICZ 2004). Poland, as concerns the volume of precipitations, holds one of the end positions in the ranking of European countries while appearance of the precipitations is characterized by high variability over time and space in individual agricultural-climatic regions. The total year precipitations in Poland are characterized by parallel zoning. The lowest volume of precipitations is recorded in central Poland (525-550 mm); the volume is higher in the north (600-700 mm) while the largest volumes are recorded in the south (600-800 mm) and in the high mountain areas (up to 1700 mm). As concerns spatial distribution of precipitations, north-eastern Poland belongs to the northern zone with precipitations exceeding 600 mm. Also the number of days with precipitations in that part of Poland increases from 160

in the southern part to 180 in Mrągowo Lake District (TAMULEWICZ, STACH 2005). Abundance, that is the volume in mm per an average day with precipitations, is another indicator for precipitations. In the north-eastern part of Mazury Lake District that value increases (36-38 mm) while in the northern part it decreases (34-30 mm) – TAMULEWICZ 1993. Precipitations in north-eastern Poland are larger than in other regions of the country and their distribution in relation to water demand of crops is unfavourable (GRABOWSKI 1994). During individual seasons, including the vegetation periods, periods with either excess of deficit of precipitations happen frequently (KAPUŚCIŃSKI, NOWAK 2003a,b,c). Most frequently precipitations higher than standard appear during the period from January to April (over 150%) which mitigates, among others, consequences of warm winters. In particular during the recent years frequent warm winters have been recorded, which leads to a decrease in soil water retention level (KAPUŚCIŃSKI, KARLIŃSKI 2003a). On the other hand, during the maximum demand of plants for water (May, June) the precipitations reach around 80% of the standard only (KAPUŚCIŃSKI, KARLIŃSKI 2003b). This leads to appearance of droughts and dry periods, mainly in the areas with the lowest precipitations (KAPUŚCIŃSKI, NOWAK 2003a). Bots excess and shortages of precipitations cause, among others losses in crops resulting in the increase of production costs.

This paper aims at presenting the trends in sums of precipitations for years and year quarters as well as assessment of appearance of extreme precipitations in three selected locations in north-eastern Poland belonging to different mesoregions of Mazury Lake District.

Materials and Methods of Study

The characteristic of precipitations' volumes and trends in north-eastern Poland are presented on the basis of results from meteorological observations conducted in three locations belonging to different mesoregions of north-eastern Poland: Bałcyny – Iława Lake District, 53° 40' N, 19° 50' E, Łężany – Mrągowo Lake District, 53° 58' N, 21° 18' E and Pozorty-Tomaszkowo – Olsztyn Lake District, 53° 42' N, 20° 26' E (Figure 1).

Those mesoregions are characterized by different physiographic and soil characteristics as well as different development of meteorological elements. The input material for this paper consisted of the results of precipitation measurements for the years:

– Bałcyny and Łężany: 1959-1995,
– Pozorty: 1953-1990 and Tomaszkowo: 1991-1995.

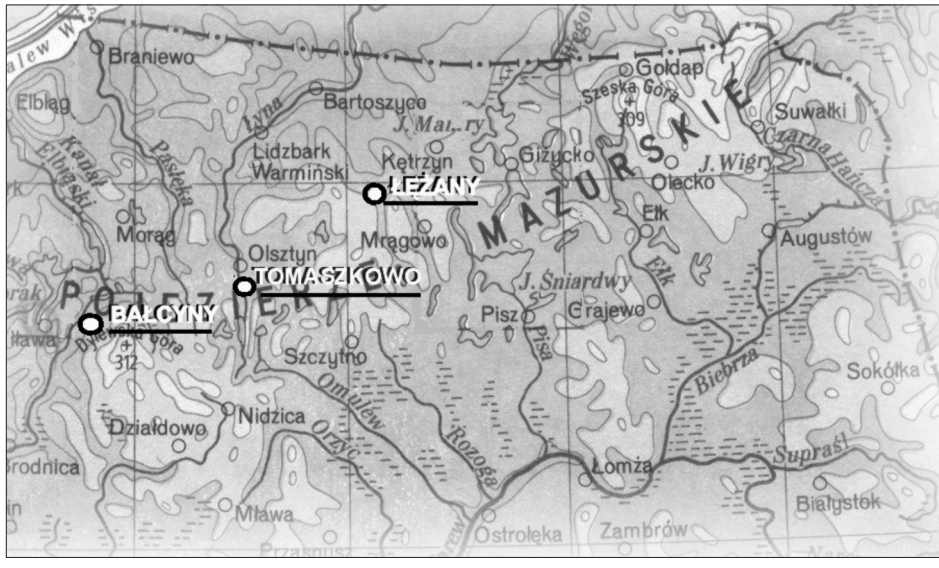


Fig. 1. Topographic map of Mazury Lake District

As a consequence of the fact that the Meteorological Station in Pozorty ceased the measurements in 1990 accomplished for the period of 1991-1995 observations made by the Meteorological Station in Tomaszkowo, around 2 km west of Pozorty were used.

In the analysis of precipitation conditions during the studied periods the following was considered:

– average total precipitations;
– month and year sums of minimum precipitations;
– month and year sums of maximum precipitations;
– trends of sums of precipitations for periods: III-V, VI-VIII, IX-XI, XII-II, I-XII.

Wet years were determined on the basis of excessive precipitations and dry ones on the basis of shortage of precipitations as compared to long-term average sums (KACZOROWSKA 1962).

The study also included computation of variability of precipitations in the localities covered.

Results and Discussion

The average sums of precipitations in the covered localities are presented in Figure 2. During the analyzed period the highest precipitations (over 125% of the standard) were diversified among the localities covered. In Bałcyny they

appeared in 1966, 1967,1970, 1974 and 1977 – 862 mm, 783 mm, 892 mm, 846 mm and 782 mm respectively. In Łężany they were recorded in 1966, 1967, 1970 and 1974 – 848 mm, 800 mm, 939 mm and 794 mm respectively. In Pozorty-Tomaszkowo the highest precipitations were recorded in 1956, 1966, 1970, 1972, 1974, 1978 and 1980 – 887 mm, 960 mm, 777 mm, 745 mm, 818 mm, 775 mm and 823 mm respectively.

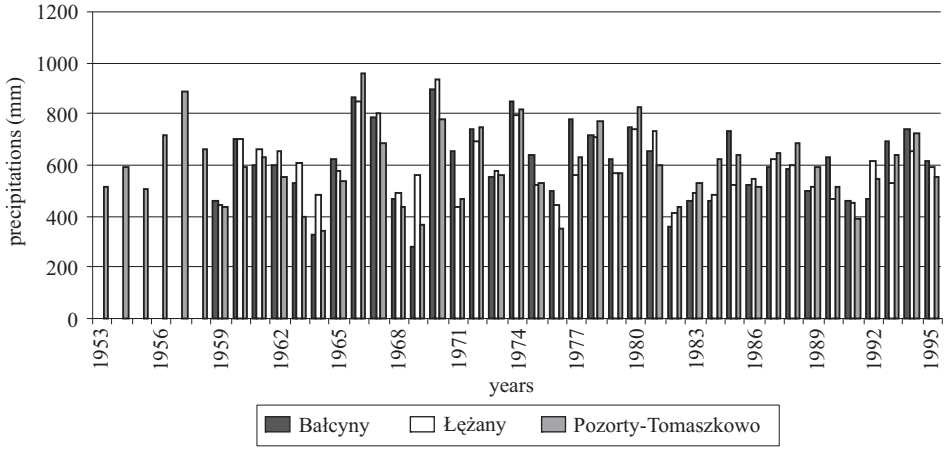


Fig. 2. Development of year sums of precipitations (mm) in selected locations during the years 1953-1995

The lowest precipitations in the analyzed locations (below 75% of the standard) occurred in Bałcyny in 1964, 1969 and 1982 – 328 mm, 280 mm and 356 mm respectively, in Łężany in 1959, 1971, 1976 and 1982 – 444 mm, 437 mm, 447 mm and 412 mm respectively and in Pozorty-Tomaszkowo in 1959, 1963, 1964, 1968, 1969, 1976, 1982, 1991 – 438 mm, 394 mm, 346 mm, 440 mm, 367 mm, 351 mm, 433 mm and 386 mm respectively.

The average sums of precipitations during the analyzed period were the highest in Bałcyny (605 mm), lower in Łężany (596 mm) and the lowest in Pozorty-Tomaszkowo (593 mm). The largest number of years with average precipitations (90-110% of the standard) was recorded in Pozorty-Tomaszkowo – 44%, lower in Łężany – 40% and the lowest in Bałcyny 32%. Additionally it was established that during the period covered the highest share of years with the lowest precipitations was recorded in Bałcyny (under 75% of the standard) – 27%, and in Pozorty-Tomaszkowo and Łężany the share was the same at 19%. The number of years with precipitations exceeding 125% of the standard was 14% in Bałcyny, 16% in Pozorty-Tomaszkowo and 19% in Łężany. The number of years with precipitations below 89% of the standard was 40%

in Bałcyny, 51% in Łężany and 63% in Pozorty-Tomaszkowo, including 19% of years with precipitations lower than 75% of the standard. For comparison, the study by GRABOWSKI (1994) concerning the distribution of precipitations in Bałcyny during the years 1972-1990 showed that the average level of precipitations for that period was 599 mm. That value corresponds to the value of average year precipitations for the region of Mazury Lake District for the years 1951-1995 (SZWEJKOWSKI et al. 2002). The variability of precipitations for the period of 1972-1990 in Bałcyny was 21% while for the years 1959 -1995 it was 23% while in case of the other locations it was from 21% in Pozorty-Tomaszkowo to 22% in Łężany. The average sums of precipitations for the studied periods were higher than the average sums of precipitations for the period of 35 years (1961-1995): in Pozorty-Tomaszkowo by 17 mm, in Bałcyny by 5 mm and in Łężany they were lower by 8 mm respectively (*Atlas*. 2001) – Figure 2.

Table 1, Table 2 and Table 3 present the highest and the lowest precipitations in the selected locations in Mazury Lake District. The data indicates high diversification over time and space of the extreme precipitations. The lowest precipitations in the specific locations were recorded during the cold half of the year. Among the analyzed locations the precipitations did not occur at all in Łężany in February, May, October and December 1959. For the period of April–September the average sum of lowest precipitations in Łężany and Pozorty-Tomaszkowo was 81 mm each and for Bałcyny – 59 mm. Analyzing the highest month precipitations during the periods covered it can be concluded that they exceeded from 2 to 10 times the standards accepted for those areas

Table 1

Extreme month sums of precipitations in Bałcyny during the years 1959-1995

Item	Month											
	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII
Minimum (mm)	3	0	2	4	2	4	24	15	10	3	10	2
Year	1963	1972	1964	1988	1971	1969	1971	1967	1982	1979	1959	1968
Maximum (mm)	49	74	68	90	144	161	201	187	120	227	82	86
Year	1976	1967	1967	1977	1962	1985	1960	1972	1971	1974	1973	1967

Table 2

Extreme month sums of precipitations in Łężany during the years 1959-1995

Item	Month											
	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII
Minimum (mm)	2	0	3	4	0	26	19	14	18	0	12	0
Year	1971	1959	1969	1988	1959	1977	1991	1984	1959	1959	1959	1960
Maximum (mm)	69	48	77	83	136	155	170	190	112	248	127	93
Year	1976	1973	1983	1970	1967	1974	1960	1970	1978	1974	1963	1967

Table 3
Extreme month sums of precipitations in Pozorty-Tomaszkowo during the years 1953-1995

Item	Month											
	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII
Minimum (mm)	7	1	2	6	13	5	10	1	22	0	18	1
Year	1972	1976	1964	1976, 1988	1963	1969	1989	1955	1982	1979	1954	1968
Maximum (mm)	80	58	89	97	134	201	193	173	160	160	85	91
Year	1970	1957	1994	1994	1967	1974	1960	1978	1984	1956	1969	1954

(*Atlas*... 2001). In Bałcyny the highest maximum month precipitations occurred in October 1974 – 227 mm and July 1960 – 201 mm, in Łężany also in October 1974 – 248 mm and August 1970 – 190 mm and in Pozorty-Tomaszkowo in June 1974 – 201 mm and August 1978 – 173 mm.

Figures 3-17 present the trends of precipitations for the studied locations during year and year-quarter periods. During the analyzed period a decreasing trend in precipitations, similar to the twenty year period of 1956-1982 for Wielkopolska occurred in all locations (KAPUŚCIŃSKI 2000). The highest decrement in the trend value for precipitations for the period of I-XII occurred in Łężany (Figure 4), the lowest in Pozorty-Tomaszkowo (Figure 5), and intermediate in Bałcyny (Figure 3). In Łężany, during the 37 years covered by the study, precipitations lower than the average sum (605 mm) for the analyzed period occurred during 18 years, in Bałcyny (596 mm) during 21 years and in case of Pozorty-Tomaszkowo for the period of 43 years precipitations lower than the computed average sum (593 mm) occurred during 24 years. During the 37 years covered by the study, in Bałcyny and Łężany, there were 35% of years with precipitations below 90% of the average long-term sum and

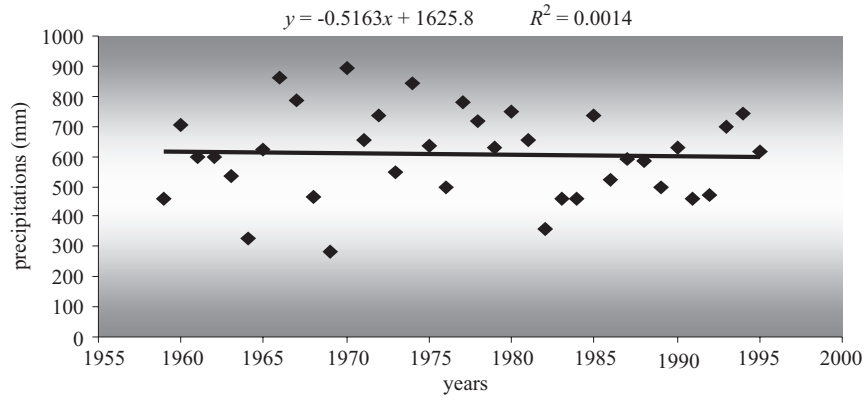


Fig. 3. Trend of the year sum of precipitations in Bałcyny during the years 1959-1995

respectively 29% and 24% with precipitations exceeding 110% of that average. At the same time in Pozorty-Tomaszkowo, for the 43 years covered by the study the share of years with precipitations below 90% of the long-term average was 46% while that with precipitations exceeding 110% of the standard was 28%. The share of years with precipitations under 75% of the standards was 45% in Bałcyny, and 58% in Pozorty-Tomaszkowo. During the analyzed period there were no extremely dry years with precipitations under 50% of the standard and extremely wet years with precipitations exceeding 150% of the standard (KACZOROWSKA 1962).

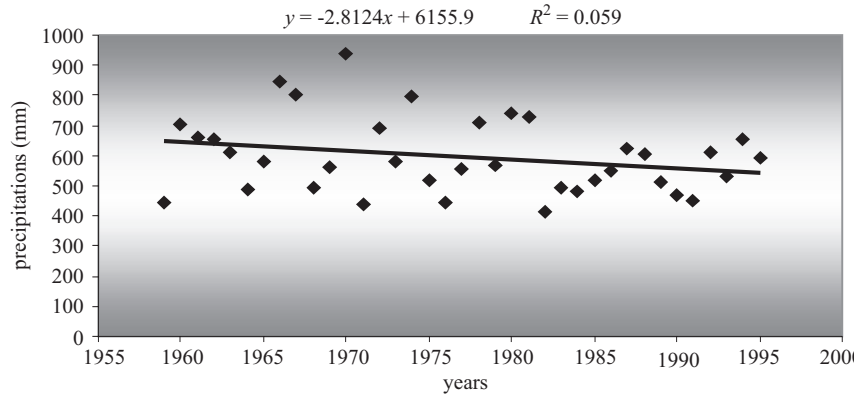


Fig. 4. Trend of the year sum of precipitations in Łężany during the years 1959-1995

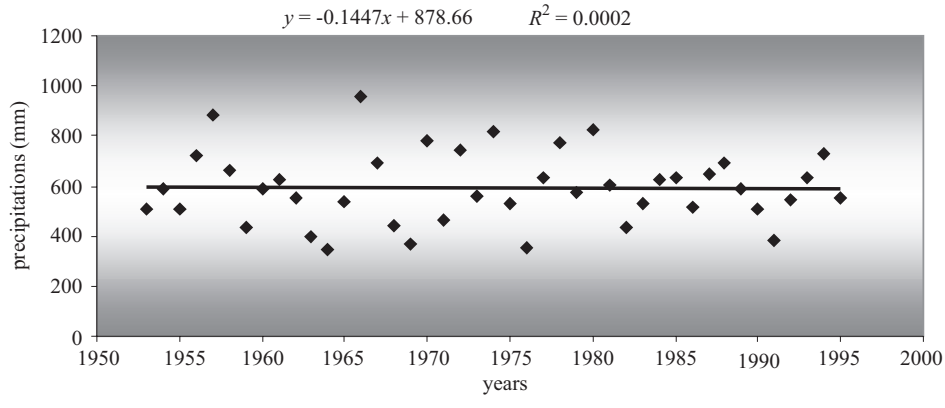


Fig. 5. Trend of the year sum of precipitations in Tomaszkowo during the years 1953-1995

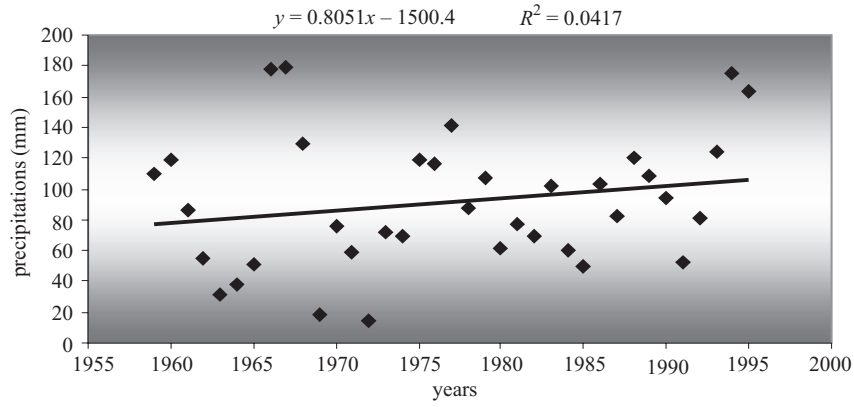
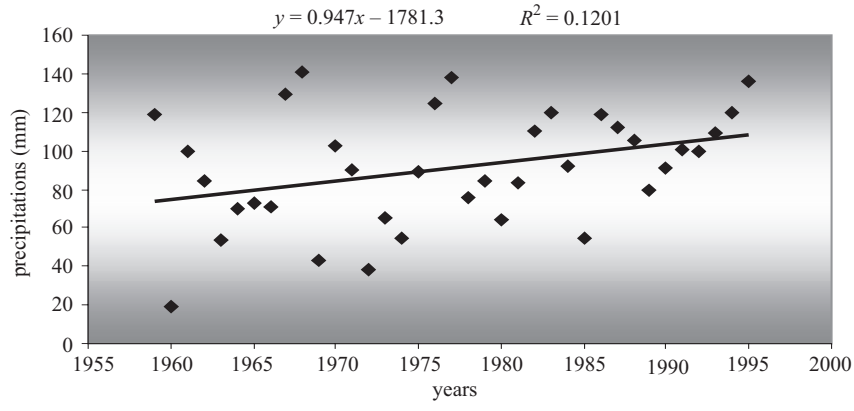


Fig. 6. Trend of the sum of precipitations in Bałcyny during the period of XII-II for years 1959-1995



*statistically significant trend

Fig. 7. Trend of the sum of precipitations in Łężany during the period of XII-II for years 1959-1995

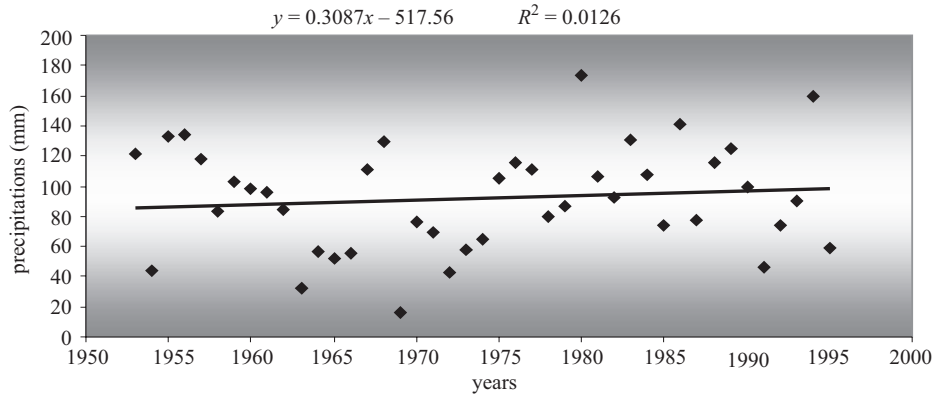


Fig. 8. Trend of the sum of precipitations in Pozorty-Tomaszkowo during the period of XII-II for years 1953-1995

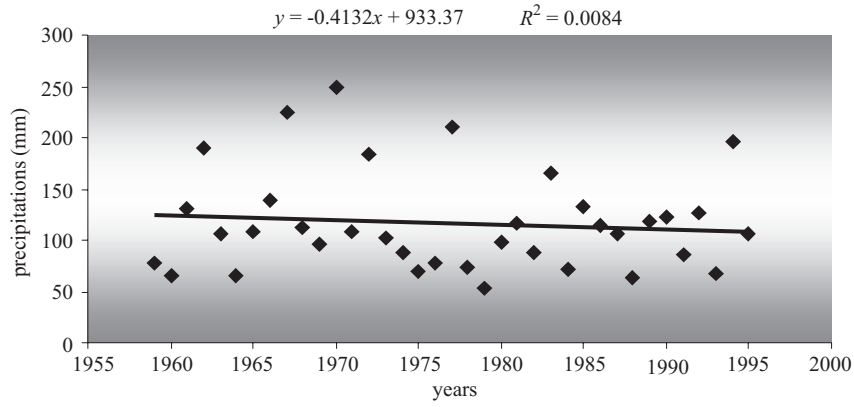


Fig. 9. Trend of the sum of precipitations in Bałcyny during the period of III-V for years 1959-1995

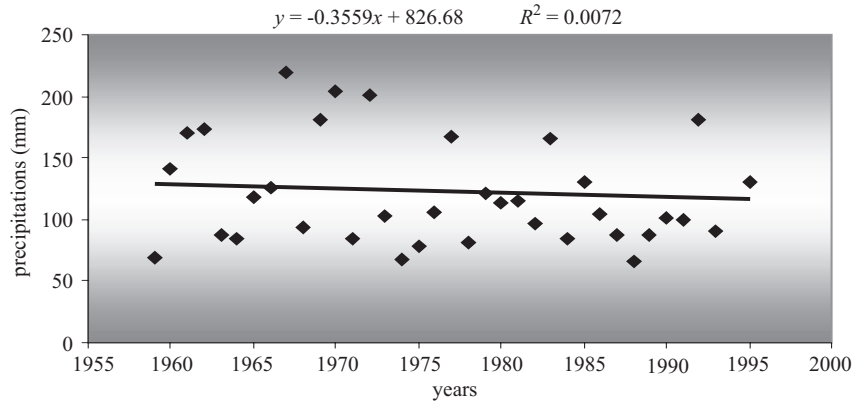


Fig. 10. Trend of the sum of precipitations in Łężany during the period of III-V for years 1959-1995

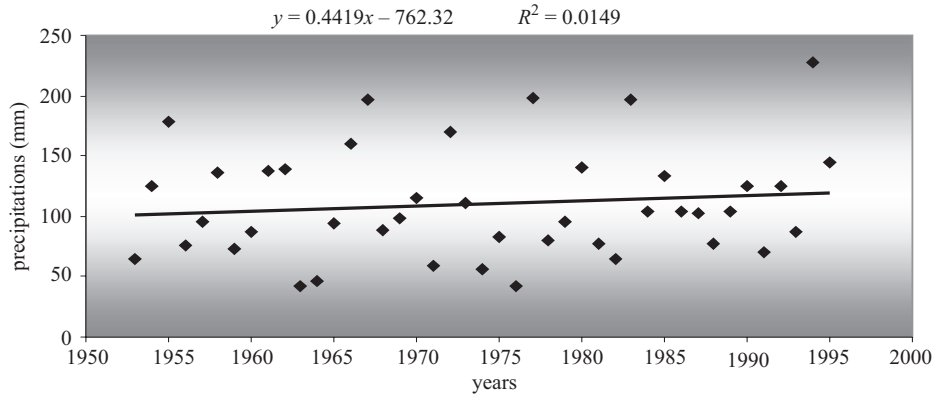


Fig. 11. Trend of the sum of precipitations in Pozorty-Tomaszkowo during the period of III-V for years 1953-1995

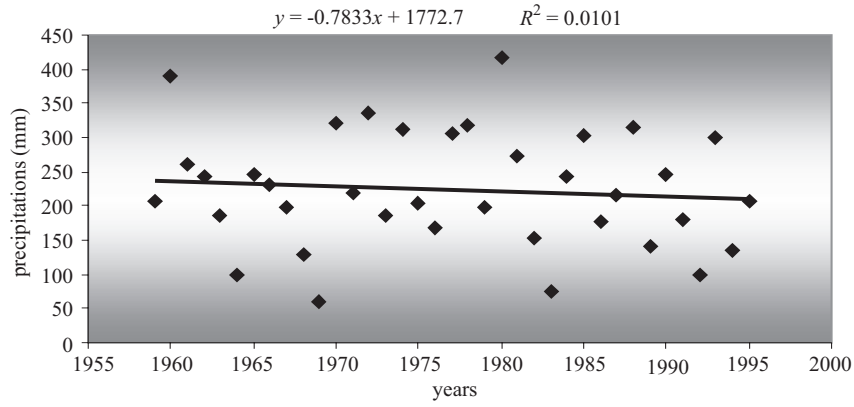
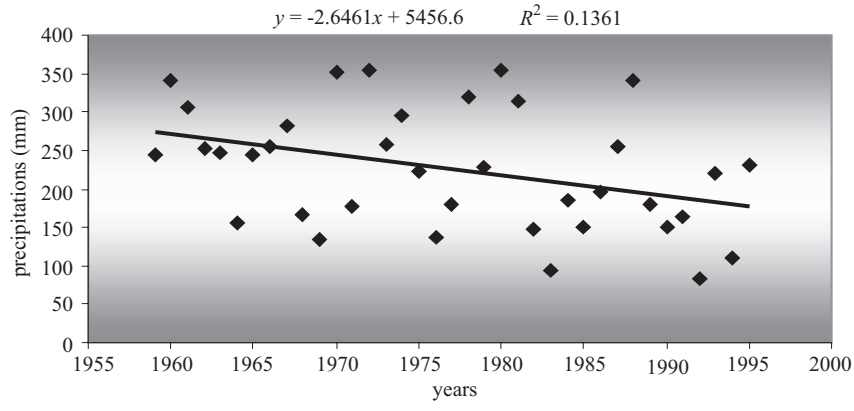


Fig. 12. Trend of the sum of precipitations in Bałcyny during the period of VI-VIII for years 1959-1995



* statistically significant trend

Fig. 13. Trend of the sum of precipitations in Łężany during the period of VI-VIII for years 1959-1995

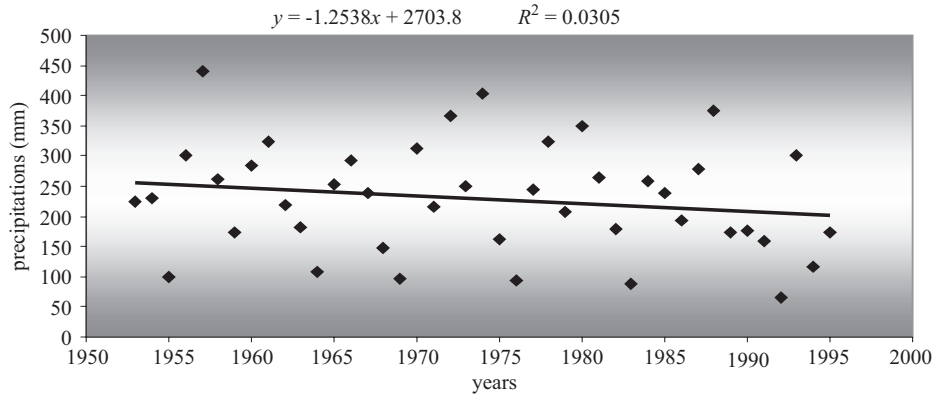


Fig. 14. Trend of the sum of precipitations in Pozorty-Tomaszkowo during the period of VI-VIII for years 1953-1995

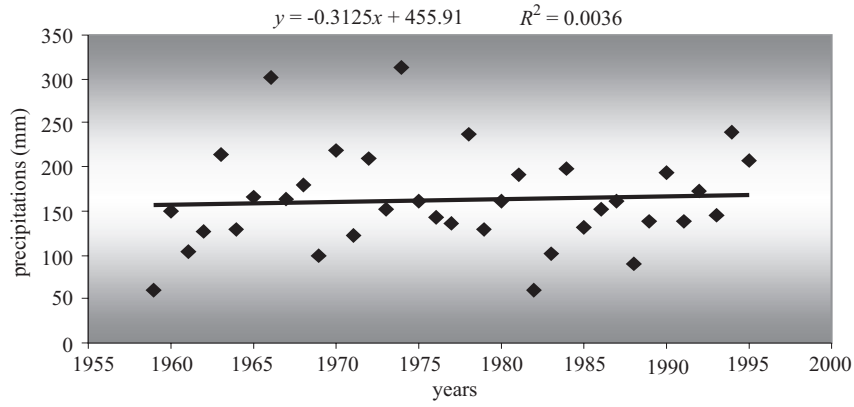


Fig. 15. Trend of the sum of precipitations in Bałcyny during the period of IX-XI for years 1959-1995

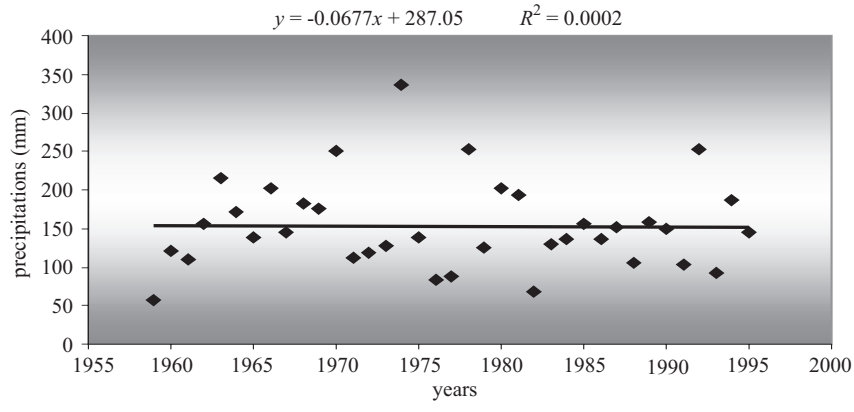


Fig. 16. Trend of the sum of precipitations in Łężany during the period of IX-XI for years 1959-1995

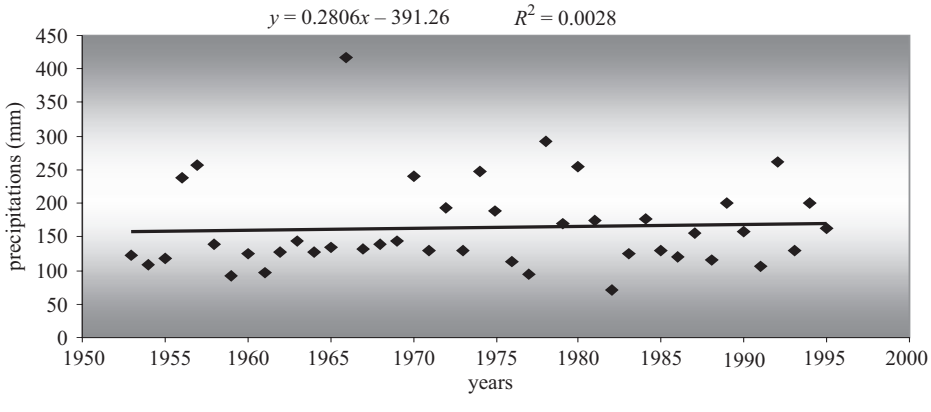


Fig. 17. Trend of the sum of precipitations in Pozorty-Tomaszkowo during the period of IX-XI for years 1953-1995

Figures 6, 7 and 8 present the development in trends of precipitations during the winter period (XII-II). The volume of those precipitations compared to the other analyzed year-quarters in the locations covered was the lowest, which results from the geographic position and the characteristics of Polish climate. At the individual locations the precipitations volume trends are increasing in value but only the increase in the trend of precipitations volume for the Meteorological Station in Łężany is statistically significant. The average volume of precipitations during the analyzed period (XII-II) during the years 1959-1995 was 91 mm in Bałcyny and Łężany and for Pozorty-Tomaszkowo for the years 1953-1995 it was 92 mm.

The presented trends of precipitations for the period III-V (Figure 9, 10) for Bałcyny and Łężany show a decreasing value, similar to Wielkopolska for the years 1956-1992 (KAPUŚCIŃSKI, NOWAK 2003c). The value of the decrease in the trend of precipitations in the analyzed mesoregions was lower compared to the decreasing trend of precipitations for Wielkopolska (KAPUŚCIŃSKI, NOWAK 2003c). In Bałcyny there were 21 years with precipitations lover than the average sum (116 mm) for the period covered and in Łężany 24 years with the quarterly volume of 123 mm. In Pozorty-Tomaszkowo (Figure 11), with the average precipitations of 110 mm, the increasing trend occurred and during the 43 years covered 26 years with precipitations below the computed average value were recorded.

In Poland the summer period, particularly the months VI-VIII are characterized by the most abundant precipitations as compared to the other year--quarters. The trends of precipitations presented in Figures 12, 13 and 14 show a decreasing trend. In Łężany the value of that trend for the period of 37 years (1959-1995) was statistically significant. The average volume of precipitations at the studied locations for the period of VI-VIII was 224 mm in Bałcyny, 225 mm in Łężany and 229 mm in Pozorty-Tomaszkowo.

Presented trends in precipitations for the period of IX-XI for Bałcyny and Łężany (Figures 15, 16) showed a decreasing value similarly to the majority of year-quarters at the studied locations while in Pozorty-Tomaszkowo the trend showed a slightly increasing value (Figure 17). The precipitations trends development in the three locations covered was statistically insignificant. As shown, in those locations the trends for periods of III-V, VI-VIII and IX-XI represented a decrease, which corresponded to the 36 years period (1956-1992) for Wielkopolska (KAPUŚCIŃSKI 2005). The exceptions are slightly increasing values of trends (statistically insignificant) for Pozorty-Tomaszkowo for the periods of III-V and IX-XI. That could be caused by the physiographic conditions of that location causing a larger thermally continental character of that area, which influences thermal and anemometric conditions of that place (GRABOWSKI 1994).

Among the analyzed year quarters for the periods of 37 and 43 years at locations covered, for the period of VI-VIII the share of years with precipitations lower than 90% of the standard in Bałcyny was 59%, in Pozorty-Tomaszkowo 39% and in Łężany 38%.

Conclusions

1. Major differentiation in the volume of precipitations during the analyzed long-term period was found between locations covered situated in different mesoregions of north-eastern Poland.

2. The largest number of years with average precipitations of from 90 to 110% of the standard was recorded in Pozorty-Tomaszkowo. The number was smaller in Łężany and the smallest in Bałcyny.

3. Among the three locations analyzed as concerns the volume of precipitations during the period covered the largest number of dry years with precipitations at 89-75% of the standard was recorded in Bałcyny.

4. The determined year trends of precipitations for the studied locations showed a decrease but the decrease was statistically insignificant; the trend of the sum of precipitations in Łężany was closest to significance.

5. The trends of the volumes of precipitations for the winter period (XII-II) were increasing but that increase was significant in case of the Meteorological Station in Łężany only. During the other year quarters for the analyzed long-term periods (37 and 43 years) the largest decreases in trends of precipitations were determined for the periods of III-V and VI-IX; in case of Pozorty-Tomaszkowo during the periods of III-V and IX-XI a slight increase in precipitations was recorded.

6. In the analyzed locations the number of years with precipitations lower than the average sum for the period covered was much higher than the number of years with precipitations higher than the average.

7. For the analyzed locations no regularity in appearance of years with precipitations close to long-term standards or extreme precipitations were determined.

Translated by JOANNA JENSEN

Accepted for print 14.09.2007

References

Atlas klimatycznego ryzyka uprawy roślin w Polsce. 2001. Wyd. AR Szczecin, Uniwersytet Szczeciński.

BORYCZKA J., STOPA-BORYCZKA M., KICIŃSKA B., ŻMUDZKA E. 1992. *Atlas współzależności parametrów meteorologicznych i geograficznych w Polsce.* VII. *Zmiany warunków klimatycznych Polski*. Wyd. UW, Warszawa.

GRABOWSKI J. 1994. *Charakterystyka opadów atmosferycznych w RZD Bałcyny w latach 1972-1990*. Abbrev. Acta Acad. Agricult. Techn. Olsztyn, 58: 59-67.

KACZOROWSKA Z. 1962. *Opady w Polsce w przekroju wieloletnim*. Instytut Geografii, Warszawa.

KAPUŚCIŃSKI J. 2000. *Struktura bilansu cieplnego powierzchni czynnej na tle warunków klimatycznych środkowo-zachodniej Polski*. Rozprawy naukowe. Roczn. AR w Poznaniu, 303, ss. 249.

KAPUŚCIŃSKI J., KARLIŃSKI M. 2003a. *Roczny przebieg średniej miesięcznej temperatury powietrza w Poznaniu w latach 2000-2002 na tle wielolecia*. [W:] *Kształtowanie i ochrona środowiska leśnego. Klimat a las*. Red. A. Miler, 7(1): 65-70.

KAPUŚCIŃSKI J., KARLIŃSKI M. 2003b. *Roczny przebieg sum miesięcznych opadów atmosferycznych w okresie 2000-2002 w Poznaniu na tle wielolecia*. [W:] *Kształtowanie i ochrona środowiska leśnego. Klimat a las*. Red. A. Miler, Wyd. AR Poznań, ss. 71-76.

KAPUŚCIŃSKI J., NOWAK R. 2003a. *The frequency of the occurrence of droughts and post-droughts periods in mid-west Poland on the example of Poznań, Wałcz and Wieluń*. [W:] *Kształtowanie i ochrona środowiska leśnego. Klimat a las*. Red. A. Miler, Wyd. AR Poznań, 77-84.

KAPUŚCIŃSKI J., NOWAK R. 2003b. *A seasonal run of actual evapotranspiration and precipitation in coniferous and deciduous forests in mid-west Poland*. [W:] *Kształtowanie i ochrona środowiska leśnego. Klimat a las*. Red. A. Miler, Wyd. AR Poznań, 7(3): 85-91.

KAPUŚCIŃSKI J., NOWAK R. 2003c. *The analysis of the climatic water balance trend of deciduous and coniferous forestsin Wielkopolska on the example of Poznań*. [W:] *Kształtowanie i ochrona środowiska leśnego. Klimat a las*. Red. A. Miler, Wyd. AR Poznań, ss. 92-99.

SADOWSKI M. 2001. *Czy grozi nam klimatyczna apokalipsa*? Przegl. Geogr., 1/2: 9-14.

STANKIEWICZ W. 2004. *„Globalne ocieplenie" jako zagrożenie współczesnego świata i stosunków międzynarodowych*. Athenaeum, 13: 101-126.

SZWEJKOWSKI Z., NOWICKA A., DRAGAŃSKA E. 2002. *Klimat Pojezierza Mazurskiego*. I. *Temperatury i opady atmosferyczne w okresie 45-lecia 1951-1995*. Fragm. Agron., 2(74): 285-296.

TAMULEWICZ J. 1993. *Struktura pola opadów atmosferycznych Polski w okresie 1951-1980*. Wyd. Nauk. UAM w Poznaniu. S. Geografia, 56: 181.

TAMULEWICZ J., STACH A. 2005. *Analiza struktury pola opadów atmosferycznych Polski metodą składowych głównych powierzchni trendu*. [W:] *Funkcjonowanie geosystemów Polski w warunkach zmian klimatu i różnokierunkowej antropopresji*. Red. A. Kostrewski, R. Kolender, Biblioteka Monitoringu Środowiska, ss. 541-562.

POLISH JOURNAL OF NATURAL SCIENCES
Abbrev.: Pol. J. Natur. Sc., Vol 23(2): 299-309, Y. 2008

DOI 10.2478/v10020-008-0023-1

FUNGI ISOLATED FROM THE ROOTS AND STEM BASES OF SPRING WHEAT GROWN AFTER DIFFERENT CRUCIFEROUS PLANTS AS FORECROPS

***Barbara Majchrzak*[1], *Tomasz Paweł Kurowski*[1], *Adam Okorski*[2]**

[1] Chair of Phytopathology and Entomology
[2] Chair of Diagnostics and Plant Pathophysiology
University of Warmia and Mazury in Olsztyn

K e y w o r d s: spring wheat, roots, stem base, forecrop, cruciferous plants, fungi.

A b s t r a c t

The present study was conducted during the years 2000-2002 at the Production-Experimental Station of the University of Warmia and Mazury in Bałcyny near Ostróda to determine the health status of the roots and stem bases of spring wheat grown after the following spring cruciferous plants: oilseed rape (*Brassica napus* ssp. *oleiferus* Metz.), Chinese mustard (*Brassica juncea* L.), white mustard (*Sinapis alba* L.), radish (*Raphanus sativus* var. *oleiferus* L.), false flax (*Camelina sativa* L.), Spanish colewort (*Crambe abbysinica* Hoechst.) and oat (*Avena sativa* L.) which served as control. A mycological analysis of the roots and stem bases of spring wheat was performed at the tillering stage (GS 28) and at the dough stage (GS 87) respectively.

It was found that the species composition and abundance of fungal communities were affected by the forecrop and crop residue management (CRM) practices. A total of 551 fungal colonies were isolated from the roots of spring wheat. Fungi of the genus *Fusarium* were isolated most frequently (30.5% of all isolates). The species *Fusarium equiseti*, *F. culmorum* and *F. oxysporum* dominated among them. A total of 1605 fungal colonies were isolated from the stem bases of spring wheat. The highest total number of isolates was obtained from treatments which involved ploughing in stubble and straw (408 colonies). Regardless of the forecrop, members of the genus *Fusarium* posed the most serious threat to the stem bases of spring wheat (69.0% of all isolates). The dominant species was *F. avenaceum* (40.6% of all isolates), but *F. culmorum* (9.5%) and *F. equiseti* (8.4%) were also relatively abundant.

Address: Barbara Majchrzak, University of Warmia and Mazury, ul. Prawocheńskiego 17, 10-722 Olsztyn, Poland, phone: (089) 523 45 46, e-mail: barbara.majchrzak@uwm.edu.pl

GRZYBY WYIZOLOWANE Z KORZENI I PODSTAWY ŹDŹBŁA PSZENICY JAREJ UPRAWIANEJ PO RÓŻNYCH PRZEDPLONACH Z RODZINY KAPUSTNYCH

***Barbara Majchrzak*[1], *Tomasz Paweł Kurowski*[1], *Adam Okorski*[2]**

[1] Katedra Fitopatologii i Entomologii
[2] Katedra Diagnostyki i Patofizjologii Roślin
Uniwersytet Warmińsko-Mazurski w Olsztynie

S ł o w a k l u c z o w e: pszenica jara, korzenie, podstawa źdźbła, przedplon, rośliny krzyżowe, grzyby.

S t r e s z c z e n i e

W latach 2000-2002 w Zakładzie Produkcyjno-Doświadczalnym Uniwersytetu Warmińsko-Mazurskiego w Bałcynach k. Ostródy badano zdrowotność korzeni i podstawy źdźbła pszenicy jarej, uprawianej po jarych roślinach kapustnych: rzepaku jarym (*Brassica napus* ssp. *oleiferus* Metz.), gorczycy sarepskiej (*Brassica juncea* L.), gorczycy białej (*Sinapis alba* L.), rzodkwi oleistej (*Raphanus sativus* var. *oleiferus* L.), lniance siewnej (*Camelina sativa* L.), katranie abisyńskim (*Crambe abbysinica* Hoechst.) oraz po owsie (*Avena sativa* L.) jako kombinacji kontrolnej. Analizę mikologiczną korzeni pszenicy jarej przeprowadzono w fazie krzewienia (GS 28), natomiast podstawy źdźbła w fazie dojrzałości woskowej ziarna (GS 87).

Na zróżnicowanie składu gatunkowego i liczebność poznanych zbiorowisk grzybów wpływ miały: ukształtowane przez przedplon środowisko oraz sposób zagospodarowania resztek pożniwnych. Z korzeni pszenicy jarej wyizolowano ogółem 551 kolonii grzybów. Dominował rodzaj *Fusarium* (30,5% wszystkich wyosobnień), najczęściej występowały *Fusarium equiseti*, *F. culmorum* i *F. oxysporum*.

Ogółem z podstawy źdźbła pszenicy jarej wyosobniono 1605 kolonii grzybów. Najwyższą ogólną liczbę izolatów uzyskano ze źdźbeł pszenicy jarej uprawianej w obiektach, których przyorywano ścierń ze słomą (408 kolonii). Największe zagrożenie dla podstawy źdźbła pszenicy jarej, niezależnie od przedplonu, stanowiły grzyby z rodzaju *Fusarium* (69,0% ogółu wyosobnień). Zdecydowanie dominował gatunek *F. avenaceum* (40,6% wszystkich wyosobnionych grzybów). Dość liczną grupę izolatów stanowiły również gatunki *F. culmorum* (9,5%) i *F. equiseti* (8,4%).

Introduction

The health of wheat stem bases can be considerably improved by the right choice and sequence of rotation crops (KUROWSKI 2002, PLĄSKOWSKA 1996). Individual forecrops have a different impact on the soil environment, contributing to the formation of specific fungal communities, composed of saprotrophic, pathogenic and antagonistic species (BOJARCZUK et al. 1991, PLĄSKOWSKA 1996). If the proportion of cereals in crop rotation is high, particular attention should be paid to the selection of appropriate forecrops (PLĄSKOWSKA 2005). Forecrops not only improve the chemical, physical and biological properties of the soil, but also exert a positive influence of the growth and development of successive crops. Legumes, crucifers and oat may play an important role

in crop rotation (BOJARCZUK et al. 1991). The *Cruciferae* are among the best forecrops for cereals, because they leave in the soil large amounts of after--harvest residues rich in glucosinolates and other biologically active compounds (OLESZEK 1997). Oat roots secrete organic compounds inhibiting the growth of soil pathogenic fungi. Moreover, oat is only sporadically and weakly infested by pathogens causing take-all diseases (BOJARCZUK et al. 1991).

The objective of this study was to determine changes taking place in the species composition of fungal communities isolated from the roots and stem bases of spring wheat, depending on the forecrop and crop residue management practices.

Materials and Methods

The study was conducted during the years 2000-2002 at the Production--Experimental Station in Bałcyny near Ostróda (NE Poland), on the experimental plots of the Department of Plant Production, University of Warmia and Mazury in Olsztyn. The experiment was established on gray-brown podsolic soil developed from light silty clay, of quality class III a, of good wheat complex (2000 and 2001) or very good rye complex (2002). Mineral fertilizers (NPK) were applied at a rate of 90: 70: 100 kg ha^{-1}. Before sowing the seeds were dressed with Raxil 020 FS. Weeds were killed at the tillering stage with Chwastox Turbo 340 SL (MCPA + dicamba) in 2000, and Granstar 75 WG (tribenuron methyl) in 2001 and 2002. Disease prevention consisted in the single application, at the stem formation stage, of the following fungicides: Charisma 207 EC (flusilazole + famoxate) in 2000, Sanazol 250 EC (propiconazole) in 2001, and Alert 375 SC (flusilazole + carbendazim) in 2002. Spring wheat cv. Torka was grown after spring cruciferous plants and oat.

The field trial was performed in a randomized block design, in three replications. The experimental factors were as follows:
factor I – forecrops
– spring oilseed rape (*Brassica napus* f. *annua*) – cv. Margo,
– white mustard (*Sinapis alba*) – cv. Heter,
– Chinese mustard (*Brassica juncea* var. *sareptana*) – cv. Małopolska,
– radish (*Raphanus sativus* var. *oleifera*) – cv. Pegletta,
– false flax (*Camelina sativa*) – cv. Borowska,
– Spanish colewort (*Crambe abyssinica*)– cv. Borowski
– oat (*Avena sativa* L.) – cv. Bajka;
factor II – crop residue management
– ploughing in stubble (S)
– ploughing in stubble + straw (S+S)
– ploughing in stubble + straw + nitrogen (30 kg ha^{-1}) (S+S+N).

A mycological analysis of spring wheat roots was conducted at the tillering stage (GS 28), according to the method proposed by MARYNIUK, MYŚKÓW (1983). At the dough stage (GS 87) 1 cm sections were cut out of spring wheat stems with spots, disinfected in 50% ethyl alcohol and 1% sodium oxochlorate, rinsed in sterile water and transferred to a glucose-potato medium. Twenty plants representative of both development stages were selected randomly from each plot. Infected parts of spring wheat plants were transferred to artificial media in order to identify pathogens causing take-all diseases.

Results

A total of 551 fungal colonies belonging to 27 species as well as non-spore forming fungi and yeast-like fungi were isolated from the roots of spring wheat over the three-year experimental period (Table 1). Members of the genus *Fusarium* dominated among them (32.1% of all colonies). Species of the order *Mucorales* (31.2%) and *Alternaria alternata* (15.6%) were also abundant. A total of 177 fungal cultures of the genus *Fusarium* were isolated. The dominant species was *F. equiseti* (9.0% of all colonies), but *F. culmorum* (5.9%), *F. oxysporum* (3.9%) and *F. sambucinum* (3.4%) were also common. The second largest group of fungi colonizing the roots of spring wheat were members of the order *Mucorales*. 172 colonies of these fungi were isolated in the experiment. The most abundant among them was *Mucor hiemalis* (10.8% of all colonies). The largest fungal community was obtained from the roots of spring wheat grown after Spanish colewort (15.2%), false flax and oilseed rape (14.7% each), while the smallest from the roots of spring wheat grown after oat and radish (13.4% of all isolates).

Crop residue management practices had no significant effect on the population size of root-dwelling fungi. The smallest fungal community was isolated from the roots of spring wheat in treatments which involved ploughing in stubble and straw (32.6% of all isolates), and the largest in treatments where stubble + straw + nitrogen (30 kg ha^{-1}) were ploughed in. The abundance of fungi of the genus *Fusarium* was considerably affected by crop residue management. The most colonies of this genus (71) were isolated from the roots of spring wheat grown in plots where stubble and straw were ploughed in, whereas the fewest (51) from plots where the extra dose of nitrogen was applied. It was found that the decrease in the abundance of the most frequently isolated species of the genus *Fusarium* (*F. culmorum*, *F. equiseti*, *F. sambucinum*, *F. tricinctum*) resulted from the fact that 30 kg nitrogen ha^{-1} was added to straw and stubble.

Table 1

Fungi isolated from roots of spring wheat (number of isolates)

Fungal species	White mustard				Chinese mustard				Spanish colewort				False flax				Oilseed rape				Radish				Oat				Sum	%
	1*	2	3	Σ	1	2	3	Σ	1	2	3	Σ	1	2	3	Σ	1	2	3	Σ	1	2	3	Σ	1	2	3	Σ		
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31
Absidia glauca Hagem	2	1		*3*			1	*1*	5		2	*7*				*0*	2		3	*5*			2	*2*		1	2	3	21	3.8
Alternaria alternata (Fr.) Keissl.	2	3	4	*9*	7	3		*10*	5	2	5	*12*	2	5	4	*11*	6	3	4	*13*	7	5	3	*15*	7	2	7	*16*	86	15.6
Aureobasidium bolleyi (R. Sprague) Arx		1	2	*3*	1		2	*3*		2	1	3	1			*1*	2			*2*				0	1	2	1	*4*	16	2.9
Bipolaris sorokiniana (Sacc.) Shoemaker				*0*		1		*1*				0	3			*3*			1	*1*	1	1		*2*			4	4	11	2.0
Botrytis cinerea Pers.				*0*			1	*1*			1	*1*				*0*				*0*			1	*1*		2		2	5	0.9
Cylindrocarpon destructans (Zinssm.) Scholten		2		*2*	2			*2*	1	2	1	*4*				*0*	2	1		*3*		1		*1*				0	12	2.2
Fusarium avenaceum (Fr.) Sacc.			1	*1*			2	*2*			1	*1*	1			*1*		1	1	2	3			*3*				0	10	1.8
Fusarium culmorum (W.G. Sm.) Sacc.	2	6	1	*9*		1		*1*		2	2	*4*		5	2	*7*			2	*2*		2	2	*4*	5		1	*6*	33	6.0
Fusarium equiseti (Corda) Sacc.	2	1	1	*4*	3	3	3	*9*	8	2		*10*	5	2	5	*12*	6	2		*8*		1		*1*	2	4		*6*	50	9.1
Fusarium oxysporum Schltdl.	1	1	1	*3*	1		5	*6*		1	2	*3*		2	2	*4*		3		*3*	1			*1*	1		1	*2*	22	4.0
Fusarium poae (Peck) Wollenw.		2	1	*3*				*0*		1		*1*				*0*				*0*				*0*				*0*	4	0.7
Fusarium sambucinum Fuckel		5		*5*			1	*1*		1		*1*	1	4		*5*			2	*2*	1	2		*3*		2		*2*	19	3.4
Fusarium solani (Mart.) Sacc.				*0*	1	1	1	*3*				*0*				*0*				*0*				*0*		1		*1*	4	0.7
Fusarium tricinctum (Corda) Sacc.	1			*1*		1	3	*4*				*0*		2		*2*		5		*5*	1			*1*	1	2	1	*4*	17	3.1
Fusarium spp.		1		*1*	3	1		*4*				*0*		1		*1*				*0*			3	*3*				*0*	9	1.6
Gliocladium penicillioides Corda	1			*1*				*0*		1		*1*				*0*				*0*				*0*				*0*	2	0.4
Microdochium nivale (Fr.) Samuels & I.C. Hallett	1			*1*				*0*				*0*			3	*3*	3		1	*4*	1			*1*				*0*	9	1.6
Mortierella alpina Peyronel	2		1	*3*	1	5	2	*8*	6		3	*9*	4	6	4	*14*	2	4	2	*8*			2	*2*				*0*	44	8.0
Mortierella isabellina Oudem.			1	*1*	1	1	2	*4*	3	3		*6*		3	1	*4*		6		*6*				*0*			1	*1*	22	4.0
Mucor circinelloides Tiegh.				*0*				*0*				*0*				*0*				*0*	1			*1*				*0*	1	0.2
Mucor hiemalis Wehmer	3	4		*7*			3	*3*		4	6	*10*	3			*3*	5			*5*	5	6	11	*22*	3	1	6	*10*	60	10.9
Penicillium spp.	1			*1*				*0*				*0*				*0*				*0*		1		*1*				*0*	2	0.4

cont. table 1

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31
Pythium debaryanum R. Hesse				*0*			5	*5*				*0*				*0*				*0*				*0*				*0*	5	0.9
Rhizopus nigricans Ehrenb.	3	1	1	*5*		1	1	*2*		4	1	*5*	1	1	1	*3*	1	0	2	*3*	4	1	2	*7*	3	5	3	*11*	36	6.5
Spicaria simplicissima Oudem.				*0*				*0*	1			*1*				*0*				*0*				*0*				*0*	1	0.2
Trichoderma harzianum Rifai				*0*				*0*				*0*				*0*				*0*	2			*2*				*0*	2	0.4
Zygorhynchus heterogamus (Vuill.) Vuill.			2	*2*	1	2		*3*	1	2		*3*				*0*				*0*				*0*			1	*1*	9	1.6
Non-sporulating fungi	1			*1*	4			*4*			1	*1*	1			*1*			*1*	1	1			*1*	1			*1*	10	1.8
Yeast-like fungi		1	10	*11*	1	1		*2*		1		*1*	1		6	*7*	1		7	*8*				*0*	0			*0*	29	5.3
Sum	22	31	24		26	24	29		30	27	27		23	31	28		30	25	26		28	20	26		24	22	28		551	100
Sum of plant	77				79				84				82				81				74				74					

1* – S, 2 – S +Ś, 3 – S+Ś+N

Table 2

Fungi isolated from stem bases of spring wheat (number of isolates)

Fungal species	White mustard				Chinese mustard				Spanish colewort				False flax				Oilseed rape				Radish				Oat				Sum	%
	1*	2	3	Σ	1	2	3	Σ	1	2	3	Σ	1	2	3	Σ	1	2	3	Σ	1	2	3	Σ	1	2	3	Σ		
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31
Acremonium strictum W. Gams			2	*2*				*0*				*0*		6		*6*		3	2	*5*				*0*				*0*	13	1.1
Alternaria alternata (Fr.) Keissl.	4	7	2	*13*		7	8	*15*	2	2	2	*6*	9		3	*12*	3	2	5	*10*	4	1	3	*8*	9	6	8	*23*	87	7.5
Aureobasidium bolleyi (R. Sprague) Arx	4	3	1	*8*		3	1	4	3			*3*	1			*1*				*0*		2		*2*	2			*2*	20	1.7
Bipolaris sorokiniana (Sacc.) Shoemaker		2	2	*4*	2			*2*	3	1		*4*	1	5	3	*9*	2	2	2	*6*		1	0	*1*	1	2		*3*	29	2.5
Epicocum purpurascens Ehrenb. ex Schlecht	1			*1*	2			*2*		4		*4*				*0*	6			*6*	2			*2*			2	*2*	17	1.5
Fusarium avenaceum (Fr.) Sacc.	19	19	23	*61*	21	24	14	*59*	37	31	18	*86*	23	22	24	*69*	17	30	13	*60*	31	33	19	*83*	23	11	17	*51*	469	40.6
Fusarium culmorum (W.G. Sm.) Sacc.	5	6	1	*12*	11		11	*22*	5	5	3	*13*	6	6	7	*19*	4	2	1	*7*	9	6	4	*19*	6	6	6	*18*	110	9.5
Fusarium equiseti (Corda) Sacc.	7		11	*18*	2	10	6	*18*	3		8	*11*	2	3	5	*10*	12	4	2	*18*	1	2	4	*7*	5	8	2	*15*	97	8.4
Fusarium fusarioides (Gonz. Frag. & Cif.) C. Booth		12		*12*		1	1	*2*		2		*2*		2		*2*		1		*1*		1	5	*6*		8	9	17	42	3.6
Fusarium oxysporum Schltdl.	2			*2*	2			*2*		1		*1*		3	2	*5*	5			*5*		4		4	4		2	*6*	25	2.2
Fusarium poae (Peck) Wollenw.			1	*1*		6		*6*			2	*2*	1			*1*			1	*1*				*0*				*0*	11	1.0
Fusarium sambucinum Fuckel				*0*	1			*1*				*0*				*0*				*0*				*0*				*0*	1	0.1
Fusarium solani (Mart.) Sacc.	5		2	*7*	2			*2*	1			*1*	2		2	*4*	1	2	2	*5*				*0*				*0*	19	1.6
Fusarium tricinctum (Corda) Sacc.				*0*			4	*4*	1			*1*		3		*3*				*0*			1	*1*		3		*3*	12	1.0
Fusarium spp.		5		*5*			1	*1*				*0*				*0*		2		*2*			1	*1*			2	*2*	11	1.0
Gliocladium penicilliodes Corda				*0*				*0*		2		*2*				*0*				*0*				*0*				*0*	2	0.2
Halophylus ater Corda				*0*		3		*3*				*0*				*0*				*0*				*0*				*0*	3	0.3
Humicola fuscoatra Traaen				*0*				*0*				*0*				*0*				*0*				*0*	1			*1*	1	0.09
Microdochium nivale (Fr.) Samuels & I.C. Hallett	4	3		*7*	8			*8*				*0*	1	5		*6*	2			*2*			4	*4*	1	5		*6*	33	2.9
Mucor hiemalis Wehmer			2	*2*		1		*1*				*0*				*0*				*0*				*0*				*0*	3	0.3
Penicillium spp.				*0*				*0*	1			*1*	2			*2*				*0*				*0*				*0*	3	0.3

cont. table 2

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31
Periconia funerea (Ces.) E.W. Mason & M.B. Ellis				*0*	1			*1*				*0*				*0*				*0*				*0*				*0*	1	0.09
Pythium debaryanum R. Hesse				*0*				*0*				*0*				*0*	2			*2*				*0*				*0*	2	0.17
Rhizoctonia solani J.G. Kühn	1	2	4	*7*			3	*3*		3		*3*	1	8	4	*13*			2	*2*		5	1	*6*		3	1	4	38	3.29
Rhizopus nigricans Ehrenb.		4		*4*		1		*1*				*0*	4			*4*		6	7	*13*	3			*3*	7		4	*11*	36	3.12
Spicaria simplicissima Oudem.				*0*				*0*				*0*				*0*		1		*1*				*0*				*0*	1	0.09
Thysanophora penicillioides (Roum.) W.B. Kendr.				*0*				*0*				*0*				*0*				*0*				*0*	1			*1*	1	0.09
Non-sporulating fungi	4		3	*7*	1	2	5	*8*			7	*7*	5		6	*11*		3	5	*8*	2	2	7	*11*	3	6	7	*16*	68	5.89
Sum	56	63	54		53	58	54		55	51	41		58	63	56		54	58	42		52	57	49		63	58	60		1155	100
Sum of plant	173				165				147				177				154				158				181					

1* – S, 2 – S+Ś, 3 – S+Ś+N

A total of 1155 fungal colonies were isolated from the stem bases of spring wheat (Table 2). 26 species and asporogenous cultures were identified. Members of the genus *Fusarium* were isolated most frequently (71.8% of all isolates). Species of the genus *Rhizoctonia* (3.3%), *Alternaria alternata* (7.5%) and asporogenous cultures (5.8%) were also relatively abundant. The most numerous fungal community was obtained from the stem bases of spring wheat grown after oat (181 cultures), while the lowest number of fungal colonies (147) was isolated from wheat spring grown after Spanish colewort. Fungi of the genus *Fusarium* accounted for 71.8% of all isolates. They were isolated at the highest percentage from the stem bases of spring wheat grown after Spanish colewort (79.6%) and radish (79.1%), and at the lowest percentage from the stem bases of spring wheat grown after oat (65.2%) and oilseed rape (65.6%). Apart from *Fusarium*, also *Alternaria alternata* (7.5%), *Rhizoctonia* spp. (3.3%) and *Bipolaris sorokiniana* (2.5%) were relatively common. The dominant species within the genus *Fusarium* were *F. avenaceum* and *F. culmorum*, which accounted for respectively 56.5% and 13.2% of all *Fusarium* spp. isolates.

Differences stemming from crop residue management practices were observed in the abundance of fungi colonizing the stem bases of spring wheat. The highest number of fungal colonies (408) was recorded in plots where stubble and straw were ploughed in, while the lowest (356) in plots where the extra dose of nitrogen was applied. The same trend was noted in the case of the abundance of *Fusarium* species (299 vs. 240 cultures). The substantial decrease in the number of colonies isolated in treatments with nitrogen application was mainly due to the decrease in the abundance of *F. avenaceum*.

Discussion

A high proportion of cereals in crop rotation has numerous negative consequences, leading to a decrease in unit plant productivity (KUŚ, SIUTA 1995). The right position of wheat in crop rotation has a significant effect on yield level and stability. Among all cereals this species responds by the highest decrease in grain yield when grown after grain crops. The main reason for this situation are high rates of infection by pathogens causing take-all diseases (SMAGACZ 1998).

The roots of spring wheat were colonized primarily by pathogenic species of the genus *Fusarium*. As reported by KUROWSKI, MAJCHRZAK (2000), they are predominantly isolated from the roots of edible crops. The common occurrence and exceptional competitive ability of these fungi result from their increased tolerance for metabolites of other microorganisms, as well as from the efficient utilization of nutrient sources (BURGIEŁ 1996). In the present study fungi

of the genus *Fusarium* were more commonly isolated from the stem bases than from the roots of spring wheat. According to MAJCHRZAK et al. (2004), POLLEY (1995), those species are the main colonizers of cereal stems. *F. culmorum* and *F. graminearum* may attack cereal crops systematically – the infection begins in the roots and then spreads to the stem base (SNIJDERS 1990).

F. culmorum, *F. avenaceum*, *F. poae* and *F. graminearum*, isolated most frequently in the current experiment, are the most dangerous to cereals grown in Poland (KUROWSKI, MAJCHRZAK 2000, MIKOŁAJSKA et al. 1996). *Fusarium culmorum* is considered the dominant species in Western and Central Europe (WAGACHA, MUTHOMI 2007).

Members of the order *Mucorales* were also relatively abundant in the roots of spring wheat. DOMSCH, GAMS (1980) demonstrated that the above fungi form a cosmopolitan group of soil microbes. The high percentage of *Aureobasidium bolleyi* cultures isolated from the roots of spring wheat may be related to the fact that this species occurs, together with *Gaeumannomyces graminis*, on plants showing symptoms of root-rot (ŁACICOWA et al. 1997).

The forecrops tested in the study had different effects on fungal communities, which is consistent with reference data (PLĄSKOWSKA 1996, SMAGACZ 1998). In the case of wheat, the recommended forecrops are Chinese mustard and Spanish colewort. These plants improve soil structure, are known for their phytosanitary properties, and considered good forecrops of cereals (SZCZEBIOT, OJCZYK 2002).

Conclusions

1. The species composition and abundance of fungal communities were affected by the forecrop and crop residue management practices.

2. The largest fungal communities were isolated from the roots of spring wheat grown after Spanish colewort, and from the stem bases of spring wheat grown after oat.

3. Fungi of the genus *Fusarium* were isolated most frequently from both the roots and stem bases of spring wheat.

4. *F. equiseti* dominated on the roots and *F. avenaceum* on the stem bases of young wheat plants.

5. The additional dose of nitrogen applied prior to ploughing in stubble and straw caused a decrease in the total number of fungal isolates, especially members of the genus *Fusarium*.

Translated by ALEKSANDRA POPRAWSKA

Accepted for print 18.09.2007

References

BOJARCZUK M., KREL E., BOJARCZUK J. 1991. *Zgorzel siewek oraz zgnilizna korzeni i podstawy źdźbła powodowana przez grzyby z rodzaju Fusarium u pszenicy ozimej*. Hod. Rośl. Aklim. Nas., 35 (5/6): 77-92.

BURGIEŁ Z.J. 1996. *Wrażliwość wybranych odmian pszenicy ozimej na zgorzel podstawy źdźbła powodowaną przez Fusarium* spp. Materiały Sympozjum *Nowe kierunki w fitopatologii*, Kraków 11-13 września, ss. 195-198.

DOMSCH K.H., GAMS W. 1980. *Compendium of Soil Fungi*. Academic Press, London, p. 859.

KUROWSKI T.P. 2002. *Studia nad chorobami podsuszkowymi zbóż uprawianych w wieloletnich monokulturach*. Rozp. Monogr., 56, UWM Olsztyn.

KUROWSKI T.P., MAJCHRZAK B. 2000. *Patogeniczność i szkodliwość wybranych gatunków z rodzaju Fusarium dla żyta, pszenicy i owsa*. Rocz. AR Poznań CCCXXI. Ogrodn., 30: 61-67.

KUŚ J., SIUTA A. 1995. *Plonowanie zbóż ozimych w zależności od przedplonu i kompleksu glebowego*. Fragm. Agron., 3: 53-58.

ŁACICOWA B., PIĘTA D., KIECANA I. 1997. *Grzyby powodujące choroby podsuszkowe jęczmienia jarego* (*Hordeum vulgare L.*) *uprawianego w monokulturze z uwzględnieniem Aureobasidium pullulans* (*de Bary*) *Arnaud*. Ann. UMCS, ser. EEE, 237-254.

MAJCHRZAK B., OKORSKI A., CHODOROWSKI B. 2004. *Zdrowotność korzeni i podstawy źdźbła pszenicy jarej uprawianej po różnych przedplonach*. Annales UMCS, Sec. E, 59(4): 1779-1788.

MARTYNIUK S., MYŚKÓW W. 1983. *Das Vorkommen der Gaeumannomyces graminis und Phialophora radicola stamte auf getreidewurzem von verschiedenen Standarten und die pektinolitische Aktivitat Pilze*. Izbl. Mikrobiol., 138: 465-473.

MIKOŁAJSKA J., MAJCHRZAK B., PSZCZÓŁKOWSKI P. 1996. *Z badań nad fuzariozami zbóż na Pojezierzu Mazurskim*. Mat. Symp. Nauk. *Nowe kierunki w fitopatologii* Kraków, 11-13 września, ss. 299-302.

OLESZEK W. 1997. *Glukozynolany – występowanie i znaczenie ekoligczne*. Wiad. Bot., 39(1/2): 49-58.

PLĄSKOWSKA E. 1996. *Badania zdrowotności pszenicy ozimej w zależności od zastosowanego przedplonu*. Zesz. Nauk. Akad. Roln. Wrocł., Roln., 47 (300): 67-91.

PLĄSKOWSKA E. 2005. *Zdrowotność pszenicy jarej uprawianej w siewie czystym i w mieszaninach odmian*. Zeszyty Naukowe AR Wrocław, 528: 142.

POLLEY R.W., TURNER J.A. 1995. *Surveys of stem base diseases and fusarium ear diseases in winter wheat in England, Wales and Scotland*, 1989-1990. Ann. Appl. Biol., 126: 45-59.

SMAGACZ J. 1998. *Plonowanie i porażenie przez patogeny podstawy źdźbła odmian pszenżyta ozimego w zależności od przedplonu*. Biul. IHAR, 205/206: 253-258.

SNIJDERS C.H.A. 1990. *Systemic fungal growth of Fusarium culmorum in stems of winter wheat*. J. Phytopathol. – Phytopatholo-gische Zeitschrift, 129: 133-140.

SZCZEBIOT M., OJCZYK T. 2002. *Wartość nawozowa resztek pożniwnych jarych roślin oleistych dla pszenicy ozimej*. Fragm. Agron., 19: 198-206.

WAGACHA J.M., MUTHOMI J.W. 2007. *Fusarium culmorum*: *Infection process, mechanisms of mycotoxin production and their role in pathogenesis in wheat*. Crop Protection, 26(7): 877-885.

POLISH JOURNAL OF NATURAL SCIENCES
Abbrev.: Pol. J. Natur. Sc., Vol 23(2): 310-325, Y. 2008

DOI 10.2478/v10020-008-0024-0

LEAF GREENNESS (SPAD) AND YIELD OF *FESTULOLIUM BRAUNII* (K. RICHT.) A. CAMUS GROWN IN MIXTURES WITH LEGUMES DEPENDING ON MULTIPLE NITROGEN RATES

Marzenna Olszewska

Chair of Grassland Management
University of Warmia and Mazury in Olsztyn

K e y w o r d s: festulolium, leaf greenness (SPAD), bird's-foot trefoil, white clover, yield.

A b s t r a c t

The effects of cultivation regime and nitrogen fertilization on leaf greenness (SPAD) and the yield of two *Festulolium braunii* cultivars (Sulino, Felopa) were determined. It was found that the tested cultivars of *Festulolium* grown in pure stand were characterized by lower SPAD values, compared to those grown in mixtures with legumes. Significantly higher leaf greenness was recorded in *Festulolium* cultivars grown with white clover than in those grown with bird's-foot trefoil. Substantially lower leaf greenness values were observed in cv. Sulino. When grown in pure stand and in mixtures with bird's-foot trefoil, *Festulolium* cultivars contained the largest quantities of chlorophyll in plots fertilized with 120 kg N ha^{-1}. When grown with white clover, these cultivars had a higher chlorophyll content in the control treatment and in plots fertilized with 60 kg N ha^{-1}. Over a three-year experimental period, the lowest yield was attained when *Festulolium* was grown in pure stand. The introduction of legumes caused a significant increase in yield. *Festulolium* grown in mixtures with bird's-foot trefoil yielded higher than *Festulolium* grown in mixtures with white clover. In addition, the yield of *Festulolium* grown in mixtures with legumes, non-fertilized with nitrogen, was significantly higher than the yield of *Festulolium* grown in pure stand, fertilized with 120 kg N ha^{-1}. Weather conditions affected the chlorophyll content of leaves and sward yield. Higher SPAD values and a lower yield were recorded under conditions of deficient rainfall and high temperatures.

Address: Marzenna Olszewska, University of Warmia and Mazury, pl. Łódzki 1, 10-727 Olsztyn, Poland, phone (089) 523 35 01, e-mail: marzenna.olszewska@uwm.edu.pl

INDEKS ZIELONOŚCI LIŚCI (SPAD) I PLONOWANIE *FESTULOLIUM BRAUNII* (K. RICHT.) A. CAMUS W MIESZANKACH Z MOTYLKOWATYMI NA TLE ZRÓŻNICOWANEGO NAWOŻENIA AZOTEM

Marzenna Olszewska

Katedra Łąkarstwa
Uniwersytet Warmińsko-Mazurski w Olsztynie

S ł o w a k l u c z o w e: festulolium, indeks zieloności liści (SPAD), komonica zwyczajna, koniczyna biała, plonowanie.

A b s t r a k t

W pracy przedstawiono wpływ sposobu uprawy i nawożenia azotem na indeks zieloności liści (SPAD) i plonowanie dwóch odmian *Festulolium braunii* (Sulino, Felopa).

Sulino i Felopa uprawiane w siewie czystym charakteryzowały się niższymi wartościami SPAD niż w mieszankach z motylkowatymi, przy czym istotnie wyższy indeks zieloności liści cechował odmiany uprawiane z koniczyną białą niż rosnące z komonicą zwyczajną. W blaszkach liściowych odmiany Sulino stwierdzono znacznie niższe wartości SPAD niż u odmiany Felopa. Odmiany festulolium uprawiane w siewie czystym i w mieszance z komonicą zwyczajną najwięcej chlorofilu zawierały w obiekcie nawożonym 120 kg N ha^{-1}, natomiast rosnące z koniczyną białą wyższe wartości wykazały w obiekcie kontrolnym i nawożonym niższą dawką azotu. W okresie 3-letnich badań najniżej plonowały odmiany festulolium uprawiane w siewie czystym. Wprowadzenie roślin motylkowatych spowodowało istotną zwyżkę plonu. Mieszanki z udziałem komonicy zwyczajnej plonowały wyżej niż z koniczyną białą. Mieszanki z udziałem roślin motylkowatych, nie nawożone azotem istotnie przewyższały plonowaniem siewy czyste festulolium, zasilane dawką 120 kg N ha^{-1}. Warunki pogodowe modyfikowały poziom chlorofilu w liściach badanych roślin oraz plonowanie runi. W okresach niedoboru opadów i wysokich temperatur powietrza stwierdzono wyższe wartości SPAD oraz znaczne ograniczenie plonowania.

Introduction

Being the most common photosynthetic pigment, chlorophyll affects the rate of photosynthesis. Chlorophyll can be also considered an indicator of the annual yield of grasses and grass regrowth, since there exists a positive correlation between its content in the leaf blades of grasses and dry matter yield (GÁBORČÍK 1996, KOZŁOWSKI et al. 2001, OLSZEWSKA 2005). Chlorophyll concentration is a reliable indicator of plant vigor and resistance to environmental stressors. The chlorophyll content of leaves depends on a variety of factors, including the genetic traits of varieties, climatic and soil conditions, nutrient availability, and development stage of a plant (MICHAŁEK, SAWICKA 2005). An important role is also played by a competitive impact of species that are sward components.

The objective of this study was to determine the effects of nitrogen fertilization on leaf greenness (SPAD) and the yield of two *Festulolium braunii* cultivars grown in pure stand and in mixtures with white clover or bird's-foot trefoil.

Materials and Methods

The study was conducted during the years 2004-2006. An exact field experiment was established in a randomized split-plot design, in four replications, at the Experimental Station in Tomaszkowo, on typical brown soil developed from light loam of quality class III b of good wheat complex. The humus content of soil was 1.50%, and its chemical properties were as follows: pH in 1 mol KCl – 7.1 dm^{-3}, total N – 0.085%, P – 54 mg, K – 108 mg, Mg – 50 mg kg^{-1}.

Experimental factor 1 was *Festulolium braunii* cultivar: two cultivars, Felopa and Sulino, were grown in pure stand and in mixtures with legumes (50% grass + 50% legume):

– *Festulolium* cv. Felopa,
– *Festulolium* cv. Sulino,
– *Festulolium* cv. Felopa + white clover cv. Rawo,
– *Festulolium* cv. Felopa + bird's-foot trefoil cv. Skrzeszowicka,
– *Festulolium* cv. Sulino+ white clover cv. Rawo,
– *Festulolium* cv. Sulino+ bird's-foot trefoil cv. Skrzeszowicka.

Experimental factor 2 was nitrogen fertilization:

– non-fertilized treatment,
– 60 kg N ha^{-1},
– 120 kg N ha^{-1}.

Nitrogen fertilizer (ammonium nitrate 34%) was applied at three equal rates to each regrowth. Phosphorus and potassium were applied at constant rates in all treatments. A single rate (80 kg P_2O_5 ha^{-1}) of phosphorus (superphosphate 46%) was applied in the spring, while two equal rates (120 kg K_2O ha^{-1}) of potassium (potash salt 60%) were applied in the spring after the first cut.

Leaf greenness was measured with a SPAD 502 chlorophyll meter (Figure 1). This device measures the difference between light absorption at a wavelength of 650 and 940 nm, and the quotient of these values is presented as leaf greenness, i.e. an indexed chlorophyll content (BLACKMER, SCHEPERS 1994). SPAD (Soil Plant Analysis Development) readings are highly positively correlated with chlorophyll content, which enables its precise determination (CHAPMAN, BARETTO 1997, SAMBORSKI, ROZBICKI 2002). Leaf greenness was meas-

ured on the youngest, fully developed leaves selected randomly in each plot. Four measurements were performed for each regrowth, and readings were taken at one week intervals. Means for regrowths are presented in the paper. The plants were cut three times over the growing season. Plot area was 10 m^2. The collected biomass was weighed, and 1 kg samples were dried at 105°C to constant mass, in order to determine dry matter yield. The proportion of legumes in the sward was estimated based on a botanical-gravimetric analysis of plant material. Results were processed statistically using STATISTICA 6.0 software.

Fig. 1. Chlorophyll meter SPAD 502

Weather conditions during the experimental period are presented in Figure 2. In 2004 mean daily air temperatures over the growing season were comparable to long-term averages, and exceeded them substantially only in August. In 2005-2006 mean air temperatures were high, particularly in July, September and October. Precipitation distribution varied greatly during the growing season. In 2004 and 2006 precipitation total over the growing season was higher (by approx. 18%) than the long-term average. In 2004 heavy rainfalls were recorded in April, May, June and August, whereas considerable deficits were noted in September. In 2006 precipitation total reached the highest level in May and August, while April and July were rain-deficient. In 2005 precipitation total was 20% lower than the long-term average. April, May, June and August were particularly dry, and July and September rainfall totals exceeded the long-term average by 25% and 33% respectively.

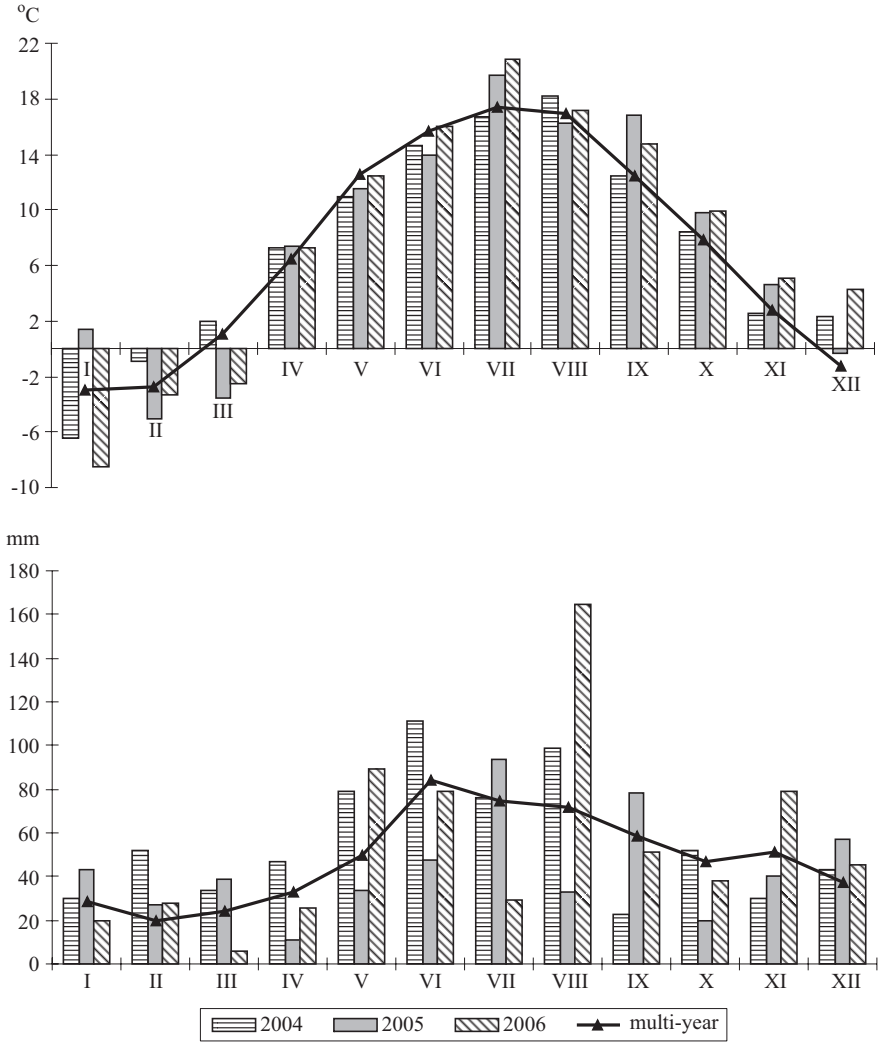


Fig. 2. Mean air temperature and rainfalls in the years 2004-2006 (data from the meteorological station in Tomaszkowo)

Results

Leaf greenness (SPAD)

Over a three-year experimental period the tested cultivars of *Festulolium* grown in pure stand were characterized by lower SPAD values, compared to those grown in mixtures with legumes. Substantially lower leaf greenness values were observed in cv. Sulino, grown in both pure stand and in mixtures

(Table 1, Table 2, Table 3). Compared with cv. Felopa, the above values were lower by 6.1% for growing in pure stand, 4.9% for growing in mixtures with white clover and 7.6% for growing in mixtures with bird's-foot trefoil. Significantly higher leaf greenness was recorded in *Festulolium* cultivars grown with white clover than in those grown with bird's-foot trefoil. Chlorophyll concentration in the leaves of grasses varied throughout the growing season. In the first two years of the experiment the highest chlorophyll content was recorded in the third regrowth, while in the third year – in the second regrowth of *Festulolium* grown in mixtures with legumes, and in the first regrowth of *Festulolium* grown in pure stand. Changes in chlorophyll content during plant growth were also reported by other authors (KOZŁOWSKI et al. 2001,

Table 1

Leaf greenness (SPAD) of *Festulolium* in 2004

Object	N fertilization	1st regrowth	2nd regrowth	3rd regrowth	Mean
Festulolium cv. Felopa	0	24.65^{ab}	28.89^{a}	35.28^{bcd}	29.60^{bc}
	60	25.40^{ab}	28.09^{a}	36.55^{def}	30.01^{bc}
	120	33.93^{fg}	33.54^{gh}	36.66^{def}	34.70^{f}
Festulolium cv. Felopa + white clover	0	29.21^{cd}	37.74^{j}	36.98^{def}	34.64^{f}
	60	32.64^{ef}	36.36^{ij}	40.40^{h}	36.47^{h}
	120	33.65^{fg}	34.08^{h}	37.41^{efg}	35.05^{fg}
Festulolium cv. Felopa + bird's-foot trefoil	0	31.81^{ef}	35.61^{i}	36.31^{cde}	34.58^{f}
	60	29.13^{cd}	32.46^{efg}	35.06^{bc}	32.22^{d}
	120	37.30^{h}	31.26^{cde}	39.09^{gh}	35.88^{gh}
Festulolium cv. Sulino	0	24.31^{a}	29.13^{ab}	31.60^{a}	28.35^{a}
	60	25.00^{ab}	31.54^{cde}	30.78^{a}	29.10^{ab}
	120	29.11^{cd}	32.96^{fgh}	33.93^{b}	32.00^{d}
Festulolium cv. Sulino + white clover	0	33.65^{fg}	33.51^{gh}	37.60^{efg}	34.92^{f}
	60	30.64^{de}	28.98^{ab}	36.42^{cde}	32.01^{d}
	120	35.30^{gh}	30.38^{bc}	38.45^{fg}	34.71^{f}
Festulolium cv. Sulino + bird's-foot trefoil	0	26.90^{bc}	31.38^{cde}	31.96^{a}	30.08^{c}
	60	27.76^{c}	30.69^{cd}	31.85^{a}	30.10^{c}
	120	32.18^{ef}	31.81^{def}	36.49^{cde}	33.49^{e}
Mean for objects					
Festulolium cv. Felopa		27.99^{b}	30.17^{a}	36.16^{c}	31.44^{b}
Festulolium cv. Felopa + white clover		31.83^{c}	36.05^{d}	38.26^{e}	35.38^{d}
Festulolium cv. Felopa + bird's-foot trefoil		32.75^{cd}	33.11^{c}	36.82^{cd}	34.23^{c}
Festulolium cv. Sulino		26.14^{a}	31.21^{b}	32.10^{a}	29.82^{a}
Festulolium cv. Sulino + white clover		33.20^{d}	30.95^{b}	37.49^{de}	33.88^{c}
Festulolium cv. Sulino + bird's-foot trefoil		28.9^{b}	31.29^{b}	33.43^{b}	31.22^{b}
Mean for fertilization					
0		28.42^{a}	32.71^{b}	34.95^{a}	32.03^{b}
60		28.43^{a}	31.35^{a}	35.18^{a}	31.65^{a}
120		33.58^{b}	32.34^{b}	37.00^{b}	34.31^{c}

Table 2

Leaf greenness (SPAD) of *Festulolium* in 2005

Object	N fertilization	1st regrowth	2nd regrowth	3rd regrowth	Mean
Festulolium cv. Felopa	0	27.24^{bc}	23.73^{a}	33.95^{c}	28.31^{ab}
	60	27.13^{b}	25.40^{b}	35.59^{de}	29.37^{cd}
	120	33.93^{j}	28.03^{c}	35.75^{ef}	32.57^{g}
Festulolium cv. Felopa + white clover	0	33.21^{i}	33.65^{fg}	36.80^{fgh}	34.55^{i}
	60	32.28^{g}	34.85^{gh}	39.23^{i}	35.45^{j}
	120	35.58^{k}	30.07^{de}	35.71^{ef}	33.79^{h}
Festulolium cv. Felopa + bird's-foot trefoil	0	32.67^{gh}	35.20^{h}	35.93^{efg}	34.60^{i}
	60	30.66^{cd}	32.63^{f}	34.09^{c}	32.46^{g}
	120	35.54^{k}	30.10^{de}	37.40^{h}	34.35^{hi}
Festulolium cv. Sulino	0	25.87^{a}	28.33^{c}	30.46^{ab}	28.22^{a}
	60	27.61^{c}	30.00^{de}	29.30^{a}	28.97^{bc}
	120	30.00^{e}	28.83^{cd}	34.43^{cd}	31.09^{f}
Festulolium cv. Sulino + white clover	0	34.23^{j}	30.75^{e}	37.79^{h}	34.25^{hi}
	60	30.84^{f}	24.30^{ab}	36.03^{efg}	30.39^{e}
	120	34.13^{j}	27.95^{c}	37.09^{gh}	33.05^{g}
Festulolium cv. Sulino + bird's-foot trefoil	0	29.37^{d}	28.88^{cd}	31.44^{b}	29.89^{de}
	60	29.74^{de}	30.58^{e}	29.64^{a}	29.34^{cd}
	120	33.00^{hi}	34.80^{gh}	35.54^{de}	34.45^{hi}
Mean for objects					
Festulolium cv. Felopa		29.43^{b}	25.72^{a}	35.10^{c}	30.08^{b}
Festulolium cv. Felopa + white clover		33.69^{e}	32.86^{e}	37.25^{e}	34.60^{f}
Festulolium cv. Felopa + bird's-foot trefoil		32.96^{d}	32.64^{e}	35.80^{d}	33.80^{e}
Festulolium cv. Sulino		27.83^{a}	29.05^{c}	31.40^{a}	29.43^{a}
Festulolium cv. Sulino + white clover		33.06^{d}	27.67^{b}	36.97^{e}	32.57^{d}
Festulolium cv. Sulino + bird's-foot trefoil		30.71^{c}	31.42^{d}	32.20^{b}	31.23^{c}
Mean for fertilization					
0		30.43^{b}	30.09^{b}	34.39^{b}	31.64^{b}
60		29.71^{a}	29.63^{a}	33.98^{a}	31.00^{a}
120		33.70^{c}	29.96^{ab}	35.99^{c}	33.22^{c}

MICHAŁEK, SAWICKA 2005, OLSZEWSKI 2004, RYKACZEWSKA 2005). SPAD values were generally higher in legumes than in grasses (Table 4, Table 5, Table 6). Compared to *Festulolium* cultivars grown in pure stand, white clover contained 54% and 62% more chlorophyll when grown with cv. Felopa and cv. Sulino respectively. In the case of bird's-foot trefoil the respective values were 51% and 58%. The introduction of legumes increased the chlorophyll content of *Festulolium* leaves and decreased the differences in chlorophyll concentration between grasses and legumes. White clover grown with cv. Felopa and cv. Sulino contained on average around 34% and 39% more chlorophyll respectively. The respective values for bird's-foot trefoil were 33% and 42%. In the first two years of the experiment white clover and bird's-foot trefoil accumulated the

largest amounts of chlorophyll in the second regrowth, while in the third year – in the first regrowth. Weather conditions also affected the chlorophyll content of legume leaves. The highest chlorophyll content was noted in the second year, characterized by deficient rainfall and high mean air temperatures. A significant impact of weather conditions on an increase in chlorophyll concentration was reported by MICHAŁEK and SAWICKA (2005), who demonstrated that low rainfall levels and high air temperatures in June through August contributed to the accumulation of this pigment. Our previous studies also revealed a higher chlorophyll content of plants grown under soil moisture deficits (OLSZEWSKA 2004a, 2004b).

Table 3

Leaf greenness (SPAD) of *Festulolium* in w 2006

Object	N fertilization	1st regrowth	2nd regrowth	3rd regrowth	Mean
Festulolium cv. Felopa	0	34.30^{cde}	29.93^{d}	28.25^{b}	30.83^{de}
	60	29.58^{a}	27.48^{bc}	28.26^{b}	28.43^{bc}
	120	41.15^{h}	31.43^{d}	31.25^{cd}	34.61^{f}
Festulolium cv. Felopa + white clover	0	37.10^{efg}	36.48^{f}	31.90^{de}	35.16^{fg}
	60	34.73^{de}	40.40^{gh}	35.25^{ghi}	36.79^{hi}
	120	38.93^{fgh}	42.43^{h}	36.13^{ij}	39.16^{j}
Festulolium cv. Felopa + bird's-foot trefoil	0	35.70^{de}	41.90^{h}	33.03^{def}	36.880^{hi}
	60	35.35^{de}	41.53^{gh}	37.38^{j}	38.08^{ij}
	120	39.25^{gh}	35.70^{ef}	35.55^{hi}	36.83^{hi}
Festulolium cv. Sulino	0	29.60^{a}	24.23^{a}	28.13^{b}	27.32^{ab}
	60	29.00^{a}	25.50^{ab}	25.78^{a}	26.76^{a}
	120	31.00^{ab}	29.20^{cd}	28.63^{b}	29.61^{cd}
Festulolium cv. Sulino + white clover	0	35.40^{de}	39.35^{g}	33.88^{fgh}	36.21^{fg}
	60	30.93^{ab}	36.10^{ef}	36.00^{ij}	34.34^{f}
	120	36.25^{def}	35.78^{ef}	34.08^{fgh}	35.37^{fg}
Festulolium cv. Sulino + bird's-foot trefoil	0	33.65^{bcd}	35.38^{ef}	33.63^{efg}	34.22^{f}
	60	31.50^{abc}	33.95^{e}	29.88^{bc}	31.78^{e}
	120	36.15^{def}	46.03^{i}	33.13^{ef}	38.43^{j}
Mean for objects					
Festulolium cv. Felopa		35.01^{b}	29.61^{b}	29.25^{b}	31.29^{b}
Festulolium cv. Felopa + white clover		36.92^{c}	39.77^{e}	34.43^{d}	37.04^{d}
Festulolium cv. Felopa + bird's-foot trefoil		36.77^{c}	39.71^{e}	35.32^{e}	37.26^{d}
Festulolium cv. Sulino		29.87^{a}	26.31^{a}	27.51^{a}	27.89^{a}
Festulolium cv. Sulino + white clover		34.19^{b}	37.08^{c}	34.65^{de}	35.31^{c}
Festulolium cv. Sulino + bird's-foot trefoil		33.77^{b}	38.45^{d}	32.21^{c}	34.80^{c}
Mean for fertilization					
0		34.29^{b}	34.54^{a}	31.47^{a}	33.43^{b}
60		31.85^{a}	34.15^{a}	32.09^{b}	32.70^{a}
120		37.12^{c}	36.76^{b}	33.13^{c}	35.67^{c}

Table 4

Leaf greenness (SPAD) of legumes in 2004

Object	N fertilization	1st regrowth	2nd regrowth	3rd regrowth	Mean
White clover + *Festulolium* cv. Felopa	0	42.04^{b}	52.60^{f}	47.71^{bc}	47.45^{cd}
	60	43.01^{bcd}	52.76^{f}	51.73^{de}	49.17^{e}
	120	42.40^{bc}	52.40^{f}	46.99^{abc}	47.26^{cd}
White clover + *Festulolium* cv. Sulino	0	44.24^{de}	50.93^{cde}	48.23^{c}	47.79^{d}
	60	43.13^{bcd}	51.10^{de}	51.91^{e}	48.71^{e}
	120	44.58^{e}	50.10^{cd}	46.54^{ab}	47.07^{cd}
Bird's-foot trefoil + *Festulolium* cv. Felopa	0	40.48^{a}	51.74^{ef}	48.24^{c}	46.82^{bc}
	60	44.49^{e}	52.03^{ef}	46.84^{abc}	47.78^{d}
	120	41.86^{b}	49.83^{c}	46.90^{abc}	46.20^{ab}
Bird's-foot trefoil + *Festulolium* cv. Sulino	0	43.49^{cde}	48.28^{b}	46.14^{a}	45.96^{a}
	60	42.85^{bc}	50.94^{cde}	47.59^{abc}	47.13^{cd}
	120	42.31^{bc}	45.30^{a}	50.21^{d}	45.94^{a}
Mean for objects					
White clover + *Festulolium* cv. Felopa		42.48^{ab}	52.59^{c}	48.81^{b}	47.96^{c}
White clover + *Festulolium* cv. Sulino		43.98^{c}	50.78^{b}	48.89^{b}	47.86^{c}
Bird's-foot trefoil + *Festulolium* cv. Felopa		42.28^{a}	51.20^{b}	47.33^{a}	46.93^{b}
Bird's-foot trefoil + *Festulolium* cv. Sulino		42.88^{b}	48.17^{a}	47.98^{a}	46.34^{a}
Mean for fertilization					
0		42.56^{a}	50.88^{b}	47.58^{a}	47.01^{b}
60		43.37^{b}	51.71^{c}	49.52^{b}	48.20^{c}
120		42.79^{a}	49.41^{a}	47.66^{a}	46.62^{a}

Table 5

Leaf greenness (SPAD) of legumes in 2005

Object	N fertilization	1st regrowth	2nd regrowth	3rd regrowth	Mean
White clover + *Festulolium* cv. Felopa	0	46.03^{de}	55.68^{gh}	50.31^{d}	50.67^{g}
	60	46.42^{ef}	56.70^{h}	46.14^{ab}	49.75^{fg}
	120	47.60^{g}	51.45^{de}	45.55^{a}	48.20^{d}
White clover + *Festulolium* cv. Sulino	0	47.34^{g}	51.85^{e}	51.54^{d}	50.24^{gh}
	60	48.40^{h}	49.83^{cd}	45.88^{a}	48.03^{d}
	120	48.15^{h}	48.88^{bc}	46.54^{ab}	47.85^{cd}
Bird's-foot trefoil + *Festulolium* cv. Felopa	0	47.64^{g}	53.88^{fg}	45.74^{a}	49.08^{ef}
	60	45.43^{bc}	52.00^{ef}	47.86^{c}	48.43^{de}
	120	45.08^{b}	51.15^{de}	45.80^{a}	47.34^{bc}
Bird's-foot trefoil + *Festulolium* cv. Sulino	0	45.61^{cd}	54.93^{gh}	47.11^{bc}	49.22^{f}
	60	46.60^{f}	47.10^{ab}	45.59^{a}	46.43^{a}
	120	43.25^{a}	45.80^{a}	50.93^{de}	46.66^{ab}
Mean for objects					
White clover + *Festulolium* cv. Felopa		46.68^{c}	54.61^{d}	47.33^{b}	49.54^{d}
White clover + *Festulolium* cv. Sulino		47.96^{d}	50.18^{b}	47.98^{c}	48.71^{c}
Bird's-foot trefoil + *Festulolium* cv. Felopa		46.05^{b}	52.34^{c}	46.47^{a}	48.29^{b}
Bird's-foot trefoil + *Festulolium* cv. Sulino		45.15^{a}	49.28^{a}	47.88^{bc}	47.43^{a}
Mean for fertilization					
0		46.65^{b}	54.08^{c}	48.68^{c}	49.80^{c}
60		46.71^{b}	51.41^{b}	46.37^{a}	48.16^{b}
120		46.02^{a}	49.32^{a}	47.20^{b}	47.51^{a}

Table 6

Leaf greenness (SPAD) of legumes in 2006

Object	N fertilization	1st regrowth	2nd regrowth	3rd regrowth	Mean
White clover + *Festulolium* cv. Felopa	0	46.80^{ab}	42.23^{ab}	42.28^{bcd}	43.77^{ab}
	60	54.00^{d}	46.10^{cd}	44.35^{ef}	48.15^{f}
	120	46.10^{ab}	45.40^{cd}	41.40^{abc}	44.30^{bcd}
White clover + *Festulolium* cv. Sulino	0	44.28^{a}	42.40^{ab}	41.03^{ab}	42.57^{a}
	60	48.00^{bc}	47.25^{d}	43.38^{cde}	46.21^{e}
	120	46.60^{ab}	44.00^{bc}	44.95^{ef}	45.18^{de}
Bird's-foot trefoil + *Festulolium* cv. Felopa	0	44.95^{ab}	41.53^{a}	44.98^{ef}	43.82^{abc}
	60	50.58^{cd}	43.23^{ab}	45.38^{f}	46.39^{e}
	120	47.30^{abc}	45.93^{cd}	42.25^{bcd}	45.16^{cde}
Bird's-foot trefoil + *Festulolium* cv. Sulino	0	48.15^{bc}	41.75^{a}	40.15^{a}	43.35^{ab}
	60	50.85^{cd}	43.25^{ab}	44.13^{def}	46.08^{e}
	120	45.35^{ab}	42.05^{ab}	41.93^{abc}	43.11^{ab}
Mean for objects					
White clover + *Festulolium* cv. Felopa		48.97^{b}	44.58^{c}	42.68^{ab}	45.41^{c}
White clover + *Festulolium* cv. Sulino		46.29^{a}	44.55^{c}	43.12^{b}	44.65^{ab}
Bird's-foot trefoil + *Festulolium* cv. Felopa		47.61^{ab}	43.56^{b}	44.20^{c}	45.12^{bc}
Bird's-foot trefoil + *Festulolium* cv. Sulino		48.12^{b}	42.35^{a}	42.07^{a}	44.18^{a}
Mean for fertilization					
0		46.04^{a}	41.98^{a}	42.11^{a}	43.38^{a}
60		50.86^{b}	44.96^{b}	44.31^{b}	46.71^{c}
120		46.34^{a}	44.34^{b}	42.63^{a}	44.44^{b}

In the present study nitrogen fertilization affected leaf greenness in the tested cultivars of *Festulolium*. In all three years of the experiment the highest chlorophyll content of leaves was observed in *Festulolium* cultivars fertilized with 120 kg N ha^{-1}. An analysis of the interactions between experimental factors showed that when grown in pure stand and in mixtures with bird's-foot trefoil, *Festulolium* cultivars contained the largest quantities of chlorophyll in plots fertilized with 120 kg N ha^{-1}, and when grown with white clover, these cultivars had a higher chlorophyll content in the control treatment and in plots fertilized with 60 kg N ha^{-1}. SPAD values varied over the experimental period. In 2004 and 2006 the highest chlorophyll content of legumes was recorded in plots fertilized with 60 kg N ha^{-1}, whereas in 2005 – in control plots.

Yield

Sward yield varied over the experimental period. The highest dry matter yield was obtained in 2004 (Table 7), while the lowest in 2005, when mean daily air temperatures during the growing season exceeded the long-term average,

Table 7

Dry matter yield in 2004 (t ha^{-1})

Object	N fertilization	1st regrowth	2nd regrowth	3rd regrowth	Total
Festulolium cv. Felopa	0	1.22^{a}	1.12^{a}	0.91^{ab}	3.25^{a}
	60	3.31^{bc}	2.17^{abc}	1.26^{abc}	6.74^{ab}
	120	6.20^{de}	3.93^{cde}	1.71^{bcd}	11.84^{d}
Festulolium cv. Felopa + white clover	0	5.93^{de}	3.57^{bcde}	1.90^{cde}	11.40^{d}
	60	6.00^{de}	4.17^{de}	2.09^{cde}	12.26^{de}
	120	8.40^{fg}	4.03^{cde}	1.31^{abc}	13.74^{def}
Festulolium cv. Felopa + bird's-foot trefoil	0	5.25^{de}	5.11^{e}	2.12^{cde}	12.48^{de}
	60	5.64^{de}	5.26^{e}	1.89^{cd}	12.79^{de}
	120	8.13^{fg}	5.32^{e}	2.53^{de}	15.98^{ef}
Festulolium cv. Sulino	0	1.82^{ab}	1.80^{ab}	0.82^{a}	4.45^{ab}
	60	3.10^{bc}	2.99^{abcd}	1.45^{abc}	7.54^{bc}
	120	6.17^{de}	4.25^{de}	1.39^{abc}	11.81^{d}
Festulolium cv. Sulino + white clover	0	5.65^{de}	4.08^{bcd}	1.81^{cd}	11.55^{d}
	60	5.58^{de}	3.61^{bcde}	1.79^{cd}	10.97^{cd}
	120	8.51^{fg}	3.84^{cde}	1.63^{abc}	13.97^{def}
Festulolium cv. Sulino + bird's-foot trefoil	0	4.85^{cd}	4.68^{de}	1.71^{bcd}	11.25^{cd}
	60	6.91^{ef}	5.05^{e}	2.11^{cde}	14.07^{def}
	120	8.88^{g}	4.95^{e}	2.80^{e}	16.62^{f}
Mean for objects					
Festulolium cv. Felopa		3.58^{a}	2.41^{a}	1.29^{a}	7.28^{a}
Festulolium cv. Felopa + white clover		6.78^{b}	3.93^{c}	1.77^{b}	12.47^{bc}
Festulolium cv. Felopa + bird's-foot trefoil		6.334^{b}	5.23^{d}	2.18^{c}	13.75^{bc}
Festulolium cv. Sulino		3.70^{a}	3.01^{ab}	1.22^{a}	7.93^{a}
Festulolium cv. Sulino + white clover		6.58^{b}	3.84^{bc}	1.74^{b}	12.16^{b}
Festulolium cv. Sulino + bird's-foot trefoil		6.88^{b}	4.89^{d}	2.21^{c}	13.98^{c}
Mean for fertilization					
0		4.12^{a}	3.39^{a}	1.55^{a}	9.06^{a}
60		5.09^{b}	3.88^{ab}	1.77^{ab}	10.73^{b}
120		7.71^{c}	4.39^{b}	1.89^{b}	13.99^{c}

and rainfall was lower by 20% and unevenly distributed (Table 8). Over the entire experimental period *Festulolium* grown in pure stand provided the lowest yield (Table 7-9). The yield of cv. Sulino was slightly higher than the yield of cv. Felopa, but this difference was statistically non-significant. The introduction of legumes had a significant, positive effect on yielding. The average dry matter yield of *Festulolium* grown in mixtures with white clover was higher by 64% (cv. Felopa) and 47% (cv. Sulino). When *Festulolium* was grown with bird's-foot trefoil, the respective values were 96% and 91%. Although the rate of photosynthesis was faster in white clover and in *Festulolium* cultivars grown in mixtures with white clover (OLSZEWSKA 2008), the average yield attained over the entire experimental period was higher in plots cropped to *Festulolium* and bird's-foot trefoil. Biomass

Table 8

Dry matter yield in 2005 (t ha^{-1})

Object	N fertilization	1st regrowth	2nd regrowth	3rd regrowth	Total
Festulolium cv. Felopa	0	2.25^{a}	0.90^{a}	0.33^{a}	3.48^{a}
	60	2.03^{a}	1.13^{abc}	0.65^{abc}	3.80^{a}
	120	2.73^{ab}	1.40^{abcd}	0.65^{abc}	4.78^{abc}
Festulolium cv. Felopa + white clover	0	4.48^{de}	1.68^{cde}	1.23^{fgh}	7.38^{def}
	60	4.18^{cde}	1.93^{defg}	1.05^{def}	7.15^{def}
	120	4.03^{bcde}	1.90^{defg}	1.08^{def}	7.00^{def}
Festulolium cv. Felopa + bird's-foot trefoil	0	4.68^{de}	2.80^{hi}	1.35^{fgh}	8.83^{fg}
	60	4.53^{de}	2.00^{efg}	0.83^{cd}	7.35^{def}
	120	4.03^{bcde}	2.15^{efg}	0.80^{bcd}	6.98^{def}
Festulolium cv. Sulino	0	2.30^{a}	0.83^{a}	0.43^{ab}	3.55^{a}
	60	2.93^{abc}	1.00^{a}	0.50^{abc}	4.43^{ab}
	120	3.40^{abcd}	1.08^{ab}	0.38^{a}	4.85^{abc}
Festulolium cv. Sulino + white clover	0	3.98^{cdef}	1.80^{def}	1.18^{def}	6.95^{def}
	60	3.40^{abcd}	1.70^{cde}	1.05^{def}	6.15^{bcd}
	120	4.20^{cde}	1.60^{bcde}	0.88^{cde}	6.68^{cde}
Festulolium cv. Sulino + bird's-foot trefoil	0	4.88^{e}	3.10^{i}	1.58^{gh}	9.55^{g}
	60	4.38^{de}	2.38^{fgh}	1.15^{def}	7.90^{defg}
	120	4.30^{cde}	2.43^{gh}	1.68^{h}	8.40^{efg}
Mean for objects					
Festulolium cv. Felopa		2.33^{a}	1.14^{a}	0.54^{a}	4.02^{a}
Festulolium cv. Felopa + white clover		4.23^{b}	1.83^{b}	1.12^{b}	7.18^{bc}
Festulolium cv. Felopa + bird's-foot trefoil		4.41^{b}	2.32^{c}	0.99^{b}	7.72^{cd}
Festulolium cv. Sulino		2.88^{a}	0.97^{a}	0.43^{a}	4.28^{a}
Festulolium cv. Sulino + white clover		3.86^{b}	1.70^{b}	1.03^{b}	6.59^{b}
Festulolium cv. Sulino + bird's-foot trefoil		4.52^{b}	2.63^{d}	1.47^{c}	8.62^{d}
Mean for fertilization					
0		3.76^{a}	1.85^{b}	1.01^{b}	6.62^{a}
60		3.57^{a}	1.69^{a}	0.87^{a}	6.13^{a}
120		3.78^{a}	1.76^{ab}	0.91^{a}	6.45^{a}

production is dependent upon photosynthesis taking place in leaves to the greatest degree, but not always a higher rate of this process per unit area guarantees a higher yield. According to STARCK (2002), RAWSON et al. (1983) and CARLSON (1985), crop yield is also affected by other factors, including the efficiency of translocation and distribution of assimilates in the plant. In the current study an important role was also played by weather conditions, which corresponds to the findings of ARDIANI et al. (1992). These authors reported that fluctuations of soil water balance had a greater influence on grassland productivity than the photosynthetic efficiency of species. White clover grown under adverse conditions rapidly utilizes the accumulated energy (FOULDS, YOUNG 1977 as cited in WARDA 1996), which can partly explain the lower

yielding of mixtures containing this species. Repair mechanisms in plants often involve enhanced respiration (STARCK 2002). Bird's-foot trefoil coped better with changing weather conditions, including uneven precipitation and high temperatures (2005 and 2006). The proportion of this species in the sward was higher, compared to white clover, even in plots fertilized with 120 kg N ha^{-1}.

Table 9

Dry matter yield in 2006 (t ha^{-1})

Object	N fertilization	1st regrowth	2nd regrowth	3rd regrowth	Total
Festulolium cv. Felopa	0	2.58^{ab}	1.53^{a}	1.71^{ab}	5.82^{abc}
	60	1.41^{a}	1.61^{a}	1.63^{a}	4.65^{a}
	120	2.79^{ab}	1.56^{a}	2.01^{abcd}	6.36^{abcd}
Festulolium cv. Felopa + white clover	0	3.07^{bc}	2.03^{ab}	2.44^{cde}	7.54^{cdef}
	60	3.53^{bcd}	1.84^{a}	3.11^{ef}	8.48^{f}
	120	3.66^{bcd}	2.14^{ab}	2.42^{cdef}	8.22^{ef}
Festulolium cv. Felopa + bird's-foot trefoil	0	4.97^{def}	5.07^{de}	3.34^{f}	13.37^{h}
	60	4.50^{cdef}	3.08^{bc}	2.86^{def}	10.43^{g}
	120	5.25^{ef}	4.08^{cd}	1.86^{abc}	11.19^{g}
Festulolium cv. Sulino	0	2.25^{ab}	1.70^{a}	1.94^{abc}	5.89^{abc}
	60	2.37^{ab}	1.61^{a}	1.62^{a}	5.60^{ab}
	120	2.29^{ab}	1.80^{a}	2.40^{cdef}	6.49^{bcde}
Festulolium cv. Sulino + white clover	0	2.80^{ab}	1.91^{ab}	2.75^{def}	7.46^{cdef}
	60	3.44^{bcd}	2.11^{ab}	2.56^{cdef}	8.11^{def}
	120	4.42^{cdef}	1.80^{a}	2.02^{bcde}	8.24^{ef}
Festulolium cv. Sulino + bird's-foot trefoil	0	3.74^{bcde}	4.09^{cd}	3.07^{ef}	10.90^{g}
	60	5.92^{f}	5.41^{e}	3.31^{f}	14.64^{h}
	120	4.84^{def}	3.55^{c}	2.41^{cdef}	10.80^{g}
Mean for objects					
Festulolium cv. Felopa		2.26^{a}	1.57^{a}	1.78^{a}	5.61^{a}
Festulolium cv. Felopa + white clover		3.42^{b}	2.00^{a}	2.66^{c}	8.08^{b}
Festulolium cv. Felopa + bird's-foot trefoil		4.90^{c}	4.08^{b}	2.68^{c}	11.67^{c}
Festulolium cv. Sulino		2.30^{a}	1.70^{a}	1.99^{ab}	5.99^{a}
Festulolium cv. Sulino + white clover		3.56^{b}	1.94^{a}	2.45^{bc}	7.94^{b}
Festulolium cv. Sulino + bird's-foot trefoil		4.84^{c}	4.35^{b}	2.93^{c}	12.11^{c}
Mean for fertilization					
0		3.23^{a}	2.72^{a}	2.541^{b}	8.50^{a}
60		3.53^{ab}	2.61^{a}	2.51^{b}	8.65^{a}
120		3.88^{b}	2.49^{a}	2.19^{a}	8.55^{a}

Nitrogen fertilization significantly increased dry matter yield only in the first year of the study, when weather conditions promoted the development of grassland plants and the contribution of legumes to mixed sward was substantially lower than in the other years. In 2005 and 2006 fertilization exerted a beneficial effect on the yield of *Festulolium* grown in pure stand,

while the yield of mixtures in fertilized and non-fertilized plots was comparable or slightly higher in the latter. The yield of *Festulolium* grown in mixtures with legumes, non-fertilized with nitrogen, was significantly higher than the yield of *Festulolium* grown in pure stand, fertilized with 120 kg N ha^{-1}. Doubtlessly, yield level was considerably affected by a high proportion of legumes in the sward. Legumes generally coped better with rainfall deficits than *Festulolium,* which is consistent with the findings of BOROWIECKI (2002). According to this author, *Festulolium* belongs to grass species sensitive to water deficiency, which is manifested in regrowth inhibition and a weaker response to nitrogen fertilization. On the other hand, many authors (STYPIŃSKI 1993, WARDA 1996, LOID, LAIDNA 1990) claim that white clover is much more sensitive to water stress than grasses. In the second year of the experiment, characterized by high temperatures and low precipitation totals over the growing season, in non-fertilized plots white clover accounted on average for 54.7% and 57.2% of sward when grown with cv. Sulino and cv. Felopa respectively. The respective values for bird's-foot trefoil were 54.6% and 64%. In 2006 the proportion of legumes in the sward was still high, reaching 50.3 to 52.2% for white clover and 54.4 to 57.4% for bird's-foot trefoil (Table 10).

Table 10

Mean share of legumes in the sward (%)

Object	N fertilization	2004	2005	2006
White clover + *Festulolium* cv. Felopa	0	25.0	57.2	50.3
	60	20.1	44.4	45.1
	120	15.5	32.1	33.7
White clover + *Festulolium* cv. Sulino	0	24.6	54.7	52.2
	60	23.3	42.3	48.6
	120	15.6	35.2	33.4
Bird's-foot trefoil + *Festulolium* cv. Felopa	0	46.0	64.0	57.4
	60	28.4	45.5	48.0
	120	33.2	40.2	34.3
Bird's-foot trefoil + *Festulolium* cv. Sulino	0	42.6	54.6	54.4
	60	30.4	45.6	49.3
	120	38.9	39.0	37.2

Conclusions

1. The tested cultivars of *Festulolium* grown in pure stand were characterized by lower SPAD values, compared to those grown in mixtures with legumes. Significantly higher leaf greenness was recorded in *Festulolium* cultivars grown with white clover than in those grown with bird's-foot trefoil.

2. Substantially lower leaf greenness values were observed in cv. Sulino.

3. When grown in pure stand and in mixtures with bird's-foot trefoil, *Festulolium* cultivars contained the largest quantities of chlorophyll in plots fertilized with 120 kg N ha^{-1}. When grown with white clover, these cultivars had a higher chlorophyll content in the control treatment and in plots fertilized with 60 kg N ha^{-1}.

4. Over a three-year experimental period, the lowest yield was attained when *Festulolium* was grown in pure stand. The introduction of legumes caused a significant increase in yield. *Festulolium* grown in mixtures with bird's-foot trefoil yielded higher than *Festulolium* grown in mixtures with white clover.

5. The yield of *Festulolium* grown in mixtures with legumes, non-fertilized with nitrogen, was significantly higher than the yield of *Festulolium* grown in pure stand, fertilized with 120 kg N ha^{-1}.

6. Weather conditions affected the chlorophyll content of leaves and sward yield. Higher SPAD values and a considerably lower yield were recorded under conditions of deficient rainfall and high temperatures.

Translated by Authoress and ALEKSANDRA POPRAWSKA

Accepted for print 24.10.2007

References

ARDIANI R., PARENTE G., SCIMONE M. 1992. *Analysis of photosynthetic and ecophysiological parameters on grassland species in a greenhouse experiment*. Proc. 14th Gen. Meet. Europ. Grassland Fed., Lahti., 416-417.

BLACKMER T.M., SCHEPERS J.S. 1994. *Techniques for monitoring crop nitrogen status in corn*. Communications in Soil Science and Plant Analysis., 25: 1791-1800.

BOROWIECKI J. 2002. *Wpływ nawożenia azotem na plon i wartość pokarmową Festulolium braunii odm. Felopa*. Pam. Puł., 131: 39-48.

CARLSON P.S. 1985. *Fotosynteza, produkcyjność i plon roślin uprawnych,* [w:] *Biologia plonowania*. PWRiL Warszawa, 14-57.

CHAPMAN S.C., BARETTO H.J. 1997. *Using a chlorophyll meter to estimate specific leaf nitrogen of tropical maize during vegetative growth*. Agron. J., 89 (4): 557-562.

FOULDS W., YOUNG L. 1977. *Effects of frosting, moisture stress and potassium cyanide on the metabolism of cyanogenic and acyanogenic phenotypes of Lotus corniculatus* L. *and Trifolium repens* L. Heredity., 38, 19-24.

GÁBORČÍK N. 1996. *Koncentrácia chlorofylu (SPAD) v listoch dominantného druhu tráv ako indicátor produkcie trávneho porastu*. Roślinna Vyroba., 42(10): 478-480.

KOZŁOWSKI S., GOLIŃSKI P., GOLIŃSKA B. 2001. *Barwniki chlorofilowe jako wskaźniki wartości użytkowej gatunków i odmian traw*. Zesz. Probl. Post. Nauk Roln., 474: 215-223.

LOID H., LAIDNA T. 1990. *Perennial forage legumes and their influence on soil fertility in Estonia*. Proc. of XIII Gen. Meet. of EGF. Banska Bystrica., I: 165-169.

MICHAŁEK W., SAWICKA B. 2005. *Zawartość chlorofilu i aktywność fotosyntetyczna średnio późnych odmian ziemniaka w warunkach pola uprawnego w środkowo-wschodniej Polsce*. Acta Agrophysica., 6(1): 183-195.

OLSZEWSKA M. 2004a. *Reakcja koniczyny białej uprawianej na dwóch typach gleb na stres wodny*. Acta Sci. Pol., Agricultura., 3(2): 203-213.

OLSZEWSKA M. 2004b. *Reakcja komonicy zwyczajnej* (*Lotus corniculatus* L.) *uprawianej na glebie mineralnej i organicznej na stres wodny*. Woda-Środowisko-Obszary Wiejskie, 4, 2b(12): 185-193.

OLSZEWSKA M. 2005. *Wpływ nawożenia azotem na parametry wymiany gazowej, indeks zieloności liści (SPAD) oraz plonowanie wybranych odmian tymotki łąkowej i kostrzewy łąkowej uprawianych na glebie mineralnej*. J. Elementol., 10(3): 561-569.

OLSZEWSKA M. 2008. Gas Exchange Parameters in *Festulolium braunii* (K. Richt.) *A. Camus Grown in Mixtures with Legumes Depending on Multiple Nitrogen Rates*, Pol. J. Nat. Sci., 23(1): 48-72.

OLSZEWSKI J. 2004. *Wpływ wybranych stresów abiotycznych i biotycznych na intensywność fotosyntezy i transpiracji, plonowanie oraz zdrowotność bobiku i grochu siewnego*. Rozprawy i Monografie. UWM Olsztyn., ss. 109.

RAWSON H.M., HINDMARSH J.H., FISCHER R.A., STOCKMAN Y.M. 1983. *Changes in leaf photosynthesis with plant ontogeny and relationships with yield per ear in wheat cultivars and 120 progeny*. Aust. J. Plant. Physiol., 10: 503-514.

RYKACZEWSKA K. 2005. *Wpływ różnych form i dawek nawozów azotowych na rozwój roślin i plon, wskaźnik zieloności liści (SPAD) oraz wydajność fotosyntetyczną dwóch średnio wczesnych odmian ziemniaka;* Cz. II. Wskaźnik SPAD. Fragmenta Agronomica, 1(85): 542-549.

SAMBORSKI S., ROZBICKI J. 2002. *Przegląd badań nad wykorzystaniem chlorofilometru SPAD-502 do oceny stanu odżywienia roślin azotem*. Nawozy i Nawożenie, 2(11): 123-136.

STARCK Z. 2002. *Mechanizmy integracji procesów fotosyntezy i dystrybucji biomasy w niekorzystnych warunkach środowiska*. Zesz. Probl. Post. Nauk Roln., 481: 113-123.

STYPIŃSKI P. 1993. *Reakcja koniczyny białej* (*Trifolium repens* L.) *na wapnowanie w siewach czystych i mieszankach z trawami w doświadczeniach pastwiskowych i wazonowych*. Rozprawy. SGGW Warszawa, s. 88.

WARDA M. 1996. *Ocena rozwoju, trwałości i plonowania wybranych odmian koniczyny białej* (*Trifolium repens* L.) *w mieszankach z trawami, użytkowanych pastwiskowo*. Rozprawy Naukowe. AR Lublin ss. 61.

POLISH JOURNAL OF NATURAL SCIENCES
Abbrev.: Pol. J. Natur. Sc., Vol 23(2): 326-335, Y. 2008

DOI 10.2478/v10020-008-0025-z

RATE OF PHOTOSYNTHESIS AND TRANSPIRATION OF WINTER WHEAT LEAVES AND EARS UNDER WATER DEFICIT CONDITIONS*

Jacek Olszewski, Agnieszka Pszczółkowska, Tomasz Kulik, Gabriel Fordoński, Krystyna Płodzień, Adam Okorski, Joanna Wasielewska

Department of Diagnostics and Plant Pathophysiology
University of Warmia and Mazury in Olsztyn

K e y w o r d s: winter wheat, water deficit, photosynthesis, transpiration, flag leaf, subflag leaf, ear.

A b s t r a c t

A pot experiment was conducted to determine the rate of photosynthesis and transpiration of leaves and ears of two cultivars (Tonacja and Sukces) of winter wheat grown under conditions of an optimum moisture content and water deficit, taking into account leaf topography. A LI-COR 6400 gas analyzer was used in the study. It was found that the flag leaf had a significant effect on grain yield, due to a long period of its photosynthetic activity. Bottom leaves were characterized by a lower rate of photosynthesis and a short duration of the process. It was also demonstrated that in wheat ears carbon dioxide evolution predominated over carbon dioxide uptake. Moreover, the rate of transpiration in bottom leaves was generally low, and further reduced by soil drought.

INTENSYWNOŚĆ FOTOSYNTEZY I TRANSPIRACJI LIŚCI I KŁOSÓW PSZENICY OZIMEJ W WARUNKACH DEFICYTU WODY

Jacek Olszewski, Agnieszka Pszczółkowska, Tomasz Kulik, Gabriel Fordoński, Krystyna Płodzień, Adam Okorski, Joanna Wasielewska

Katedra Diagnostyki i Patofizjologii Roślin
Uniwersytet Warmińsko-Mazurski w Olsztynie

S ł o w a k l u c z o w e: pszenica ozima, deficyt wodny, fotosynteza, transpiracja, liść flagowy i podflagowy, kłos.

Address: Jacek Olszewski, University of Warmia and Mazury, ul. Plac Łódzki 5, 10-727 Olsztyn, Poland, phone: (089) 523 35 11, e-mail: jacek.olszewski@uwm.edu.pl
* The work was conducted within the framework of Research Project no. PBZ-KBN-097/P06/2003/2.2

A b s t r a k t

Mierzono intensywność fotosyntezy i transpiracji dwóch odmian roślin pszenicy ozimej (Tonacja i Sukces), z uwzględnieniem topografii liści, uprawianej w warunkach optymalnego oraz deficytowego zaopatrzenia w wodę. Wykonano ponadto pomiary intensywności fotosyntezy i transpiracji kłosów. W analizach posłużono się przenośnym analizatorem gazowym LI-COR 6400. Wykazano, że liść flagowy w znacznym stopniu decyduje o plonie ziarna ze względu na długi okres jego aktywności fotosyntetycznej. Dolne liście charakteryzowały się niższym poziomem fotosyntezy oraz krótkim czasem jej aktywności. Wykazano również, że w kłosach następuje wyższe wydzielanie CO2 do atmosfery niż jego pobieranie. Ponadto transpiracja dolnych liści była niska, a deficyt wody w glebie ograniczał znacząco jej intensywność.

Introduction

One of the key yield-forming factors is leaf surface area which affects the photosynthetic efficiency of plants. Leaf damage may lead to considerable yield loss, reaching even 60% (KĄKOL et al. 1997). An important role is also played by leaf arrangement on the plant. Bottom leaves receive less sunlight, and so they wither and dye relatively early. The very first symptom of this process is a change in their color, from green to yellow, in consequence of chlorophyll breakdown. The rate of photosynthesis in such leaves decreases substantially. The flag leaf stays green longest. The prevailing opinion on the significant impact on the flag leaf on grain yield seems to be justified, since damage to this organ caused by fungal diseases results in yield decrease (KĄKOL et al. 1997). In addition, the distinguishing feature of the flag leaf is a long period of photosynthetic activity.

Global climate changes, observed recently, contribute to periodic soil water deficits. Water is indispensable for the life-processes and vital functions of plants – it serves as a solvent of biologically active substances and participates in photosynthesis as, among others, a hydrogen donor. Moreover, water fills up the vacuole, thus maintaining a plant's turgor, and it transports minerals, secondary metabolites and products of assimilation. Plant dehydration reaching 20% causes changes in the polar arrangement of membrane lipids: bilayer structure turns into hexagonal structure, semi-permeable properties are abolished due to damage to aquaporins – integral membrane proteins which selectively conduct water molecules in and out. Water deficit increases the content of ABA and ethylene and decreases the amounts of cytokinins and gibberellin, inhibits starch synthesis and stimulates its degradation, as well as inhibits elongation growth (ARSENIUK 1995, GRZESIUK et al. 1999, HUBICK et al. 1986, LEVITT 1980). Drought leads to photosystem (mostly PS II) damage, which in turn decreases the rate of photosynthesis. Plants respond to water stress by closing their stomata to prevent water loss, which hinders the uptake of CO2 indispensable for photosynthesis (SKRABKA

1992). Thus, water availability is a factor determining the yield and quality of crops.

The objective of the present study was to determine the dependence between leaf arrangement on the plant and the rate of photosynthesis and transpiration in leaves and ears of two cultivars of winter wheat grown under varying levels of soil moisture.

Materials and Methods

A two-factorial pot experiment was conducted in six replications in the greenhouse of the University of Warmia and Mazury in Olsztyn, during the years 2004-2005. The experimental factors included two cultivars of winter wheat, Sukces and Tonacja, and two levels of soil moisture content: corresponding to 60-70% (optimal) and 30-35%. Both of the tested cultivars are widely grown in Poland, and known for their high yielding potential. The difference between them is that Sukces is a high-quality cultivar, while Tonacja is a bread cultivar.

The rate of photosynthesis and transpiration was measured in all leaves five times, at several-day intervals, using a LI-COR 6400 gas analyzer (Portable Photosynthesis System, DMP AG S.A. LTD). The leaves were marked as they appeared on the plant. The parameters were determined at a constant CO_2 concentration of 400 ppm and illumination of 1000 μmol m^{-2} s^{-1}. The source of photons was a LED Light Source lamp, emitting wide-spectrum light at a peak wavelength of between 670 nm and 465 nm. Measurements were performed at selected development stages of winter wheat plants (BBCH--scale), giving mean values for each stage. Gas exchange parameters within ears were measured in a 6400-05 Conifer Chamber. In tables presenting the values of net photosynthesis of leaves and ears CO_2 uptake is given in μmol m^{-2} s^{-1} and in μmol m^{-2} s^{-1} respectively.

Experimental results were processed statistically based on a multiple range test involving mean values in homogenous groups, at a significance level of $\alpha = 0.01$, with the use of STATISTICA ver. 6.0 software.

Results and Discussion

It was found that the rate of photosynthesis in leaves depends on their arrangement on the plant (Table 1 and Table 2). Regardless of the year of the study, the flag and subflag leaves of both wheat cultivars (Sukces and Tonacja) were characterized by the highest photosynthetic activity. The rate of photo-

Table 1
Rate of photosynthesis in leaves of the tested winter wheat cultivars grown under varying levels of soil moisture μmol CO_2 m^{-2} s^{-1} (2004)

Development stage	Leaf							
	bottom		middle		subflag		flag	
	control	water stress	control	water stress	control	water stress	control	water stress
cv. Sukces								
Stem elongation (BBCH 37)	7.9	4.6	9.4	6.2	13.9	7.0	16.4	7.6
Stem elongation (BBCH 39)	5.9	2.6	11.0	3.4	16.7	5.3	18.7	4.7
Beginning of heading (BBCH 51)	8.3	2.5	12.3	3.7	17.4	4.9	17.8	5.0
Middle of heading (BBCH 55)	4.7	0.7	8.2	3.0	13.6	5.8	15.5	6.4
Mean	6.7^{B*}	2.6^{A}	10.2^{B}	4.1^{A}	15.4^{B}	5.8^{A}	17.1^{B}	5.9^{A}
cv. Tonacja								
Stem elongation (BBCH 37)	7.0	4.4	8.2	5.1	13.9	6.2	15.5	6.3
Stem elongation (BBCH 39)	6.6	1.9	11.1	4.2	16.6	8.2	18.1	8.9
Beginning of heading (BBCH 51)	4.2	2.2	11.7	4.8	15.0	7.6	15.7	7.3
Middle of heading (BBCH 55)	4.0	0.5	9.1	3.3	14.3	6.0	15.0	7.0
Mean	5.4^{B}	2.2^{A}	10.0^{B}	4.4^{A}	14.9^{B}	7.0^{A}	16.1^{B}	7.4^{A}

* mean values with identical superscript letters form homogeneous groups ($\alpha = 0.01$)

Table 2
Rate of photosynthesis in leaves of the tested winter wheat cultivars grown under varying levels of soil moisture μmol CO_2 m^{-2} s^{-1} (2005)

Development stage	Leaf							
	bottom		middle		subflag		flag	
	control	water stress	control	water stress	control	water stress	control	water stress
cv. Sukces								
Stem elongation (BBCH 37)	9.7	5.6	11.4	7.6	16.9	8.6	20.0	9.2
Stem elongation (BBCH 39)	7.2	3.2	13.5	4.2	20.4	6.5	22.9	5.7
Beginning of heading (BBCH 51)	10.1	3.1	15.1	4.5	21.2	5.9	21.8	6.1
Middle of heading (BBCH 55)	5.7	0.9	10.0	3.6	16.6	7.0	18.9	7.8
Mean	8.2^{B*}	3.2^{A}	12.5^{B}	5.0^{A}	18.8^{B}	7.0^{A}	20.9^{B}	7.2^{A}
cv. Tonacja								
Stem elongation (BBCH 37)	8.6	5.4	10.0	6.3	16.9	7.6	18.9	7.7
Stem elongation (BBCH 39)	8.0	2.3	13.5	5.2	20.2	10.0	22.1	10.9
Beginning of heading (BBCH 51)	8.2	2.6	14.3	5.8	18.4	9.2	19.1	8.9
Middle of heading (BBCH 55)	4.8	0.6	11.1	4.0	17.5	7.4	18.4	8.6
Mean	6.7^{B}	2.7^{A}	12.2^{B}	5.3^{A}	18.3^{B}	8.6^{A}	19.6^{B}	9.0^{A}

* mean values with identical superscript letters form homogeneous groups ($\alpha = 0.01$)

synthesis in bottom leaves was substantially lower. Moreover, their photosynthetic activity was considerably reduced due to the fast progress in withering and senescing. It was also demonstrated that under the optimum soil moisture content the rate of photosynthesis was much higher, compared to water stress conditions, and depended on leaf topography irrespective of soil humidity levels. However, bottom leaves of plants subjected to water stress (deficit) sooner lost their photosynthetic activity due to their faster senescing.

A decrease in the rate of photosynthesis in leaves of various plant species, induced by soil water deficit, was also observed by other authors (SHARKEY, SEEMANN 1989, GRIEU et al. 1991, WYSZYŃSKI et al. 2002, PSZCZÓŁKOWSKA et al. 2003, OLSZEWSKA 2004). There are no literature data concerning the rate of photosynthesis in leaves as dependent on their arrangement on the plant. Our current knowledge is based on speculations regarding the role of the flag leaf in yield formation. However, the present study confirmed that the flag and subflag leaves had a significant effect on grain yield, due to a long period of their photosynthetic activity.

The level of transpiration was also determined in this experiment (Table 3 and Table 4). The rate of this process was very low in bottom leaves of both tested cultivars under water stress conditions. The rate of transpiration in leaves further decreased during plant growth and development. Excessive moisture loss makes the plant close the stomata, which reduces CO_2 assimila-

Table 3
Rate of transpiration in leaves of the tested winter wheat cultivars grown under varying levels of soil moisture mmol H_2O m^{-2} s^{-1} (2004)

Development stage	Leaf							
	bottom		middle		subflag		flag	
	control	water stress	control	water stress	control	water stress	control	water stress
cv. Sukces								
Stem elongation (BBCH 37)	3.8	0.8	6.2	2.1	8.6	3.3	8.4	3.2
Stem elongation (BBCH 39)	2.3	0.4	5.8	1.7	9.4	1.8	9.9	1.9
Beginning of heading (BBCH 51)	2.5	0.5	5.9	1.4	7.0	1.2	8.0	1.6
Middle of heading (BBCH 55)	0.9	0.3	1.2	0.2	2.5	0.4	3.0	0.5
Mean	2.4^{B*}	0.5^{A}	4.7^{B}	1.3^{A}	6.9^{B}	1.7^{A}	7.3^{B}	1.8^{A}
cv. Tonacja								
Stem elongation (BBCH 37)	4.4	0.4	5.9	1.9	7.9	2.6	7.3	2.5
Stem elongation (BBCH 39)	2.3	0.4	4.2	0.8	7.4	1.7	9.1	2.2
Beginning of heading (BBCH 51)	1.2	0.3	3.9	1.0	5.7	1.8	5.9	1.7
Middle of heading (BBCH 55)	0.6	0.2	1.0	0.3	1.8	0.3	2.6	0.4
Mean	2.1^{B}	0.3^{A}	3.7^{B}	1.0^{A}	5.7^{B}	1.6^{A}	6.2^{B}	1.7^{A}

* mean values with identical superscript letters form homogeneous groups (α = 0.01)

Table 4
Rate of transpiration in leaves of the tested winter wheat cultivars grown under varying levels of soil moisture mmol H_2O m^{-2} s^{-1} (2005)

Development stage	Leaf							
	bottom		middle		subflag		flag	
	control	water stress	control	water stress	control	water stress	control	water stress
cv. Sukces								
Stem elongation (BBCH 37)	4.6	1.0	7.6	2.5	10.5	4.1	10.2	3.9
Stem elongation (BBCH 39)	2.9	0.4	7.0	2.1	11.4	2.2	12.1	2.3
Beginning of heading (BBCH 51)	3.1	0.6	7.2	1.7	8.6	1.4	9.8	2.0
Middle of heading (BBCH 55)	1.2	0.3	1.4	0.2	3.1	0.4	3.6	0.7
Mean	2.9^{B*}	0.6^{A}	5.8^{B}	1.6^{A}	8.4^{B}	2.0^{A}	8.9^{B}	2.2^{A}
cv. Tonacja								
Stem elongation (BBCH 37)	5.4	0.4	7.2	2.3	9.7	3.2	8.9	3.1
Stem elongation (BBCH 39)	2.8	0.4	5.2	1.0	9.0	2.1	11.1	2.6
Beginning of heading (BBCH 51)	1.4	0.3	4.7	1.2	6.9	2.2	7.2	2.1
Middle of heading (BBCH 55)	0.8	0.2	1.2	0.3	2.2	0.3	3.2	0.4
Mean	2.6^{B}	0.4^{A}	4.6^{B}	1.2^{A}	7.0^{B}	2.0^{A}	7.6^{B}	2.1^{A}

* mean values with identical superscript letters form homogeneous groups ($\alpha = 0.01$)

tion as well as photosynthesis. According to reference data, also the rate of transpiration decreases in plants grown under water deficit (ZBIEĆ et al. 1998, RAKOWSKI 2003). Other authors observed intensive transpiration in cereal crops grown in irrigated plots. STARCK (2002) reported that in order to prevent water loss plants close their stomata, which is accompanied by internal resistance to CO_2 diffusion and – in consequence – a decrease in the rate of photosynthesis. Such a trend was also recorded in the present study.

An important yield-forming factor is photosynthesis duration. The photosynthetic activity of senescing bottom leaves is taken over by ears at the heading stage. However, the present study revealed that the process of respiration – which is the opposite of photosynthesis – also takes places in these organs (Table 5, Table 6). This phenomenon is observed throughout the day, with the participation of photosynthetically active radiation (PAR), and lasts incessantly in all live cells containing active mitochondria (KLECZKOWSKI et al. 1988). In C3 plants respiration in cells containing chloroplasts is inhibited by light, and gives way to a process known as photorespiration. Photorespiration is in many aspects similar to respiration, but involves different metabolic pathways. It occurs primarily in peroxisomes, lying between chloroplasts in mesophyll cells. KLECZKOWSKI et al. (1988) confirmed that photorespiration is closely related to photosynthesis. This results from the fact that the enzyme responsible for CO_2 assimilation in the Calvin cycle, i.e. ribulose 1.5-biphosphate carboxylase (RuBisCO), is also the first enzyme to

initiate photorespiration (as ribulose 1.5-biphosphate oxygenase). The process of photorespiration is dependent on changes in the values of such external conditions and factors as oxygen concentration, carbon dioxide concentration, temperature and light intensity. Photorespiration decreases the efficiency of photosynthesis because certain intermediate compounds used in the Calvin cycle are eliminated in this process, which slows down carbon dioxide assimilation (SOLOMON et al. 1996). That is why various methods are applied to reduce photorespiration, and it seems that the most effective strategy involves control over external factors affecting this process. According to PISKORNIK (1994), the best results can be achieved when the oxygen content of air is reduced to a level of around 2% or lower. Under such conditions the rate of net photosynthesis increases by 40 to 50% or more, which has a positive impact on yielding.

Table 5
Rate of photosynthesis (respiration) in ears of the tested winter wheat cultivars grown under varying levels of soil moisture μmol CO_2 m^{-2} s^{-1} (2004)

Development stage	Ear		Mean
	control	water stress	
cv. Sukces			
Beginning of flowering (BBCH 61)	–4.3	–1.4	-2.8^{A}
Full flowering (BBCH 65)	–2.6	–1.6	-2.1^{B}
Mean	-3.5^{A*}	-1.5^{B}	
cv. Tonacja			
Beginning of flowering (BBCH 61)	–5.0	–3.9	-4.5^{A}
Full flowering (BBCH 65)	–5.5	–3.2	-4.4^{A}
Mean	-5.3^{A}	-3.6^{B}	

* mean values with identical superscript letters form homogeneous groups (α = 0.01)

Table 6
Rate of photosynthesis (respiration) in ears of the tested winter wheat cultivars grown under varying levels of soil moisture μmol CO_2 m^{-2} s^{-1} (2005)

Development stage	Ear		Mean
	control	water stress	
cv. Sukces			
Beginning of flowering (BBCH 61)	–5.3	–1.7	-3.5^{A}
Full flowering (BBCH 65)	–3.2	–2.0	-2.6^{B}
Mean	-4.2^{A*}	-1.8^{B}	
cv. Tonacja			
Beginning of flowering (BBCH 61)	–6.2	–4.7	-5.4^{A}
Full flowering (BBCH 65)	–6.7	–4.0	-5.3^{A}
Mean	-6.4^{A}	-4.3^{B}	

* mean values with identical superscript letters form homogeneous groups (α = 0.01)

The level of transpiration in ears of the tested wheat cultivars was also determined in the current experiment (Table 7, Table 8). Similarly as in leaves, the rate of this process was very low under water stress conditions. Both cultivars (Sukces and Tonacja) responded to soil moisture deficiency by a significant decrease in the rate of transpiration. The response was stronger in the case of Tonacja, which could result from individual differences between the cultivars.

Wheat yield attained under conditions of an optimum moisture content was relatively high (Table 9). Water deficit caused a significant decrease in kernel weight per plant, thousand grain weight and plant height in both tested wheat cultivars.

Table 7

Rate of transpiration in ears of the tested winter wheat cultivars grown under varying levels of soil moisture mmol H_2O m^{-2} s^{-1} (2004)

Development stage	Ear		Mean
	control	water stress	
cv. Sukces			
Beginning of flowering (BBCH 61)	3.1	2.5	2.8^{B}
Full flowering (BBCH 65)	1.5	0.5	1.0^{A}
Mean	2.3^{B*}	1.5^{A}	
cv. Tonacja			
Beginning of flowering (BBCH 61)	2.9	2.4	2.7^{B}
Full flowering (BBCH 65)	1.3	0.5	0.9^{A}
Mean	2.1^{B}	1.4^{A}	

* mean values with identical superscript letters form homogeneous groups (α = 0.01)

Table 8

Rate of transpiration in ears of the tested winter wheat cultivars grown under varying levels of soil moisture mmol H_2O m^{-2} s^{-1} (2005)

Development stage	Ear		Mean
	control	water stress	
cv. Sukces			
Beginning of flowering (BBCH 61)	3.7	3.1	3.4^{B}
Full flowering (BBCH 65)	1.9	0.6	1.3^{A}
Mean	2.8^{B*}	1.8^{A}	
cv. Tonacja			
Beginning of flowering (BBCH 61)	3.5	3.0	3.3^{B}
Full flowering (BBCH 65)	1.5	0.6	1.1^{A}
Mean	2.5^{B}	1.8^{A}	

* mean values with identical superscript letters form homogeneous groups (α = 0.01)

Table 9
Some functional traits of the tested winter wheat cultivars grown under varying levels of soil moisture (mean of 2004-2005)

Cultivar	Plant height (cm)			TKW (g)			Grain yield per plant (g)		
	control	water stress	mean	control	water stress	mean	control	water stress	mean
Sukces	83.7	72.1	77.9^{B*}	28.9	19.3	24.1^{A}	1.72	0.55	1.14^{A}
Tonacja	74.1	62.4	68.3^{A}	30.4	22.7	26.6^{B}	1.58	0.64	1.11^{A}
Mean	78.9^{B}	67.3^{A}		29.7^{B}	21.0^{A}		1.65^{B}	0.60^{A}	

* mean values with identical superscript letters form homogeneous groups ($\alpha = 0.01$)

Conclusions

1. Water stress (deficit) significantly decreased the rate of photosynthesis and transpiration, as well as grain yield, thousand grain weight and plant height in both investigated wheat cultivars.

2. The subflag and flag leaves of both tested wheat cultivars were characterized by the highest rate of photosynthesis and transpiration.

3. In wheat ears carbon dioxide evolution predominated over carbon dioxide uptake.

Translated by Aleksandra Poprawska

Accepted for print 10.09.2007

References

Arseniuk E. 1995. *Odporność roślin na stresy biologiczne i fizyczne*. Mat. Konf. II Krajowego Symp.: *Odporność roślin na choroby, szkodniki i niesprzyjające czynniki środowiska*, IHAR Radzików, 12-14 września.

Grieu P., Robin H., Guckert A. 1991. *Sensitivity of net photosynthesis to soil drought in white clover* (*Trifolium repens* L.), <www.fao.org/docrep/V2350E/v2350e0y.htm>.

Grzesiuk S., Koczowska I., Górecki R. J. 1999. *Fizjologiczne podstawy odporności roślin na choroby*. Wyd. II. ART Olsztyn.

Hubick K.T., Taylor J.S., Reid D.M., 1986. *The effect of drought on levels of abscisic acid, cytokinins, gibberellins and ethylene in aeroponically grown sunflower plants*. Plant Growth Regulators, 4: 139-151.

Kąkol E. Stankiewicz CZ., Steć E. 1997. *Wpływ porażenia pszenicy ozimej przez Erysiphe graminis Dc Ex Merat i Puccinia triticina Erikss na skład chemiczny liści flagowych i wybrane parametry fizyko-chemiczne ziarniaków*. Fragm. Agron., 3 (55): 79-88.

Kleczkowski L., Łoboda T., Nalborczyk E. 1988. *Pomiary fotooddychania u roślin wyższych*. Wiad. Bot., 32 (4): 227-240.

Levitt J. 1980. *Response of plants to environmental stresses*. Acad. Press. New York.

Olszewska M. 2004. *Reakcja koniczyny białej uprawianej na dwóch typach gleb na stres wodny*. Acta Sci. Pol., Agricultura 3(2): 203-213.

Piskornik Z. 1994. *Fizjologia roślin dla wydziałów ogrodniczych*. AR Kraków.

PSZCZÓŁKOWSKA A., OLSZEWSKI J., PŁODZIEŃ K., KULIK T., FORDOŃSKI G., ŻUK-GOŁASZEWSKA K. 2003. *Effect of the water stress on the productivity of selected genotypes of pea* (*Pisum sativum* L.) *and yellow lupin* (*Lupinus luteus* L.). Elect. J. Pol. Agric. Univ., Agronomy 6(1), www.ejpau.media.pl/series/volume6/issue1/agronomy/art-02.

RAKOWSKI D. 2003. *Wpływ deszczowania i nawożenia mineralnego na plonowanie wybranych odmian pszenicy jarej i pszenżyta jarego uprawianych na glebie lekkiej*. Cz. II. *Aktywność procesów fizjologicznych w roślinach*. Acta Sci. Pol., Agricultura, 2(2): 33-42.

SHARKEY T.D., SEEMANN J.R. 1989. *Mild water stress effects on carbon-reduction-cycle intermediates, ribulose biphosphate carboxylase activity and spatial homogeneity of photosynthesis in intact leaves*. Plant Physiol. 89: 1060-1065.

SOLOMON E., VILLE C., BERG L., MARTIN D. 1996. *Biologia*. Multico Oficyna Wydawnicza, Warszawa.

SKRABKA H. 1992. *Roślina a środowisko. Wybrane działy z fizjologii roślin*. Skrypt AR Wrocław.

STARCK Z. 2002. *Fizjologia plonowania roślin*. Praca zbiorowa pod redakcją R. J. Góreckiego i S. Grzesiuka. Wyd. UWM Olsztyn.

WYSZYŃSKI Z., GOZDOWSKI D., ŁOBODA T., PIETKIEWICZ S., WOŁEJKO E. 2002. *Reakcja jęczmienia jarego browarnego w latach o zróżnicowanych opadach przy różnym nawożeniu azotem*. Zesz. Probl. Post. Nauk Roln., 481: 349-355.

ZBIEĆ I.I., WOJTASIK D., RUMASZ E., PODSIADŁO C. 1998. *Wpływ deszczowania i nawożenia na procesy fizjologiczne i plonowanie jęczmienia uprawianego na glebie lekkiej*. Fragm. Agron., 2(58): 63-71.

POLISH JOURNAL OF NATURAL SCIENCES
Abbrev.: Pol. J. Natur. Sc., Vol 23(2): 336-346, Y. 2008

DOI 10.2478/v10020-008-0026-y

THE CONTENT OF NITRATES(V) IN POTATO TUBERS IN DEPENDING ON THE CULTIVATION SITE AND STORAGE CONDITIONS

Jarosław Pobereżny

University of Technology and Life Sciences in Bydgoszcz
Chair of Storage and Processing of Plant Products

K e y w o r d s: nitrates(V), potato tubers, cultivar, cultivation site, long term storage.

A b s t r a c t

A potato tuber of four medium-early variations: two Polish ('Lena' and 'Mors') and two German ones ('Agria' and 'Satina') constituted the material for studies. The field tests were conducted in the years 1998-2000 in Poland (Kujawsko-Pomeranian region) and in Germany (Lower Saxony). The aim of the undertaken studies was to compare the variations of potatoes and determine the impact of the place of cultivation on the content of nitrates(V) in tubers after the harvest and after the lapse of six months of storage in temperature +4 and +8°C and relative humidity of air 95%. The content of nitrates(V) in potato tubers was determined with the application of ionoselective method with the use of a multifunctional computer device CX-721, Elmetron make. The highest content of nitrates(V), exceeding the permissible standard (according to the Ministry of Health and Social Services as of 2003) of 200 mg kg^{-1} of fresh matter, there characterized the potatoes tubers of 'Satina' variation, irrespective of the year and place of cultivation. In 2000, characterized by a rather high volume of precipitations and low temperature of the air in the period of vegetation, the content of nitrates(V) in tubers of all the variations of potatoes was much lower in comparison to the previous years and in the Kujawsko-Pomeranian region amounted on average for the studied variations of potatoes – to 159.1 mg kg^{-1} of fresh matter, and in the region of Lower Saxony – to 182.0 mg kg^{-1} of fresh matter. Potatoes of all the studied variations, after six months of storage, comprised for 7.2% (stored in temp. +4°C) and for 13.2% (stored in temp. +8°C) less nitrates(V) in tubers in comparison to the assessment made after the harvest.

Address: Jarosław Pobereżny, University of Technology and Life Sciences, ul. ks. Kordeckiego 20 bl.A, 85-225 Bydgoszcz, Poland, phone: (052) 374 93 45, e-mail: poberezny@utp.edu.pl

ZAWARTOŚĆ AZOTANÓW(V) W BULWACH ZIEMNIAKA W ZALEŻNOŚCI OD MIEJSCA UPRAWY I WARUNKÓW PRZECHOWYWANIA

Jarosław Pobereżny

Uniwersytet Technologiczno-Przyrodniczy w Bydgoszczy
Katedra Przechowalnictwa i Przetwórstwa Produktów Roślinnych

S ł o w a k l u c z o w e: azotany(V), bulwy ziemniaka, odmiana, miejsce uprawy, długotrwałe przechowywanie.

A b s t r a k t

Badano bulwy ziemniaka 4 odmian średniowczesnych – dwie polskie ('Lena' i 'Mors') i dwie niemieckie ('Agria' i 'Satina'). Doświadczenia polowe przeprowadzono w latach 1998-2000 w Polsce (region kujawsko-pomorski) i w Niemczech (Dolna Saksonia). Celem podjętych badań było porównanie odmian ziemniaka i określenie wpływu miejsca uprawy na zawartość azotanów(V) w bulwach po zbiorze i po 6 miesiącach przechowywania w temperaturze +4, +8°C oraz w wilgotności względnej powietrza 95%. Zawartość azotanów(V) w bulwach ziemniaka określono metodą jonoselektywną za pomocą wielofunkcyjnego przyrządu komputerowego CX-721 firmy Elmetron. Najwyższym poziomem tych związków, przekraczającym dopuszczalną normę (wg Ministerstwa Zdrowia i Opieki Społecznej z 2003 r.) 200 mg kg^{-1} świeżej masy, charakteryzowała się odmiana 'Satina', niezależnie od roku i miejsca uprawy. W 2000 r., charakteryzującym się dość dużą sumą opadów i niską temperaturą powietrza w okresie wegetacji, zawartość azotanów(V) w bulwach wszystkich uprawianych odmian ziemniaka była dużo niższa w porównaniu z pozostałymi latami. W regionie kujawsko-pomorskim wynosiła średnio 159,1, a w regionie Dolnej Saksonii – 182,0 mg kg^{-1} świeżej masy. Ziemniaki wszystkich badanych odmian po 6 miesiącach przechowywania zawierały mniej azotanów(V) w bulwach niż po zbiorze – składowane w temp. +4°C o 7,2%, a przechowywane w temp. +8°C o 13,2%.

Introduction

More and more attention is paid not only to the nutritive value of farm products, but also to the content of compounds harmful for a man. Low content of antinutrional substances, in this nitrates(V), is regarded to be among others, an indicator of good quality potatoes tubers.

From the point of view of a consumer's health, it is very important, that the tubers of potatoes include the smallest possible content of nitrates(V) (CIEŚLIK 1992, ROGOZIŃSKA et al. 2000). These compounds, under the influence of the stomach's bacterial flora, may undergo reduction to nitrates(III), and these in turn are the precursors of nitrosamine, that may have carcinogenic and mutagenous action. By oxidation of bivalent hemoglobin iron to a trivalent form, not being able to fix reversibly the oxygen in a human being's organism, results in formation of methemoglobin. The presence of nitrates(V) in plants is a consequence of their natural vital process connected with the conversion

of nitrogen into amino acids and proteins, and their surplus is most often connected with irrational fertilization and improper agricultural science (ROGOZIŃSKA et at. 2000, WADAS et al. 2005). Fertilization with nitrate (ROGOZIŃSKA, WOJDYŁA 1999, ŻOŁNOWSKI 2000) is the factor that most modifies the volume of nitrates(V) in potato tubers.

FAO/WHO (2002) Food Committee, fixed daily intake of an adult of nitrates(V) on the level of 0-3.7 mg and nitrates(III) 0-0.7 mg per 1 kg of body mass. From the fixed values it results, that the acceptable daily intake (ADI) by an adult of the weight 70 kg, cannot exceed 260 mg of nitrates(V) and 49 mg nitrates(III). However, the dose of nitrates(V) exceeding 8-11 mg kg^{-1} body weight /day is lethal (BURT et al. 1993).

As tubers of potatoes are consumed at the time considerably diverge from harvests, attention should be paid to the content of nitrates(V) after storage. CIEŚLIK (1995) and ROGOZIŃSKA (2003) pay attention to the fact, that contrary to other vegetables, in potato tubers the increase of nitrates(V) was observed only in the preliminary period of their storage (up to the 2nd month), and then gradual lowering of their content took place, while FRYDECKA-MAZURCZYK (1996) observed a reversible relation in relation to the time of storage.

The aim of the conducted studies was to compare and define the impact of the place of cultivation of different variations of potato on the content of nitrates(V) in tubers, after harvest and after six months of their storage in temperatures +4 and +8°C and relative humidity of air 95%.

Material and Methods

The results of the tests were based on triennial field experiences (1998-2000) that took place in Poland (Kujawsko-Pomeranian region) and in Germany (Lower Saxony). Four, medium-early variations of potatoes: two Polish ('Lena' and 'Mors') and two German ('Agria' and 'Satina') were used for the study. Polish variations of eatable potatoes were characterized by a general functional suitability for direct consumption and for processing, while German variations: 'Satina' only for direct consumption and 'Agria' for production of chips and French fries.

Potatoes were planted on silos made of clay soil classified to the very good rye complex, productivity class R IVa. In particular years of the studies, the selection of experimental areas was conducted with consideration of possibly slight soil's differentiation as far as physical and chemical properties were concerned. In all the years, cereals were the pioneer crop of potatoes. In autumn, every year preceding planting of potatoes, manure was used At the amount of 25 t ha^{-1}.

All the doses of mineral fertilizers were used in the spring before planting potatoes, at the amounts considering the nutritional requirements of plants:

Nitrogen – 120 kg N ha^{-1} in the form of ammonium nitrate (34%),

Phosphorus – 110 kg P_2O_5 ha^{-1} in the form of triple superphosphate (46%),

Potassium – 120 kg K_2O ha^{-1} in the form of potassium sulfate (50%).

The weather conditions or the whole period of conducting of the experiment in Poland (Bydgoszcz) and in Germany (Gottingen), are presented in the graphic form according to WALTER (1976) (Figure 1, Figure 2, Figure 3).

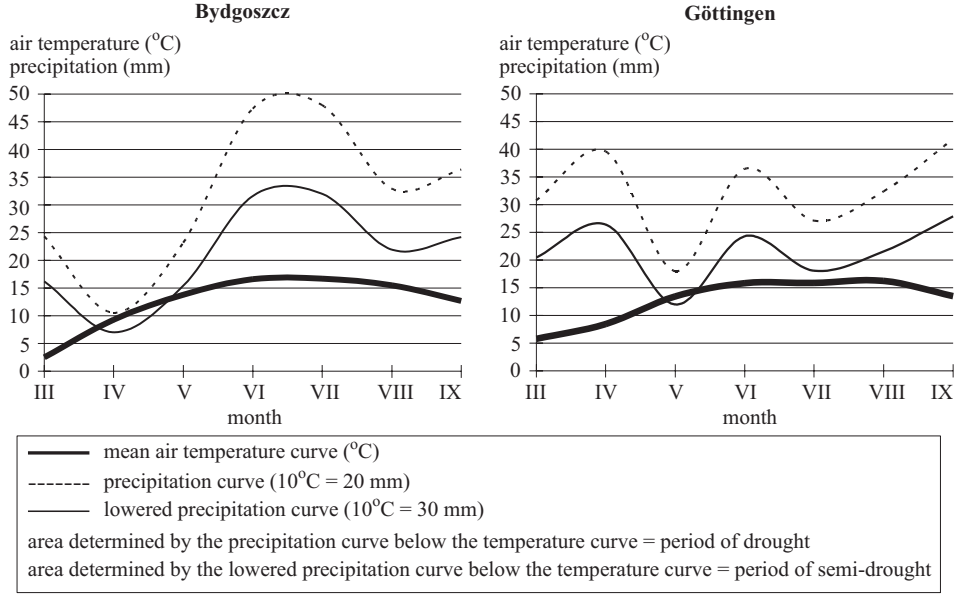


Fig. 1. Meteorological conditions in 1998

The process of storage was conducted in chambers with controlled atmosphere in the Institute of Agricultural Chemistry of the Georg-August University in Gottingen. In the period of six months of storage, a constant temperature and relative humidity of air was maintained, that was adjusted to the manner of use of potato tubers. Tubers of potato variations 'Satina', 'Lena' and 'Mors' designer for direct consumption were stored in temperature +4°C and relative air humidity 95%, and for processing purposes ('Agria', 'Lena' and 'Mors') were stored in temperature +8°C and air relative humidity 95%.

The content of nitrates(V) both directly after harvest and after six months of storage, was determined with the use of the ionselective method (KUNSCH et al. 1981) with the application of the multi-purpose computer device CX-721, Elmetron make.

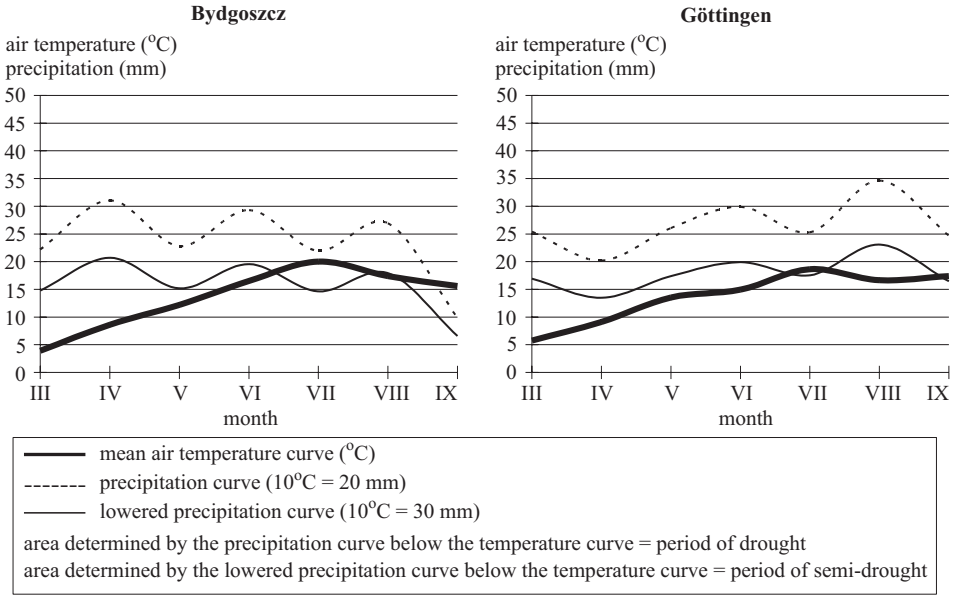


Fig. 2. Meteorological conditions in 1999

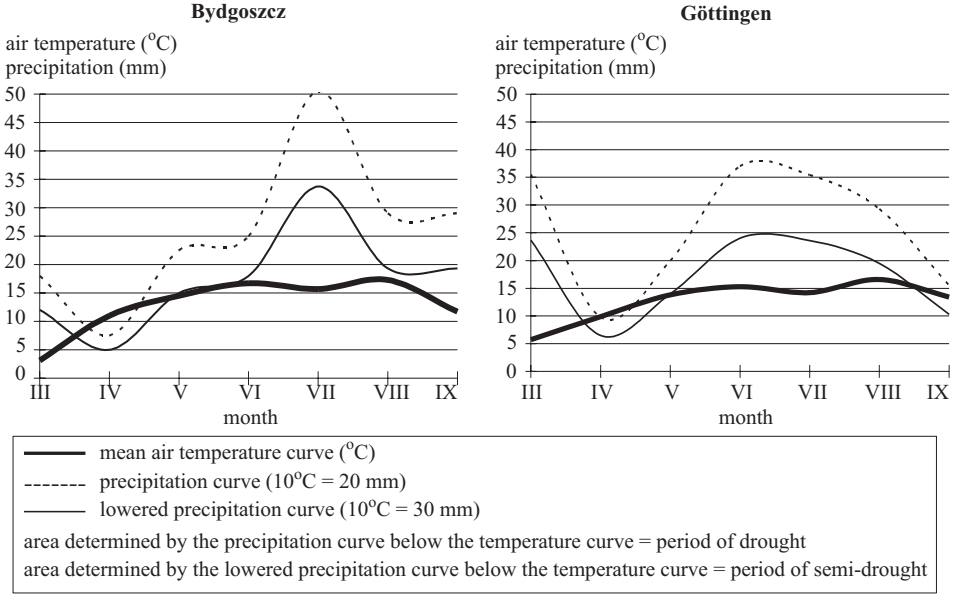


Fig. 3. Meteorological conditions in 2000

The results of 3-year studies were subject to statistical computations with the use of the method of analysis of variations for bifactor experiments, applying the Tukey's test to assess the importance of differences. In order to present the stability of studied features, the coefficients of variation were calculated.

Results and Discussion

Cultivating potatoes in comparable soil and climate conditions (Kujawsko--Pomeranian region and Lower Salony) and applying the same agricultural science it was found, that variations used in the experiment and places of cultivation considerably differentiated the content of nitrates(V) in tubers after the harvest (Table 1). The least volume of nitrates(V) there cumulated in potato tubers the 'Agria' variation, suitable for production of purified goods, cultivated in Kujawsko-Pomeranian region, and the average content of nitrates(V) amounted to 141.9 mg kg^{-1} of fresh matter.

Table 1

Nitrates(V) content in the fresh matter of potato tubers after harvest in mg kg^{-1} (3-year mean)

Cultivation site (*A*)	Cultivars (*B*)	Year			Mean
		1998	1999	2000	
Bydgoszcz	Agria	131.2	146.1	148.4	*141.9*
	Satina	200.1	256.4	217.9	*224.8*
	Lena	150.6	160.1	103.8	*138.2*
	Mors	173.6	204.9	166.1	*181.5*
	mean	*163.9*	*191.9*	*159.1*	*171.5*
Getynga	Agria	134.5	156.8	151.9	*147.7*
	Satina	247.5	266.6	254.4	*256.2*
	Lena	205.6	182.3	168.6	185.5
	Mors	193.5	226.6	153.1	*191.1*
	mean	*195.3*	*208.1*	*182.0*	*195.1*
Mean		*179.6*	*200.0*	*170.5*	*183.3*

$LSD_{p=0.05}$ *A* – 7.10 *B* – 16.96 *A* × *B* – 16.60 year – 12.80

The Ministry of Health and Social Services (*Rozporządzenie*... Dz.U. nr 37, poz. 326), determined the border permitted content of nitrates(V) in potato tubers per 200 mg kg^{-1} of fresh matter. The content of nitrates(V) in tubers exceeded the permitted standard in potatoes of variations: 'Satina' (useful only for direct consumption) and 'Mors' (for general use) cultivated in the Kujawsko-Pomeranian region respectively for: 56.4 and 4.9 mg kg^{-1} of fresh

matter and Lower Saxony for 66.6 and 26.6 mg kg^{-1} of fresh matter in 1999. The period of vegetation in 1999 was characterized by a higher air temperature with the period of "drought" (Figure 2). In 2000 the content of nitrates(V) in potatoes of all the cultivated variations was considerably lower in comparison to previous years and amounted in the Kujawsko-Pomeranian region on average for variations – 159.1 mg kg^{-1} of fresh matter and in the region of Lower Saxony – 182.0 mg kg^{-1} of fresh matter. A lower content of nitrates(V) was obtained(V) on average 179.6 and 170.5 mg kg^{-1} of fresh matter of potato tubers when the period of "draught" appeared only in the preliminary period of vegetation, that was characterized by a big volume of precipitations and rather low temperature, but it did not have a big impact on the further growth and development of a potato (Figure 1, Figure 3). A similar dependence in the content of nitrates(V) in potato tubers on the temperature and precipitations along the whole period of vegetation there obtained GISLASON et al. (1984), MIĘDZYBRODZKA et al. (1992), FARGASOWA (1994), CIEŚLIK (1995).

The studies showed, that not only particular species, but also within one specie – variations may reveal different ability for nitrates(V) accumulation. This phenomenon pertains also to a potato, what was confirmed by numerous studies conducted by authors, just as KOLBE (1990), ROGOZIŃSKA (2003) and GRUDZIŃSKA with ZGÓRSKA (2005). Just the same dependency was noticed by (1990) conducting many-years' studies on the variations of potatoes cultivated in comparable conditions but in different places.

In the studies conducted directly after the harvest, the German variation 'Agria' – useful only for purified goods, for which the coefficient of variation amounted to 6.9%, was characterized by the best stability. However, the 'Lena' variation of general usability, was characterized by the highest value of the coefficient of variation amounting to 21.2%, what proves low stability of the studied feature (Table 2). Low stability calculated in the studies, points

Table 2

Variation coefficients (%) of nitrates(V) content in potato tubers before and after storage (3-year mean)

Cultivars	After harvest	After storage	
		the temperature +4°C	the temperature +8°C
Agria[1]	6.9*	–	6.4*
Satina[2]	10.8	9.3	–
Lena[1,2]	21.2**	24.3**	25.7**
Mors[1,2]	16.2	21.4	23.0

[1] – cultivar applicable to chips and French fries production
[2] – cultivar applicable to direct consumption
* – the lowest variation
** – the highest variation

out at bigger dependency of the studied feature on weather conditions than the potato's genotype. According to MAZURCZYK and LIS (1999), the content of nitrogen substances in potato's tubers is ranked to features of low stability.

Potato's tubers studied after the period of six months of storage, considerably differed with the content of nitrates(V) depending on the genotype and the place of field cultivation (Table 3, Table 4). All the variations of potato used in the experiment reacted with lowering of the content of nitrates(V) in tubers after six months of storage on average from 4.8% (temperature +4°C) for 'Satina' variation – designed only for direct consumption – coming from field cultivation in the Kujawsko-Pomeranian region to 14.4% (temperature +8°C) – 'Agria' – designed for production of purified goods – cultivated in Lower Saxony (Figure 4). Numerous studies (GISLASON et al. 1984, KOLBE 1990, FARGASOWA 1994, CIEŚLIK 1995, FRYDECKA-MAZURCZYK, ZGÓRSKA 1996) showed, that the content of nitrates(V) in potatoes after their storage lowered even 50% in relation to the preliminary level. Such a big discrepancy of results between own studies and the ones of the above quoted authors was most probably the result of different storage conditions. In own studies, depending on the direction of storage, constant temperature and air relative humidity was applied in storage chambers, Chile the above mentioned authors stored tubers in different conditions than the ones described in this work.

Table 3

Nitrates(V) content in the fresh matter of potato tubers after 6 months of storage at the temperature +4°C in mg kg^{-1} (3-year mean)

Cultivation site (*A*)	Cultivars (*B*)	Year			Mean
		1998	1999	2000	
Bydgoszcz	Satina	192.2	238.1	212.1	*214.1*
	Lena	140.2	152.2	100.7	*131.0*
	Mors	161.7	203.5	152.1	*172.4*
	mean	*164.7*	*197.9*	*155.0*	*172.5*
Getynga	Satina	240.3	248.7	224.5	*237.8*
	Lena	203.6	179.7	129.2	*170.8*
	Mors	217.1	205.5	120.4	*181.0*
	mean	220.3	211.3	158.0	*196.6*
Mean		*192.5*	*204.6*	*156.5*	*184.5*

$LSD_{p=0.05}$ *A* – 1.53 *B* – 4.71 *A* × *B* – 6.66 year – 8.41

Variations designed for general usage 'Lena' and 'Mors' showed low stability of the studied feature. The calculated coefficient of variation of nitrates(V) content in tubers of the studied variations of potatoes after six months of storage in temp. +4°C amounted respectively to 24.3 and 21.4% and in temp. of storage +8°C the obtained coefficients of variation amounted

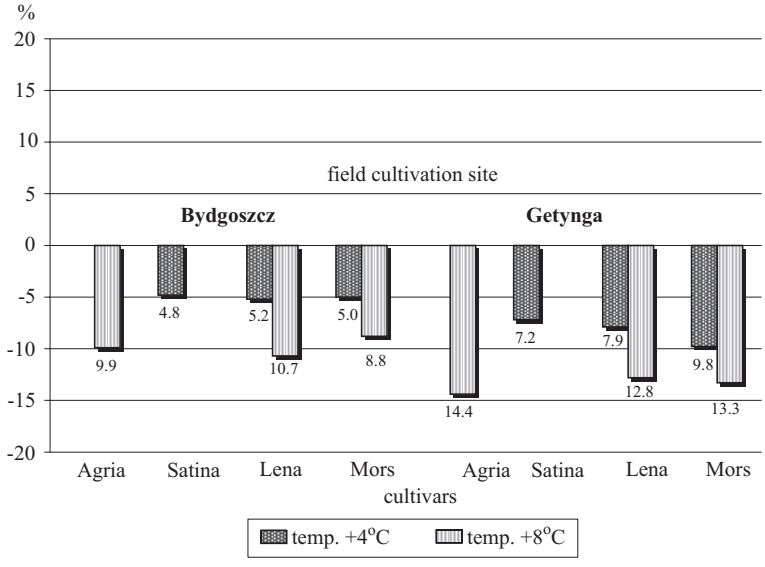


Fig. 4. The percentage losses of nitrates(V) in potato tubers in depending on the field cultivation site and storage temperature (3-year mean)

Table 4

Nitrates(V) content in the fresh matter of potato tubers after 6 months of storage at the temperature +8°C in mg kg^{-1} (3-year mean)

Cultivation site (*A*)	Cultivars (*B*)	Year			Mean
		1998	1999	2000	
Bydgoszcz	Agria	127.2	136.6	119.6	*127.8*
	Lena	133.4	138.4	98.4	*123.4*
	Mors	154.7	197.9	144.1	*165.6*
	mean	*138.4*	*157.6*	*120.7*	*138.9*
Getynga	Agria	126.0	136.1	117.1	*126.4*
	Lena	197.3	173.0	115.0	*161.8*
	Mors	210.2	200.2	111.5	*174.0*
	mean	177.8	169.8	114.5	*154.0*
Mean		*192.5*	*158.1*	*163.7*	*117.6*

$LSD_{p=0.05}$ *A* – 3.17 *B* – 4.86 *A* × *B* – 6.88 year – 3.04

respectively to 25.7 and 23.0%. However, variations of a limited manner of use 'Agria' and 'Satina' showed big stability of studied features after storage – the achieved coefficient of variation was on the level of 6.4 and 9.3% (Table 2).

Apart from raw material's storage, also other factors have an influence on the final content of nitrates(V) in products of plant origin. Nitrates(V) and nitrates(III) are water-soluble compounds, that is why during peeling off

of potatoes, precise washing and boiling, are washed out in about 43-66% (MOZOLEWSKI. et al. 2004, GRUDZIŃSKA, ZGÓRSKA 2005). Moreover, frying results in considerable lowering of the volumes of these compounds, even up to 80% (ROGOZIŃSKA 2003, MOZOLEWSKI et al. 2004). Assuming, that before consumption tubers are always subject to culinary treatment, the studied variations, even though they cumulated rather high volumes of nitrates(V), did not constitute any threat to man's health.

Conclusions

1. The highest content of nitrates(V), exceeding the permitted standard 200 mg kg^{-1} of fresh matter characterized the tubers of potato of 'Satina' variation designed only for direct consumption irrespective of the season of the year and place of cultivation. However, the least content of nitrates(V) cumulated in tubers of the potato of 'Agria' variation designer for the production of purified goods, cultivated in the Kujawsko-Pomeranian region, for which the average content of nitrates(V) amounted to 141.9 mg kg^{-1} of fresh matter.

2. Weather conditions prevailing in particular years of plants vegetation influence the content of nitrates(V) in tubers. In 1999, that in the period of vegetation was characterized by a higher air temperature, the content of nitrates(V) in all the cultivated variations of potatoes was considerably higher in comparison to the remaining years and amounted in the Kujawsko--Pomeranian region on average for variations – 191.9 mg kg^{-1} of fresh matter, and in Lower Saxony – 208.1 mg kg^{-1} of fresh matter.

3. As a result of long-lasting storage, all the potatoes of studied variations, comprised less nitrates(V) in tubers in comparison to the volume after the harvest – for 7.2% (stored in temp. +4°C) and for 13.2% (stored in temp. +8°C).

4. Variations of potato of the limited manner of use – 'Agria' and 'Satina', were characterized by the best stability of the content of nitrates(V) in tubers in comparison to the variations of general use – 'Lena' and 'Mors'.

Translated by EWA OŻGA

Accepted for print 5.10.2007

References

BURT T.P., HEATHWAITE A.L., TRUDGILL S.T. 1993. *Nitrate Process, pattern and management*. Chichester, England, Wiley, 10-28.

CIEŚLIK E. 1992. *Zmiany azotanów i azotynów podczas obróbki kulinarnej ziemniaków*. Przem. Spożywczy., 46(92): 266-267.

CIEŚLIK E. 1995. *Czynniki kształtujące zawartość azotanów i azotynów w ziemniakach*, Post. Nauk Rol., 6: 67-71.

FARGASOWA A. 1994. *The effect of environment storage on nitrate content in various potato cultivars from two localities*. Biologia, 49: 917-922.

FRYDECKA-MAZURCZYK A., ZGÓRSKA K. 1996. *Czynniki wpływające na zawartość azotanów w bulwach ziemniaka*, Biuletyn Instytutu Ziemniaka, 47: 111-122.

GISLASON J., DAHLE H.K., RAERUG R., ROER L., RONSEN K. 1984. *Nitrate in potatoes. The effect of fertilization and storage on the nitrate content in 5 genotypes grown in widely separated localities*. Potato Research, 27: 331-337.

GRASSERT V., VOGEL J., NEUBAUER W., BARTEL W. 1990. *Aspekte des Nitratgehalts von Speisekartoffeln unter Berücksichtigung mehrjährigen Ergebnisse*. Kartoffelbau, 41(10): 398-400.

GRUDZIŃSKA M., ZGÓRSKA K. 2005. *Wpływ obróbki wstępnej oraz metod gotowania na zawartość azotanów w warzywach*. Wyd. Środkowo-Pomorskiego Towarzystwa Naukowego Ochrony Środowiska, Koszalin, pp. 233-241.

KOLBE H. 1990. K*artoffeldüngung unter differenzierten ökologischen Bedingungen*. Dissertation, Institut für Agrikulturchemie der Universität, Göttingen, pp. 116-119.

KUNSCH U., SCHÄRER H., TEMPERLI A. 1981. *Eine Schnellmethode zur Bestimmung von Nitrat in Frischgemüsen mit Hilfe der ionensensitiven Elektrode*. (In:) *Mitt. Der Eidgenossischen Forschungsanstalt für Obst-Wein-und Gartenbau Wadenswill Schweiz*. Flugschrift, 46.

MAZURCZYK W., LIS B. 1999. *Zmienność składu chemicznego dojrzałych bulw ziemniaka odmian jadalnych*. Mat. Konf. Nauk. *Ziemniak jadalny i dla przetwórstwa spożywczego – czynniki agrotechniczne i przechowalnicze warunkujące jakość. Radzików*, 23-25.02. ss. 17-19.

MIĘDZYBRODZKA A., CIEŚLIK E., SIKORA E. 1992. *The effect of environment conditions on the level of nitrates and nitrites in various varieties potato*. Polish Journal Food Nutrition Sci., 4: 45-56.

MOZOLEWSKI W., SMOCZYŃSKI S. 2004. *Effect of culinary processes on the content of nitrates and nitrites in potato*. Pakistan J. of NUTR., 3(60): 357-361.

ROGOZIŃSKA I., WOJDYŁA T. 1999. *Der Einfluss variierter Düngung, eines Pflanzenschutzmittel – Anwendung und der Lagerung auf den Glycoalkaloid – Gehalt in Kartoffelknollen zweier Sorten*. Potato Research, 42: 79-88.

ROGOZIŃSKA I., WOJDYŁA T., POBEREŻNY J. 2000. *Contamination of edible potato tubers with compounds decreasing their health status as a result of mineral fertilization*. Pol. J. Env. St. Vol. 10, Suppl. I-Eco-med, 38-41.

ROGOZIŃSKA I. 2003. *Czynniki kształtujące zawartość azotanów w wybranych produktach pochodzenia roślinnego*. Żywienie człowieka, Inż. Maszyn Vol. XX, Zeszyt, 1: 67-72.

Rozporządzenie Ministra Zdrowia z dnia 13.01.2003 r. w sprawie maksymalnych poziomów zanieczyszczeń chemicznych i biologicznych, które mogą znajdować się w żywności, składnikach żywności, dozwolonych substancjach dodatkowych, substancjach pomagających w przetwarzaniu albo na powierzchni żywności. Dz.U. Nr 37, poz. 326.

WADAS W, JABŁOŃSKA-CEGLAREK R, KOSTERNA E. 2005. *The nitrates content in early potato tubers depending on growing conditions*, El. J. Pol. Agric. Univ., Horticulture, 8(1), <http://www.ejpau.media.pl/volume8/issue1/art-26.html>.

WALTER H. 1976. *Strefy roślinności a klimat*. [W:] *Diagramy klimatyczne*. PWRiL, Warszawa, ss. 26-30.

ZGÓRSKA K. 2003. *Zawartość azotanów w bulwach wybranych odmian jadalnych*. Ziemniak Polski, 4: 14-17.

ŻOŁNOWSKI A. 2000. *Oddziaływanie nawożenia na zawartość glikoalkaloidów w ziemniaku podczas wegetacji i przechowywania* (praca doktorska, Uniwersytet Warmińsko-Mazurski), ss. 32-34.

POLISH JOURNAL OF NATURAL SCIENCES
Abbrev.: Pol. J. Natur. Sc., Vol 23(2): 347-365, Y. 2008

DOI 10.2478/v10020-008-0027-x

THE EFFECT OF VARIOUS CALCIUM, MAGNESIUM, POTASSIUM AND HYDROGEN SATURATION OF CEC ON THE YIELD AND MINERAL COMPOSITION OF SUNFLOWER

Marta Zalewska

Chair of Agricultural Chemistry and Environmental Protection
University of Warmia and Mazury in Olsztyn

K e y w o r d s: sunflower, calcium, magnesium, potassium saturation of the soil CEC, cation ratios in the soil, mineral composition.

A b s t r a c t

A pot experiment was performed to determine the effect of varying levels of calcium, magnesium, potassium and hydrogen saturation of the soil CEC (cation exchange capacity) on the yield and mineral composition of sunflower green matter.

A high yield of plants with a balanced mineral composition was recorded when the percentage saturation of the soil CEC was around 60% for calcium, 8.4% for magnesium and 4.2% for potassium. A drop in the potassium saturation of the soil CEC below 3% and its rise above 8% resulted in a significant decrease in sunflower yield. A drop in the magnesium saturation of the soil CEC below 5% also caused a significant decrease in the yield of sunflower green forage.

The potassium content of sunflower green matter depended primarily on soil potassium saturation. Increased magnesium or calcium saturation of the soil CEC limited excessive potassium accumulation in plant tissues to a slight degree only. The ratio of exchangeable magnesium to exchangeable potassium in the soil was found to be a more reliable indicator of magnesium availability than the percentage saturation of the soil CEC with this element or exchangeable magnesium content. The recommended Mg : K ratio in the soil, ensuring a high yield of sunflower with a desirable mineral composition, is 2:1 on a percent exchangeable basis.

Address: Marta Zalewska, University of Warmia and Mazury in Olsztyn, ul. Michała Oczapowskiego 8, 10-744 Olsztyn, Poland, phone 48 (089) 523 49 64, e-mail: marta.zalewska@uwm.edu.pl

WPŁYW ZRÓŻNICOWANEGO WYSYCENIA KOMPLEKSU SORPCYJNEGO GLEBY WAPNIEM, MAGNEZEM, POTASEM I WODOREM NA PLONOWANIE I SKŁAD MINERALNY SŁONECZNIKA

Marta Zalewska

Katedra Chemii Rolnej i Ochrony Środowiska
Uniwersytet Warmińsko-Mazurski w Olsztynie

S ł o w a k l u c z o w e: słonecznik, wysycenie kompleksu sorpcyjnego gleby wapniem, magnezem, potasem, skład mineralny, stosunki kationów.

A b s t r a k t

W doświadczeniu wazonowym badano wpływ zróżnicowanego wysycenia kompleksu sorpcyjnego gleby wapniem, magnezem, potasem i wodorem na plonowanie oraz skład mineralny zielonki słonecznika.

Wysoką masę roślin o zrównoważonym składzie mineralnym uzyskano na glebie o wysyceniu kompleksu sorpcyjnego wapniem ok. 60%, magnezem – 8,4% i potasem – 4,2%. Zmniejszenie udziału potasu w kompleksie sorpcyjnym poniżej 3% oraz jego wzrost powyżej 8% był przyczyną istotnego spadku plonów słonecznika. Obniżenie wysycenia kompleksu sorpcyjnego magnezem poniżej 5% także spowodowało istotne zmniejszenie zielonej masy słonecznika.

Zawartość potasu w zielonce słonecznika zależała głównie od udziału tego pierwiastka w kompleksie sorpcyjnym gleby. Zwiększenie wysycenia kompleksu sorpcyjnego gleby magnezem bądź wapniem w małym stopniu ograniczyło nadmierną akumulację potasu w tkankach roślin.

Wartość stosunku Mg : K wymiennego w glebie okazała się lepszym wskaźnikiem do określenia przyswajalności Mg niż stopień wysycenia kompleksu sorpcyjnego tym pierwiastkiem oraz zawartość Mg wymiennego. W celu uzyskania wysokich plonów słonecznika o prawidłowym składzie mineralnym stosunek Mg : K wymiennego w glebie powinien kształtować się na poziomie zbliżonym do 2 : 1.

Introduction

Fertilization levels are usually determined based on soil tests, following one of the two basic approaches, namely the SLAN (the sufficiency level of available nutrients) concept and the BCSR (basic cation saturation ratios) concept. The former (applied more frequently) involves the determination of the sufficient level of each nutrient in the soil, while the latter involves the determination of the optimum cation saturation of the soil CEC, leading to high yields of good-quality crops (MCLEAN, CARBONELL 1972, LIEBHARDT 1981, OLSON et al. 1982, MCLEAN et al. 1983).

Studies on the BCSR concept have revealed that there is no one optimum level of calcium, magnesium, potassium and hydrogen that would meet the requirements of all plant species and soil types. Sometimes considerable changes in the cation saturation of the soil CEC have no impact on crop yield. However, it should be stressed that differences in soil saturation with cations are followed by substantial differences in the mineral composition of plants,

particularly of their vegetative parts (McLean, Carbonell 1972, Eckert, McLean 1981, McLean et al. 1983, Zalewska 2005a, Zalewska 2005b).

The optimum cation saturation of the soil CEC, indispensable for attaining high yields of good-quality crops, should reach 65 to 85% for Ca, 10 to 15% for Mg and 5% for K (McLean, Carbonell 1972, Zalewska 2003). An increase in the potassium saturation of the soil CEC above 5% often contributes to yield increment, but at the same time it causes undesirable changes in the mineral composition of plants, due to excessive accumulation of potassium accompanied by a decrease in the concentrations of magnesium and calcium. This concerns in particular fodder crops grown for green forage. Most authors share the opinion that the Mg : K ratio in the soil should not be lower than 2:1 in order to achieve high yields of plants with the desired mineral composition (McLean, Carbonell 1972, Zalewska 2003). A decrease in the value of the Mg : K ratio below 2 generally leads to a decrease in the quality of green forage due to excessive accumulation of potassium and a decrease in magnesium concentration (Zalewska 2005a, 2005b). A yield decrease usually occurs at greater disproportions between those elements in the soil. Research results have shown that the Ca:Mg ratio has only a limited influence on the yield and mineral composition of plants grown on soil with an optimum reaction (McLean, Carbonell 1972, Fox, Piekiełek 1984, Zalewska 2003).

The ratios between the cations K^+, Mg^{2+} and Ca^{2+}, both in the soil and plant, may provide a basis for estimating the degree of acid soil degradation caused by cationic unbalance of the soil CEC (Filipek 2001).

Materials and Methods

A pot experiment was carried out on 14 fertilizer treatments (each in four replications) with varied cation saturation of soil CEC. The experiment consisted of three parts. In A part the ratio value of Ca to the other cations increased. In B part the ratio value of Mg to the other cations increased, while in C part the ratio value of K : Ca and K : Ca increased.

The pots were filled with 6 kg of light silty loam (terminology based on Polish Norm *Gleby...* BN-78/9180-11). Total cation exchange capacity amounted to 114.4 mmol(+) kg^{-1} of soil. Other physical and chemical properties of the soil are presented in Table 1. The experimental plant was fodder sunflower (cv. Record) which was harvested at the beginning of flowering.

In order to obtain different Ca, Mg and K saturation of CEC in the particular experimental treatments, adequate amounts of the above cations were applied in the soil prior to sowing (Table 2). Calcium and magnesium as oxides and potassium in equal parts as KCl and K_2SO_4 were added to the soil.

Table 1

Physico-chemical properties of soil used in the experiment

Soil fractions (%)			Humus (%)	pH_{KCl}	Hh mmol H^+ kg^{-1} of soil	Exchangeable cations mg kg^{-1} of soil			Saturation of CEC (%)				T mmol(+) kg^{-1} of soil
diameter (mm)													
1.0-0.1	0.1-0.02	< 0.02				Ca	Mg	K	Ca	Mg	K	H	
36	37	27	4.51	4.2	80.6	550	52	86	24.0	3.7	1.9	70.5	114.4

Table 2

The soil Ca : Mg : K : H ratios, cation saturation of CEC and other chemical properties of soil after incubation with fertilizers

Cation ratios in soil mmol(+)				Saturation of CEC (%)				V (%)	Hh mmol H^+ kg^{-1} of soil	pH_{KCl}	Exchangeable cations mg kg^{-1} of soil			Fertilization mg kg^{-1} of soil		
Ca	Mg	K	H	Ca	Mg	K	H				Ca	Mg	K	Ca	Mg	K
6.5	1.0	0.50	19.2	24.0	3.7	1.9	70.5	29.6	80.6	4.2	550	52	86	–	–	–
Series *A*																
19.7	1.0	0.49	6.7	70.7	3.6	1.8	24.0	76.1	34.1	5.8	2026	62	98	1476	11	12
15.2	1.0	0.51	5.7	68.0	4.5	2.3	25.2	74.8	35.6	5.7	1954	79	129	1439	28	43
10.7	1.0	0.52	4.3	64.9	6.1	3.2	25.9	74.2	35.8	5.6	1820	111	176	1270	59	90
7.0	**1.0**	**0.50**	**3.4**	**58.8**	**8.4**	**4.2**	**28.6**	**71.4**	**39.8**	**5.5**	**1637**	**153**	**246**	**1087**	**102**	**160**
2.8	1.0	0.52	2.5	41.3	14.7	7.6	36.3	63.6	49.0	5.2	1124	272	438	574	221	352
Series *B*																
6.6	0.5	0.50	3.1	61.4	4.8	4.6	29.2	70.8	40.2	5.5	1733	81	260	1184	29	174
6.4	1.4	0.50	3.4	54.6	12.3	4.3	28.7	71.2	38.2	5.5	1553	217	231	1003	166	145
6.4	2.6	0.50	3.8	48.1	19.6	3.7	28.6	71.4	39.0	5.5	1343	375	203	793	324	117
6.4	5.7	0.50	4.7	37.1	32.7	2.9	27.3	72.7	36.0	5.6	1020	618	152	470	567	67
Series *C*																
6.6	1.0	0.26	3.2	59.8	9.0	2.3	28.9	71.1	38.4	5.6	1683	157	125	1133	105	39
6.8	1.0	0.75	3.5	56.6	8.3	6.2	29.0	71.1	40.2	5.5	1593	148	360	1043	97	274
6.7	1.0	0.95	3.5	54.9	8.2	7.8	29.1	70.9	40.8	5.5	1553	145	465	1003	93	379
6.4	1.0	1.41	4.1	49.8	7.7	10.9	31.6	68.4	44.4	5.3	1475	137	665	925	86	579

In addition, 0.7 g of N and 0.44 g of P per pot (in the form of $(NH_4)_2HPO_4$ and NH_4NO_3) were applied before sowing in equal doses in all the treatments. During the growing season ammonium saltpeter was added at the amount of 0.7 g N/pot. After three-week soil incubation with fertilizers, seeds of suflower were sown. After germination, 5 plants were left in each pot. Soil moisture was maintained at the level of 60% of maximum water capacity during the experiment.

Simultaneously, another experiment was carried out, in which soil samples with the same amounts of Ca, Mg and K as those used in the vegetative experiment (expressed per 1 kg of soil) were incubated for 3 weeks. The results of the analysis of the soil collected after incubation provided the basis for determination the initial cation saturation of the soil CEC. The doses of Ca, Mg and K used in the experiment and the concentration of exchangeable cations obtained after soil incubation with fertilizers and Ca, Mg and K saturation of CEC in individual fertilizer treatments are presented in Table 2.

Plant samples were dried, ground and mineralised in concentrated H_2SO_4 with an addition of 30% H_2O_2. Potassium and calcium were determined by the atomic emission spectrometry, magnesium by the atomic absorption spectrometry, nitrogen concentration by Kjeldahl's method and phosphorus by the vanadium-molybdenum method. Soil samples were taken from each pot after incubation with fertilizers, dried and analysed for exchangeable K, Ca and Mg (leaching with 1M CH_3COONH_4 at pH 7.0). Hydrolytic acidity was determined by Kappen's method and soil pH in 1 M KCl.

The statistical analysis of the experimental results involved the following:

– an analysis of variance for one-factor pot experiment in a completely random orthogonal design' the significance of differences between treatment means was verified using Student's *t*-test.

– an analysis of correlation and regression; the significance of determination coefficient was evaluated with Student's *t*-test and Snedecor's F-test.

Results and Discussion

Varying levels of Ca, Mg, K and H saturation of the soil CEC had a significant effect on the yield of sunflower plants harvested at the beginning of flowering (Table 3). In fertilization series *A*, where calcium saturation of the soil CEC increased from 41.3% to 70.7% while soil saturation with magnesium, potassium and hydrogen decreased, the highest yield of sunflower green forage was obtained in the treatment with the following levels of cation saturation of the soil CEC: Ca – 58.8%, Mg – 8.4% and K – 4.2% (the Ca : Mg : K ratio was 7 : 1 : 0.5). This was one of the highest yields attained over the entire

experiment. A further increase in the ratio between Ca and the other cations caused a decrease in plant mass. A highly significantly lower yield was achieved in the treatments where the percentage saturation of the soil CEC was 68 to 71% for Ca, 4.5 to 3.6% for Mg and 2.3 to 1.8% for K (which corresponded to the Ca : Mg : K ratio of (15-20) : 1 : 0.5). It seems that the main reason for yield decrease in the above treatments was lower potassium saturation of the soil CEC (below 3%) which resulted in insufficient supply of this element to sunflower plants.

Table 3

Effect of Ca, Mg, K and H saturation of CEC on yield and mineral composition of sunflower

Fertilizer treatments Saturation of CEC (%)				Yield d.m. g pot^{-1}	Concentration g kg^{-1} of d.m.					K Ca + Mg mmol(+)	Uptake mg pot^{-1}				
Ca	Mg	K	H		N	P	Ca	Mg	K		N	P	Ca	Mg	K
24.0	3.7	1.9	70.5	36.4	26.2	2.1	16.5	7.2	10.9	0.20	954	76	601	262	397
Series *A*															
70.7	3.6	1.8	24.0	37.1	25.9	1.9	19.9	7.3	14.1	0.23	961	70	738	271	523
68.0	4.5	2.3	25.2	39.4	26.3	1.9	18.8	8.1	15.0	0.24	1036	75	741	319	591
64.9	6.1	3.2	25.9	44.4	25.3	1.9	17.9	7.6	19.5	0.33	1123	84	795	337	866
58.8	**8.4**	**4.2**	**28.6**	**46.6**	**26.3**	**2.0**	**17.6**	**7.5**	**26.7**	**0.46**	**1226**	**93**	**820**	**350**	**1244**
41.3	14.7	7.6	36.3	45.9	26.0	1.7	15.0	7.1	48.2	0.92	1193	78	689	326	2212
Series *B*															
61.4	4.8	4.6	29.2	41.7	26.5	1.8	18.9	6.3	30.1	0.53	1105	75	788	263	1255
54.6	12.3	4.3	28.7	45.4	26.0	2.0	17.1	9.0	25.2	0.40	1180	91	776	409	1144
48.1	19.6	3.7	28.6	44.4	28.0	2.1	15.6	0.6	21.9	0.34	1243	93	693	470	972
37.1	32.7	2.9	27.3	44.0	28.0	2.5	10.9	2.1	16.6	0.28	1232	110	480	532	730
Series *C*															
59.8	9.0	2.3	28.9	40.5	26.7	2.3	19.0	1.1	15.3	0.21	1081	93	770	450	620
56.6	8.3	6.2	29.0	46.6	27.1	2.2	17.1	6.6	39.3	0.72	1263	103	797	308	1831
54.9	8.2	7.8	29.1	46.7	28.2	2.1	17.6	5.2	50.7	0.99	1317	98	822	243	2368
49.8	7.7	10.9	31.6	42.0	30.8	2.3	17.6	3.6	72.1	1.57	1294	97	739	151	3028
		$LSD_{0.01}$		**2.5**											

In fertilization series *B*, where an increase in the magnesium saturation of the soil CEC was accompanied by a decrease in soil saturation with calcium and potassium, a significant yield-forming effect of magnesium was observed when the level of soil saturation with this element increased from 4.8% to 8.4%. A further increase in the Mg saturation of the soil CEC accompanied by a decrease in the Ca and K saturation of the soil CEC, resulted in a decrease in plant mass. However, a highly significantly lower yield was recorded only when soil saturation with magnesium increased to 32.7%. In this treatment the

percentage saturation of the soil CEC for potassium and calcium was 2.9% and 37.1%, respectively (the Ca : Mg : K ratio was 6.4 : 5.7 : 0.5).

In fertilization series C, where the potassium saturation of the soil CEC increased from 2.3% to 10.9%, a significant decrease in plant mass was observed in the treatments where the potassium saturation of the soil CEC was 2.3% (the lowest level) and 10.9% (the highest level).

It may be concluded that in the case of sunflower the percentage saturation for each of the cations should oscillate around the following values: Ca – 58.8%, Mg – 8.4%, K – 4.2% (which corresponds to the Ca:Mg:K ratio of 7 : 1 : 0.5) A significant decrease in plant mass can be expected as the potassium saturation of the soil CEC drops below 3% or rises above 8%, or as the magnesium saturation of the soil CEC decreases to a level of 5%.

The mineral composition of sunflower green forage underwent substantial alternations along with changes in the proportions of exchangeable Ca, Mg, K and H in the soil (Table 3). Potassium concentration changed to the greatest extent (Table 4). It was found that sunflowers can accumulate large amounts of this element. In the control treatment, where soil K saturation was 1.9%, the potassium content of plants was 10.9 g kg^{-1} d.m. In the treatment with the highest soil K saturation – 10.9%, the concentration of this nutrient in plants increased to 72.1 g kg^{-1} d.m.

Table 4
Values of variability coefficients calculated for Ca, Mg and K concentration in sunflower

Value of variability coefficients V_x		
Ca	Mg	K
13.5	30.2	57.8

An analysis of correlations between potassium concentration in sunflower plants and the percentage saturation of the soil CEC with Ca, Mg and K, the content of exchangeable forms of those cations as well as the Ca : K, Mg : K and (Ca + Mg) : K ratios in the soil (Table 5) revealed that potassium concentration in the sunflower depended primarily on the quantity of exchangeable K (r^2 = 0.997***) or on the percentage saturation of the soil CEC with this element (r^2 = 0.993***). The courses of regression curves (Figures 1a, 1b) indicate that a rise in the above parameters was followed by a proportional increase in potassium concentration in plants. The correlation between the Mg and Ca saturation of the soil CEC and the potassium content of sunflower plants was low and non-significant. This shows that potassium can successfully compete with calcium and magnesium in the uptake process. Thus, it would

Table 5

Values of correlation coeffiecients between chemical properties of soil, concentration and uptake of Ca, Mg, K by sunflower

Plant (y)	Soil (x)									
	Exchangeable cations mg kg^{-1} of soil			Saturation of CEC (%)			Cation ratios in soil mmol(+)			
	Ca	Mg	K	Ca	Mg	K	Ca/K	Mg/K	Ca/Mg	(Ca+Mg)/K
K in d.m.	n	n	0.997^{***}	n	n	0.993^{***}	0.867^{***}	n	n	0.976^{***}
Mg in d.m.	n	0.423*	0.557^{**}	n	0.423*	0.534^{**}	n	0.925^{***}	n	0.645^{**}
Ca in d.m.	0.886^{***}	0.912^{***}	n	0.876^{***}	0.912^{***}	n	n	0.635^{***}	0.867^{***}	n
K uptake	n	n	0.990^{***}	n	n	0.992^{***}	0.899^{***}	n	n	0.987^{***}
Mg uptake	n	0.533^{**}	0.419*	n	0.535^{**}	0.391*	n	0.905^{***}	n	0.595^{**}
Ca uptake	0.850^{***}	0.870^{***}	n	0.881^{***}	0.869^{***}	n	n	0.773^{***}	0.671^{***}	n
K/(Ca+Mg)	n	n	0.997^{***}	n	n	0.995^{***}	0.852^{***}	n	n	0.957^{***}

* significant at $p = 0.05$, ** significant at $p = 0.01$, *** significant at $p = 0.001$
n – non-significant

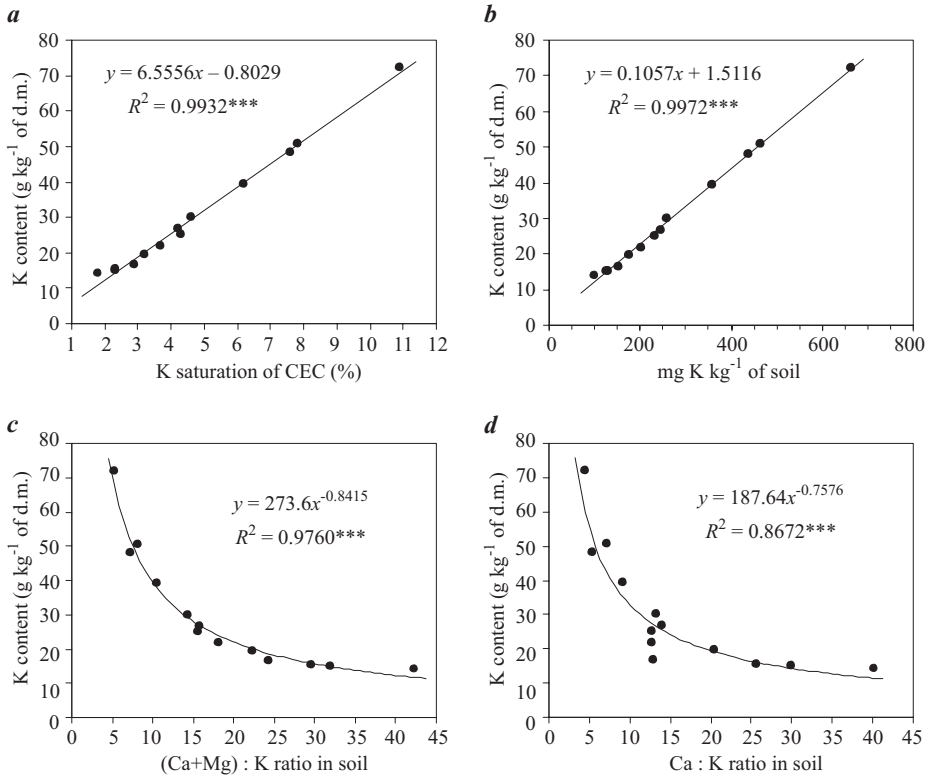


Fig. 1. Potassium concentration in sunflower as affected by percentage K saturation of CEC (a), content of exchangeable K in soil (b), (Ca+Mg) : K ratio [mmol(+)] in soil (c) and Ca : K ratio [mmol(+)] in soil (d)

be difficult to considerably limit excessive potassium accumulation by supplementary fertilization with magnesium or calcium. There was also a strong correlation between the potassium content of sunflower and the (Ca + Mg) : : K ratio (r^2 = 0.976***) and the Ca : K ratio (r^2 = 0.867***) in the soil. The courses of regression curves (Figures 1c, 1d) show that a rise in the values of those ratios from around 5 to 15-20 caused a substantial drop in potassium concentration in sunflower plants (from 72 to 20 g kg^{-1} d.m.). A further increase in the above proportions had an insignificant effect on potassium accumulation in plants. The correlations between the chemical properties of soil and potassium uptake were similar to those observed for the content of this nutrient in plants (Table 5, Figure 2).

Sunflower green forage was characterized by a very high magnesium content (3.6-12.1 g Mg kg^{-1} d.m.). It should be emphasized that the concentration of this element underwent great changes under the influence of different

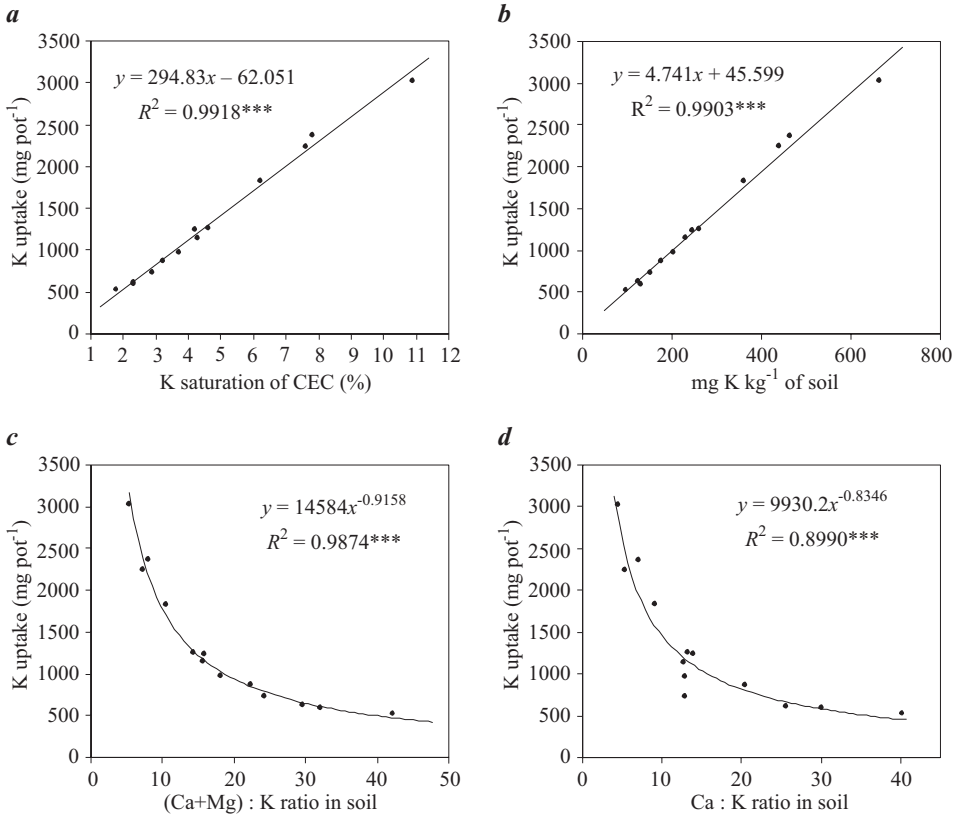


Fig. 2. Potassium uptake by sunflower as affected by percentage K saturation of CEC (a), content of exchangeable K in soil (b), (Ca+Mg) : K ratio [mmol(+)] in soil (c) and Ca : K ratio [mmol(+)] in soil (d)

Mg, K and Ca rates. Previous experiments conducted by the author (ZALEWSKA 1995, 2005a, 2005b) demonstrated that Mg content is much more stable than K concentration, and that it is related to the genetic properties of a given species to a higher extent. A low and stable concentration of this nutrient was noted in carrot roots (1.1-1.5 g kg^{-1} d.m.), sugar beet roots (1.4-1.8 g Mg kg^{-1} d.m.) and wheat grain (1.1 g Mg kg^{-1} d.m.). The magnesium content of spring and winter rape seeds was also affected by fertilization to a very low degree, but the level of this element was much higher (approx. 3.5 g Mg kg^{-1} d.m.) (PANAK, ZALEWSKA 1988, ZALEWSKA 1995). In some crops, e.g. oat harvested at the milk-ripe stage (1.8-3.0 g Mg kg^{-1} d.m.), lupine green matter (2.0-3.1 g Mg kg^{-1} d.m.), carrot leaves and beet leaves (3.1-6.4 g Mg kg^{-1} d.m.), magnesium concentration may be increased though fertilizer application (ZALEWSKA 1995, 2003, 2005a, 2005b). Among the crops tested by the author, the highest Mg levels were recorded in sunflower green matter. Moreover, in experiments on sunflower magnesium concentration increased threefold (from 3.6 to 12.1 g Mg kg^{-1} d.m.) following changes in soil cation ratios. This confirms that plant species and organs differ considerably with regard to magnesium content as well as the possibility to increase the concentration of this nutrient through fertilization. This should be taken into account while determining the composition of fodder crop mixtures. According to SHOCKEY, REID (1984), not only total Mg content but also the proportions of different chemical forms of this element in plants are determined by genetic properties.

The values of determination coefficients (Table 3) suggest that the magnesium content of sunflower plants depended to a much greater extent on the Mg : K ratio in the soil (r^2 = 0.925***) than on the magnesium saturation of the soil CEC (r^2 = 0.423*). Magnesium concentration in plants was also affected to a higher degree by the level of exchangeable K (r^2 = 0.534**) than by that of exchangeable Mg. An increase in the potassium saturation of the soil CEC was related to a gradual decrease in Mg concentration (Figure 3d). In fertilization series *C* the magnesium content of plants decreased over threefold (from 11.1 to 3.6 g Mg kg^{-1} d.m.) as a result of an increase in the potassium saturation of the soil CEC, despite high magnesium abundance in the soil (137-157 mg exchangeable Mg kg^{-1} of soil). This indicates a strong antagonistic effect of potassium on magnesium uptake, widely discussed in literature (STĘPIEŃ et al. 2001, WYSZKOWSKI 2001, ZALEWSKA 2003, 2005a, 2005b, SIENKIEWICZ et al. 2004). Therefore, it should be stressed that even in soil rich in magnesium the application of high potassium rates may substantially reduce magnesium accumulation in the vegetative parts of plants.

The course of the curve illustrating the correlation between magnesium concentration in sunflower plants and the (Ca + Mg) : K ratio in the soil is also interesting (Figure 3f). Plants in the treatment where the (Ca + Mg) : K ratio

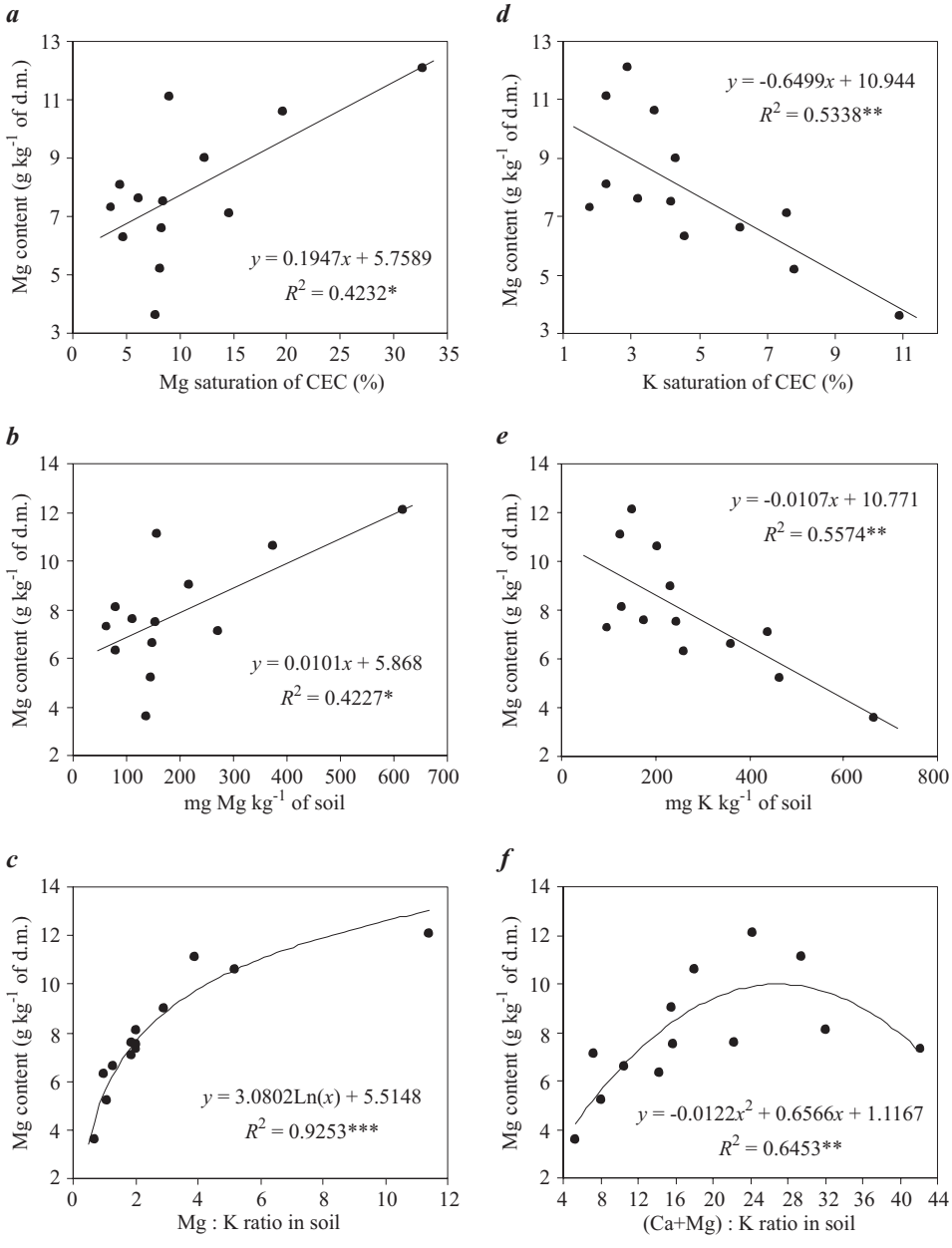


Fig. 3. Magnesium concentration in sunflower as affected by percentage Mg saturation of CEC (a), content of exchangeable Mg in soil (b), Mg : K ratio [mmol(+)] in soil (c), percentage K saturation of CEC (d), content of exchangeable K in soil (e) and (Ca+Mg):K ratio [mmol(+)] in soil (f)

in the soil was 25 : 1 were characterized by the highest magnesium content. A decrease in magnesium concentration was observed above and below this value. It may be concluded that magnesium uptake was limited not only by potassium, but also by calcium, though to a much smaller degree. The correlations between the chemical properties of soil and magnesium uptake were similar to those observed for the Mg content of plants (Table 5, Figure 4).

Sunflower green forage was also characterized by a high calcium content (10.9-19.9 g Ca kg^{-1} d.m.). The concentration of this element was more stable than the levels of potassium or magnesium (Table 4), but it also underwent changes induced by varying soil saturation with cations. An analysis of determination coefficients (Table 5) and regression equations (Figure 5), reflecting the impact of different chemical properties of soil on calcium concentration revealed that an increase in the calcium saturation of the soil CEC was followed by a significant increase in the calcium content of sunflower (r^2 = 0.876***). The concentration of this nutrient in plants was distinctly reduced by magnesium (Figures 5d, 5e). The antagonistic effect of Mg^{2+} on Ca^{2+} uptake was also observed by FILIPEK et al. (1989) in an experiment on spring barley. Those authors claim that Mg and Ca compete for negative charges in the cell during cation uptake.

It is interesting that potassium – a strong antagonist of magnesium – has no such influence on calcium. This was confirmed by a very low and non-significant coefficient of determination reflecting the correlation between calcium concentration in the sunflower and the potassium saturation of the soil CEC. A decrease in calcium concentration along with an increase in the Mg : K ratio in the soil is the evidence of a distinct, antagonistic effect of magnesium on calcium uptake (Figure 5f). A high correlation was also noted between the Ca : Mg ratio in the soil and calcium concentration in sunflower plants (r^2 = 0.867***). The course of the regression curve (Figure 5c) indicates that calcium content increased at a fast rate as the value of the Ca : Mg ratio rose from around 1 to 7. Above the value of 7, calcium concentration in plants remained at a stable level.

The relationships between the tested chemical properties of soil and calcium uptake were similar as in the case of the calcium content of plants (Table 5, Figure 6). Only the pattern of changes in the effect of soil Ca saturation was slightly different. Calcium concentration rose along with an increase in soil saturation with this element, whereas calcium uptake decreased to some degree in soil whose saturation with this cation exceeded 60%. As a result, yield decreased in treatments with high calcium saturation of the soil CEC.

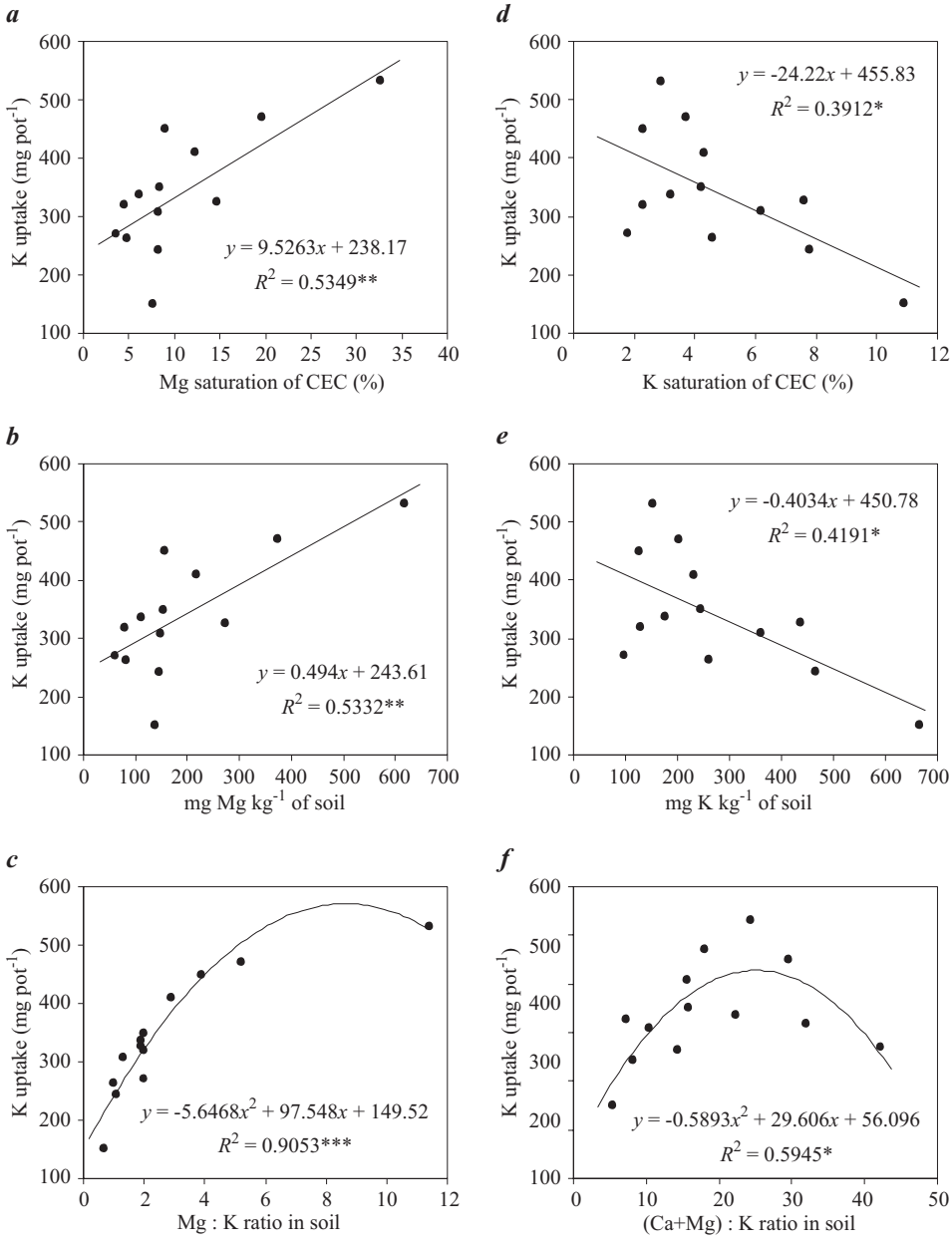


Fig. 4. Magnesium uptake by sunflower as affected by percentage Mg saturation of CEC (a), content of exchangeable Mg in soil (b), Mg : K ratio [mmol(+)] in soil (c), percentage K saturation of CEC (d), content of exchangeable K in soil (e) and (Ca+Mg):K ratio [mmol(+)] in soil (f)

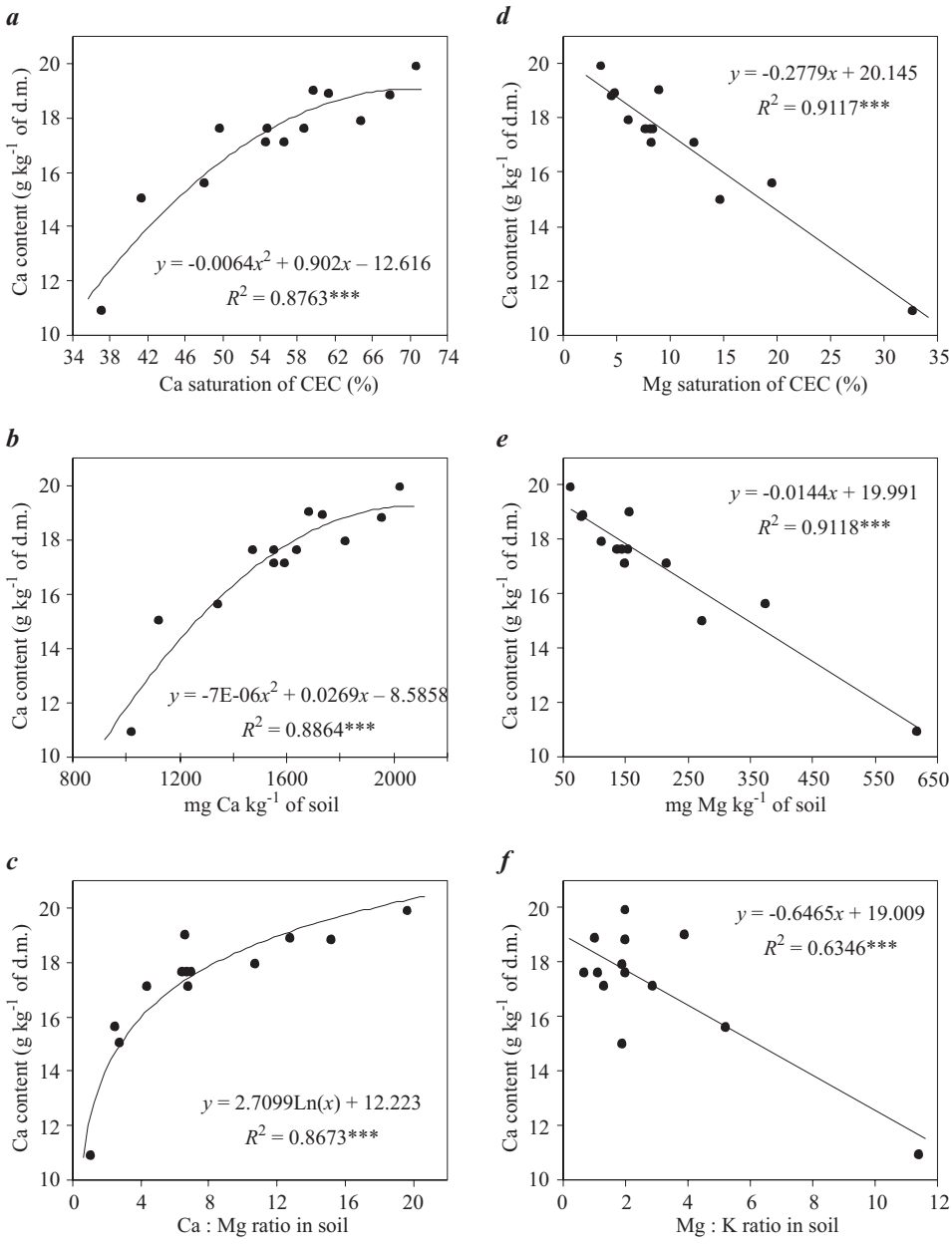


Fig. 5. Calcium concentration in sunflower as affected by percentage Ca saturation of CEC (a), content of exchangeable Ca in soil (b), Ca : Mg ratio [mmol(+)] in soil (c), percentage Mg saturation of CEC (d), content of exchangeable Mg in soil (e) and Mg : K ratio [mmol(+)] in soil (f)

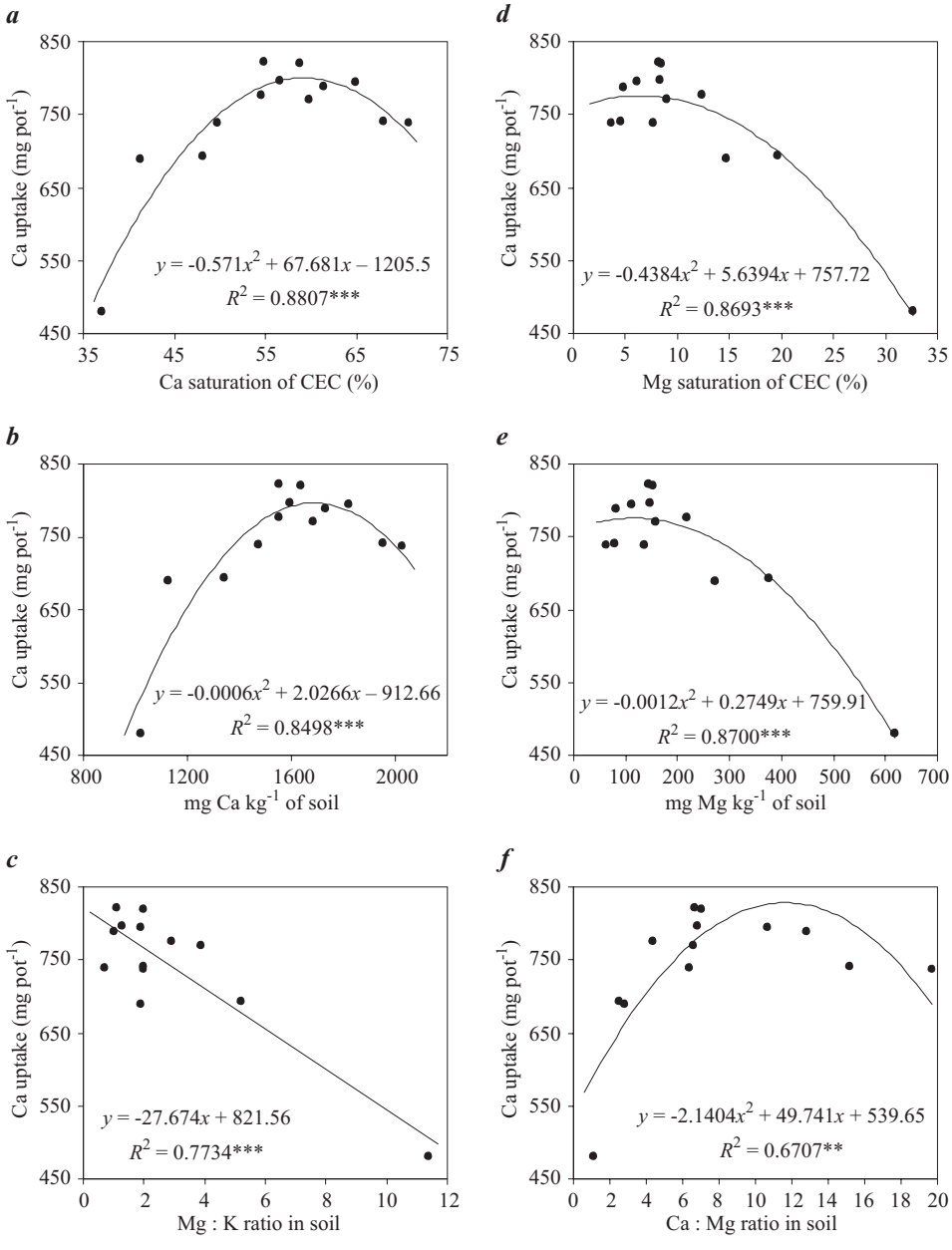


Fig. 6. Calcium uptake by sunflower as affected by percentage Ca saturation of CEC (a), content of exchangeable Ca in soil (b), Mg : K ratio [mmol(+)] in soil (c), percentage K saturation of CEC (d), content of exchangeable K in soil (e) and Ca : Mg ratio [mmol(+)] in soil (f)

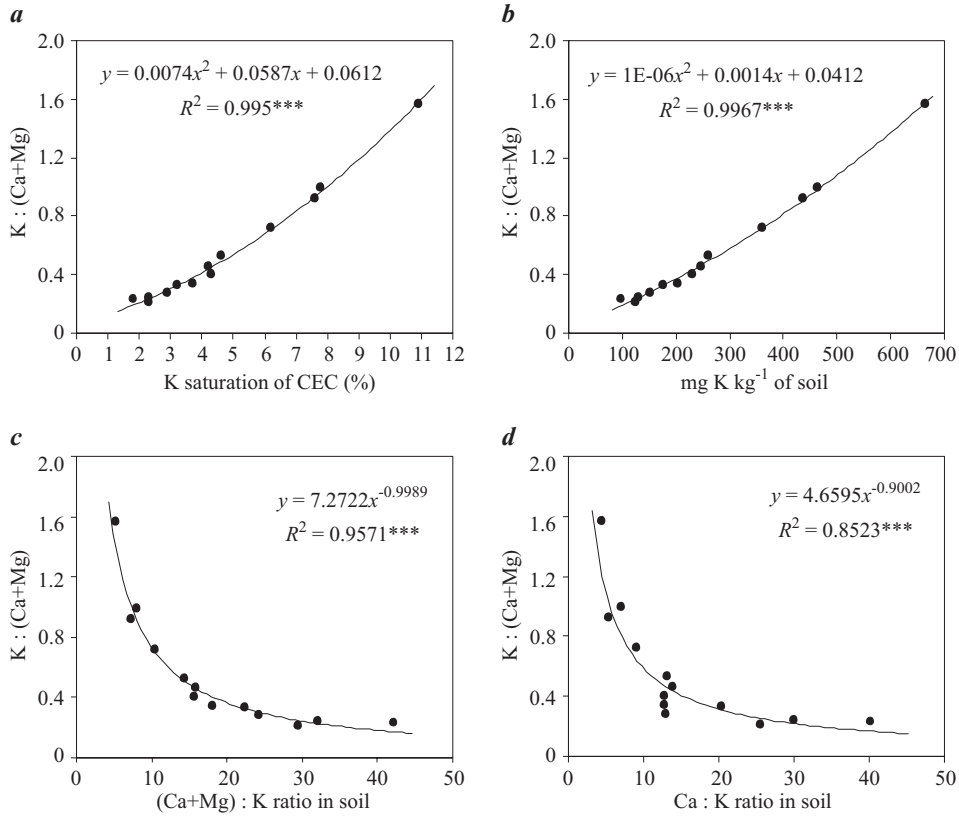


Fig. 7. K : (Ca+Mg) ratio in dry matter of sunflower as affected by percentage K saturation of CEC (a) content of exchangeable K in soil (b), (Ca+Mg) : K ratio in soil (c) and Ca : K ratio in soil (d); ratios expressed in mmol(+)

One of the criteria of fodder quality is the K : (Ca + Mg) ratio in the soil. If this ratio exceeds 2.2, the utilization of Mg and Ca contained in fodder is hindered, which in consequence leads to grass tetany in cattle. In the present experiment the K : (Ca + Mg) ratio remained below 2.2, despite a very high concentration of potassium in sunflower green forage (Table 3). Well-balanced ratios between K and two divalent cations (Ca + Mg) were determined by high concentrations of calcium and magnesium in sunflower green matter. An analysis of correlation and regression (Table 5, Figure 7) revealed that the K : (Ca + Mg) ratio was correlated to the highest degree with the percentage potassium saturation of the soil CEC (r^2 = 0.995***) or with exchangeable potassium content in soil (r^2 = 0.997***). The regression curve shows (Figure 7a) that a substantial increase in the K : (Ca + Mg) ratio took place in treatments where the potassium saturation of the soil CEC exceeded 5%. This ratio

depended also on the soil (Ca + Mg) : K ratio to a very high degree (r^2 = 0.957***) and on the soil Ca : K ratio to a slightly lower extent (r^2 = 0.852***). An increase in the above ratios in the soil from around 5 to 20 contributed to a considerable decrease in the K : (Ca + Mg) ratio in plant tissues. A further increase in the (Ca + Mg) : K and Ca : K ratios in the soil had no significant effect on the K : (Ca + Mg) ratio in sunflower green matter.

Conclusions

1. A high yield of sunflower green forage with a balanced mineral composition may be attained when the percentage saturation of the soil CEC is around 60% for calcium, 8.4% for magnesium and 4.2% for potassium. A drop in the potassium saturation of the soil CEC below 3% and its rise above 8% results in a significant decrease in plant mass. A drop in the magnesium saturation of the soil CEC below 5% also causes a significant decrease in crop yield.

2. The ratio of exchangeable Mg : K in the soil is a more reliable indicator of magnesium availability than the percentage saturation of the soil CEC with this element or exchangeable magnesium content. The recommended Mg : K ratio in the soil, ensuring a high yield of sunflower with a desirable mineral composition, is 2 : 1 on a percent exchangeable basis.

3. The potassium content of sunflower green matter depends primarily on the potassium saturation of the soil CEC. Increased magnesium or calcium saturation of the soil CEC limits excessive potassium accumulation in plant tissues to a slight degree only.

Translated by Aleksandra Poprawska

Accepted for print 27.08.2007

References

Eckert D.J., McLean E.O. 1981. *Basic cation saturation ratios as a basis for fertilizing and liming agronomic crops*. I. Growth chamber studies. Agron. J., 73: 795-799.

Filipek T. 2001. *Wpływ zakwaszenia na zawartość potasu i magnezu oraz stosunek K : Mg w glebach i roślinach zbożowych*. Zesz. Probl. Post. Nauk Rol., 480: 43-49.

Filipek T., Łabuda S., Dechnik I. 1989. *Równowaga jonowa w jęczmieniu jarym w warunkach zróżnicowanej wilgotności i wysycenia kompleksu sorpcyjnego gleb kationami wapnia i magnezu*. Ann. Univ. Mariae Curie-Skłodowska, sectio E, vol. XLIV, 12: 83-90.

Fox R.H., Piekielek W.P. 1984. *Soil magnesium level, corn* (*Zea mays* L.) *yield, and magnesium uptake*. Comm. Soil Sci. Plant Anal., 15(2): 109-123.

Gleby i utwory mineralne. Podział na frakcje i grupy granulometryczne. BN-78/9180-11.

Liebhardt W.C. 1981. *The base cation saturation ratio concept and lime and potassium recommendations on Delaware's Coastal Plain soils*. Soil Sci. Soc. Am. J., 45: 544-549.

McLean E.O., Carbonell M.D. 1972. *Calcium, magnesium and potassium saturation ratios in two*

soils and their effects upon yields and nutrient contents of German millet and alfalfa. Soil Sci. Soc. Am. Proc., 36: 927-931.

McLean E.O., Hartwig R.C., Eckert D.J., Triplett G. B. 1983. *Basic cation saturation ratios as a basis for fertilizing and liming agronomic crops*. II. *Field studies*. Agron. J., 75: 635-639.

Olson R.A., Frank K.D., Grabouski P.H., Rehm G.W. 1982. *Economic and agronomic impacts of varied philosophies of soil testing*. Agron. J., 74: 492-499.

Panak H., Zalewska M. 1988. *Wpływ zróżnicowanych stosunków kationów w glebie na plon i skład mineralny rzepaku jarego*. Rocz. AR w Poznaniu, CXCVII: 117-128.

Shockey W.L., Reid R.L. 1984. *Partition of Mg in forages as affected by fertilization, species, growth stage, and sample preparation*. Agron. J., 76 (4): 565-568.

Sienkiewicz S., Krzebietke S., Sienkiewicz J. 2004. *Równowaga jonowa kukurydzy i jej wpływ na plon w warunkach zróżnicowanego nawożenia organiczno-mineralnego i mineralnego*. J. Elementology, 9(4): 749-758.

Stępień W., Mercik S., Turemka E. 2001. *Działanie wzrastających dawek potasu na plonowanie i skład chemiczny roślin na glebach o zróżnicowanych właściwościach fizykochemicznych*. Zesz. Probl. Post. Nauk Rol., 480: 141-148.

Wyszkowski M. 2001. *Wpływ magnezu na kształtowanie plonów i wzajemnych relacji między niektórymi jonami w roślinach*. Wyd. UWM Olsztyn. Rozprawy i Monografie, 52: 7-92.

Zalewska M. 1995 *Wpływ nawożenia potasem i magnezem na skład chemiczny roślin*. Acta. Acad. Agrikult. Tech. Olst. Agricult., 61: 167-176.

Zalewska M. 2003. *The effect of various calcium, magnesium, potassium and hydrogen saturation of CEC on the yield and mineral composition of yellow lupine*. Pol. J. Natur. Sc., 15(3): 321-334.

Zalewska M. 2005a. *Wpływ zróżnicowanego wysycenia kompleksu sorpcyjnego gleby wapniem, magnezem, potasem i wodorem na plonowanie i skład mineralny marchwi*. J. Elementology, 10(3): 597-614.

Zalewska M. 2005b. *The effect of various calcium, magnesium, potassium and hydrogen saturation of CEC on the yield and mineral composition of oat*. J. Elementology, 10(4): 1137-1148.

POLISH JOURNAL OF NATURAL SCIENCES
Abbrev.: Pol. J. Natur. Sc., Vol 23(2): 366-373, Y. 2008

DOI 10.2478/v10020-008-0028-9

CHARACTERIZATION OF PROBIOTIC PROPERTIES OF *LACTOBACILLUS* STRAINS

***Monika Modzelewska-Kapituła*[1], *Lucyna Kłębukowska*[2], *Kazimierz Kornacki*[2], *Wioletta Łukaszuk*[2]**

[1] Chair of Meat Technology and Chemistry
[2] Chair of Industrial and Food Microbiology
University of Warmia and Mazury in Olsztyn

K e y w o r d s: probiotics, *Lactobacillus* sp., pH, bile salts, antibiotics.

A b s t r a c t

Twenty strains of *Lactobacillus* sp., previously isolated from infants' faeces (MODZELEWSKA et al. 2003) were evaluated for their probiotic properties such as resistance to rifampicin, neomycin, kanamycin, chloramphenicol, ampicillin, penicillin, nalidixic acid, gentamycin, colistin, oxaciclin and nitrofurantoin, survivability in the environment similar to the present in the gastrointestinal tract – pH 2 and 3 and bile salts concentration 1.2, 2.5 and 5%. All strains were resistant to colistin and nalidixic acid and susceptible to rifampicin, nitrofurantoin, chloramphenicol and ampicillin. Strains showed high survivability in pH 2 and 3. After 6 h incubation bacterial counts were at the level of 8.3-7.2 log cfu cm^{-3} compared to initial 8.7-7.2 log cfu cm^{-3}. In the environment containing 1.2-5% of bile salts bacterial counts after incubation were from below 1 to 6.6 log cfu cm^{-3}. Nine strains, which survived in the highest numbers in bile salts solutions, were considered as capable of surviving passage through the gastrointestinal tract, which is one of the basic requirements which probiotic strains must fulfil.

CHARAKTERYSTYKA WŁAŚCIWOŚCI PROBIOTYCZNYCH SZCZEPÓW *LACTOBACILLUS*

***Monika Modzelewska-Kapituła*[1], *Lucyna Kłębukowska*[2], *Kazimierz Kornacki*[2], *Wioletta Łukaszuk*[2]**

[1] Katedra Technologii i Chemii Mięsa
[2] Katedra Mikrobiologii Przemysłowej i Żywności
Uniwersytet Warmińsko-Mazurski w Olsztynie

S ł o w a k l u c z o w e: probiotyki, *Lactobacillus* sp., pH, sole żółci, antybiotyki.

Address: Monika Modzelewska-Kapituła, University of Warmia and Mazury in Olsztyn, Plac Cieszyński 1, 10-718 Olsztyn, Poland, phone: (089) 523 33 39,
e-mail: monika.modzelewska@uwm.edu.pl

A b s t r a k t

Scharakteryzowano 20 szczepów z rodzaju *Lactobacillus*, wyizolowanych z kału niemowląt (MODZELEWSKA i in. 2003). Zbadano oporność izolatów na rifampicynę, neomycynę, kanamycynę, chloramfenikol, ampicylinę, penicylinę, kwas nalidyksowy, gentamycynę, kolistynę, oxacyklinę i nitrofurantoinę oraz ich przeżywalność w środowisku zbliżonym do panującego w przewodzie pokarmowym – pH 2 i 3 oraz w obecności soli żółciowych w stężeniach 1.2, 2.5, 5%. Wszystkie szczepy były oporne na działanie kolistyny oraz kwasu nalidyksowego i wrażliwe na rifampicynę, nitrofurantoinę, chloramfenikol i ampicylinę. Ich przeżywalność w roztworach o pH 2 i 3 była wysoka. Liczba komórek bakterii po 6 godzinach inkubacji wynosiła zależnie od szczepu 8.3-6.3 log jtk cm^{-3} przy początkowej liczebności populacji na poziomie 8.7-7.2 log jtk cm^{-3}. W środowisku zawierającym 1.2-5.0% soli żółciowych liczebność szczepów po inkubacji wynosiła od poniżej 1 do 6.6 log jtk cm^{-3}. 9 szczepów, które wykazały najwyższą przeżywalność w obecności soli żółciowych, uznano za zdolne do przeżycia podczas pasażu przez przewód pokarmowy, co jest jednym z podstawowych wymogów stawianych szczepom probiotycznym.

Introduction

Probiotics are defined as live micro-organisms, which have a beneficial influence on the host health (MATTILA-SANDHOLM et al. 2002). Although probiotic cultures has been applied in food production since the beginning of XX century, still there is an unflagging interest in isolation and characterization of new probiotic strains. The strains are isolated from human organisms (NGUYEN et al. 2007), as well as from food products such as fermented meat (PENNACCHIA et al. 2006) and olives (RÖNKÄ et al. 2003), raw milk and dairy products (MARAGKOUDAKISA et al. 2006). Beneficial influence of probiotics on the human body includes: regulation of microbial balance in the gastrointestinal tract, lowering the level of faecal enzymes, reduction of cholesterol level in the blood, alleviation of lactose intolerance symptoms, enhancing the immune system and improvement of calcium absorption (ZIEMER, GIBSON 1998).

All probiotic strains, regardless their origin, must not be pathogenic and possess an ability to cause infections and disorders of the gastrointestinal tract (SAARELA et al. 2000, LIBUDZISZ 2003). They can not carry transmissible antibiotics resistance genes either (GOMES, MALCATA 1999). Another basic criteria which probiotics must fulfil is their ability to survive passage through the gastrointestinal tract, where they are subjected to low pH in the stomach and bile salts (PARK et al. 2002). An essential feature of probiotic bacteria is also their antagonistic activity against pathogens.

Lactobacillus sp. are bacteria regarded as safe (they possess the GRAS status) and widely used in production of probiotic food (HOLZAPFEL, SCHILLINGER 2002). In the earlier studies (MODZELEWSKA et al. 2003) 20 *Lactobacillus* strains were isolated from faeces of 24 infants, who had never been on antibiotic treatment. All strains, identified using API CHL 50 (bioMerieux),

showed strong antibacterial activity against potentially pathogenic and technological harmful Gram-positive bacteria such as: *Staphylococcus aureus*, *Enterococcus faecalis*, *Listeria monocytogenes*, *Bacillus cereus*, *B. subtilis* and Gram-negative: *Salmonella* sp., *Klebsiella* sp., *Escherichia coli*, *Proteus* sp., *Pseudomonas* sp., *Yersinia enterocolitica*, *Morganella morgani*. Experiments reviled that antimicrobial effect was caused by the acidification of the environment, not by specific antimicrobial agents such as bacteriocins produced by isolated strains. The aim of the present work was further characteristics of isolates, including chemotherapeutics resistance and survivability in low pH and elevated bile salts concentration.

Materials and Methods

The test bacteria were *Lactobacillus* rods belonging to *L. plantarum* – strains marked as 1, 4b, 14, 18a, 20a, 20b, 21, 22a, 22b, 23b, 24, *L. fermentum* – 4a, 7, 11b, 16, 18b, 22c, 23a, *L. casei* 6 and *L. paracasei* ssp. *paracasei* 11a. Bacteria were grown in MRS broth (Merck) at 37°C for 24 h.

For antibiotics and nitrofurantoin resistance tests a diffusion method with paper disks containing chemotherapeutic agents (bioMerieux) was used. After inoculation of Müller-Hinton (Oxoid) agar plates with bacterial suspensions containing 10^5 cfu cm^{-3} the disks were placed. Zones of growth inhibition were measured after 48 h incubation at 37°C in anaerobic conditions (Anaerocult C, Merck) and interpreted according to the disks manufacturer guidelines.

Survivability of the strains in the environment similar to the present in the stomach was proceeded in physiological salt solutions of pH 2 and 3. As a control sample solution of pH 5 was used. Bacterial cultures (1 cm^3) containing 10^9 cfu cm^{-3} were inoculated into solutions (9 cm^3) of different pH values and incubated at 37°C. Viable cells were estimated after inoculation and 2, 4 and 6 h of incubation on MRS agar (Merck) plates.

Tolerance of the strains to 1.2, 2.5, 5.0% of bile salts was proceeded in physiological salt solutions with bile salts addition (Bile Salts No 3, BTL). A 0.5 cm^3 aliquot of the bacterial cultures, containing 10^9 cfu cm^{-3} was inoculated into 5 cm^3 of different bile salts solutions and incubated at 37°C. Viable cells were estimated using plate method after inoculation and after 0.5, 1, 3 and 6 h of incubation.

Results and discussion

The study showed that all strains were resistant to colistin and nalidixic acid (Table 1). All isolates except *Lactobacillus fermentum* 22c were also resistant to neomycin and kanamycin. Resistance to gentamycin exhibited 85% of strains, whereas to penicillin only 15%. Isolated strains showed different resistance to oxaciclin: 9 were resistant, 3 marginally susceptible and 8 susceptible to the antibiotic. All tested strains were susceptible to rifampicin, nitrofurantoin, chloramphenicol and ampicillin. These finding are similar to results obtained by CHARTERIS et al. (1998), XANTHOPOULOS et al. (2000) and ARICI et al. (2004) who showed that *Lactobacillus* sp. isolated from the gastrointestinal tract of humans were resistant to penicillin, kanamycin and streptomycin and susceptible to chloramphenicol, penicillin G and tetracyclins.

Table 1

Susceptibility of isolated strains on chemotherapeutics

Chemotherapeutics	Strain number																			
	1	4a	4b	6	7	11a	11b	14	16	18a	18b	20a	20b	21	22a	22b	22c	23a	23b	24
RA	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
N	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	S	R	R	R
F/M	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
K	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	±	R	R	R
C	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
AM	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
P	S	S	S	S	S	S	S	S	S	S	S	S	R	S	R	R	S	S	S	S
NA	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
GM	R	S	R	R	R	R	R	R	S	R	R	R	R	R	R	R	S	R	R	R
CL	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
OX	±	S	R	R	R	R	S	R	S	R	R	±	S	S	R	±	S	S	±	R

RA – rifampicin 30 μg, N – neomycin 30 μg, F/M – nitrofurantoin 300 μg, K – kanamycin 30 μg, C – chloramphenicol 30 μg, AM – ampicillin 10 μg, P – penicillin G 10 IU, NA – nalidixic acid 30 μg, GM – gentamycin 10 μg, CL – colistin 50 μg, OX – oxaciclin 1 μg. R – resistant, ± marginally susceptible, S – susceptible)

Lactobacillus rods can effectively affect microbial balance of the gastrointestinal tract during and after antibiotic treatment only if they are resistant to the used antibiotic. On the other side, strains which possess antibiotic resistance genes might transfer them to the other bacteria, including pathogens, inhabiting the gastrointestinal tract. The situation takes place when resistance to antibiotic is coded by genes located in plasmids and that is the reason why in the food industry only strains without such plasmids can be used. However, it was shown that presence of plasmids containing antibiotic resistance genes is very rare in lactic acid bacteria and in most cases antibiotic

resistance can not be transmissible in conjugation process (TEMMERMAN et al. 2003). According to BORRIELIO et al. (2003) strains susceptible to at least 2 frequently used antibiotics should be chosen. This requirement was fulfilled by all tested in the present study strains, which were susceptible to at least 4 chemotherapeutics.

Isolated strains were resistant to low pH values (Table 2). Viable cells numbers of 18 strains after incubation in solution of pH 2 and 3 remained at similar level as in control solution of pH 5 (the optimal pH for lactic acid bacteria). The only strains susceptible to low pH, were *L. casei* 6 and *L. plantarum* 22b, which populations decreased about 2 log cycles after incubation in pH 2. Comparing results obtained in the present work to the ones reported by others (ERKKILÄ and PETÄJÄ 2000, XANTHOPOLOUS et al. 2000) it might be said that all isolated strains exhibit desirable tolerance to low pH. Even in the case of the described above the least tolerant strains: *L. casei* 6 and *L. plantarum* 22b, no strong decrease of population in pH 3 was noted. Moreover, consumption of probiotic cultures with milk or another food increases survivability of the bacteria (SANDERS, KLAENHAMMER 2001).

Table 2

Bacterial numbers (log cfu cm^{-3}) of isolated strains during incubation in acidic conditions

Strain	pH											
	5				3				2			
	Incubation time (hours)											
	0	2	4	6	0	2	4	6	0	2	4	6
1	8.5	8.5	8.4	8.3	8.5	8.4	8.3	8.2	8.0	8.2	8.0	8.0
4a	7.6	7.6	7.6	7.7	7.8	7.6	7.5	7.4	8.0	7.8	7.8	7.8
4b	8.0	7.8	7.7	7.6	7.8	7.7	7.5	7.3	7.6	7.6	7.6	7.6
6	8.0	7.7	7.3	7.5	7.7	7.6	7.4	7.2	8.3	8.1	8.3	6.5
7	8.5	8.4	8.1	8.0	8.3	8.3	8.1	8.3	8.1	8.3	8.2	8.3
11a	8.2	7.8	7.5	7.2	8.3	8.2	8.0	7.4	8.1	7.6	7.6	7.6
11b	8.5	8.3	8.0	8.1	8.4	8.4	8.3	8.0	8.3	8.2	8.2	8.3
14	7.7	7.8	7.7	7.7	7.7	7.5	7.4	7.3	7.7	7.6	7.5	7.0
16	7.7	7.6	7.2	7.1	7.6	7.6	7.6	7.0	7.6	7.4	7.2	7.0
18a	7.5	7.6	7.3	7.0	7.7	7.4	7.1	7.0	8.0	8.0	7.9	7.8
18b	7.5	7.5	7.4	7.3	7.3	7.0	7.3	7.1	7.2	7.1	7.0	7.2
20a	8.5	8.2	8.2	8.2	8.6	8.2	8.1	8.2	8.4	8.1	8.0	8.0
20b	8.6	8.6	8.4	8.3	7.9	7.9	7.4	7.0	8.3	8.3	8.2	8.0
21	8.4	8.3	8.2	8.0	8.1	7.8	7.8	7.7	8.7	8.5	8.6	8.0
22a	7.7	7.7	7.6	7.4	7.6	7.5	7.5	7.1	7.8	7.3	7.3	7.2
22b	8.3	8.0	7.4	7.4	8.5	8.3	7.2	7.0	8.0	7.6	7.0	6.3
22c	7.8	7.7	7.7	7.4	7.7	7.5	7.6	7.5	7.6	7.5	7.4	7.4
23a	7.7	7.7	7.5	7.5	7.7	7.2	7.2	7.1	8.0	7.8	7.7	7.6
23b	7.9	7.9	7.8	7.9	7.8	7.6	7.5	7.6	7.7	7.5	7.0	7.1
24	8.1	7.7	7.6	7.6	7.8	7.5	7.3	7.3	7.8	7.8	7.7	7.6

Tested strains were characterized by different resistant to bile salts (Table 3). The most resistant strain was *L. plantarum* 14, which number of viable cells after 6 h incubation was about 10^6 cfu cm^{-3}. Medium susceptibility to bile salts exhibited 8 strains (1, 4a, 4b, 18a, 20a, 20b, 22b, 24), which populations after incubation ranged from 10^2 to 10^4 cfu cm^{-3}. The most susceptible strains were: 6, 11a, 11b, 16, 18b, 22a, which counts after incubation in all used bile salts solution were equal or lower than 10 cfu cm^{-3}. There are some reports confirming differential survivability of lactic acid bacteria in bile salts solutions (PENNACCHIA et al. 2004). The bile salts tolerance tested *in vitro* is not equal to survivability of the bacteria in the human body (RÖNKA et al. 2003). Studies of PRASAD et al. (1998) showed better survivability of the isolates of human origin in low pH and bile salts presence compared to strains isolated from dairy products. Also, particular strains belonging to the same specie might exhibit different tolerance to bile salts (VINDEROLA, REINHEIMER 2003). The phenomenon was observed in the present study in spite of the common origin of tested strains.

Table 3

Bacterial numbers (log cfu cm^3) of isolated strains during incubation in bile salts solutions

Strain	Bile salts concentration (%)														
	1.2					2.5					5				
	Incubation time (hours)														
	0	0.5	1	3	6	0	0.5	1	3	6	0	0.5	1	3	6
1	3.8	3.6	3.6	3.4	3.4	3.6	3.6	3.5	3.3	3.3	3.9	3.5	3.5	3.3	3.2
4a	4.1	4.1	2.8	2.7	2.4	3.7	3.6	2.9	2.7	2.7	4.1	4.0	4.3	4.1	3.9
4b	3.6	3.2	2.7	2.5	2.4	3.9	2.9	2.8	2.8	2.6	3.6	3.5	3.5	3.4	3.4
6	< 1	< 1	< 1	< 1	< 1	< 1	< 1	< 1	< 1	< 1	< 1	< 1	< 1	< 1	< 1
7	3.6	1.0	1.3	1.0	1.0	3.4	1.7	1.8	1.6	1.3	3.9	3.1	3.0	3.0	3.0
11a	< 1	< 1	< 1	< 1	< 1	< 1	< 1	< 1	< 1	< 1	< 1	< 1	< 1	< 1	< 1
11b	< 1	< 1	< 1	< 1	< 1	< 1	< 1	< 1	< 1	< 1	< 1	< 1	< 1	< 1	< 1
14	6.8	6.5	6.3	6.2	6.1	6.9	6.4	6.4	6.3	6.0	< 1	6.9	6.9	6.8	6.6
16	5.4	1.0	1.0	1.0	1.0	5.7	1.0	1.3	< 1	< 1	5.3	1.3	1.0	1.0	1.0
18a	4.8	3.6	3.5	3.5	3.5	4.8	3.6	3.4	3.5	3.3	4.4	3.0	3.0	2.7	2.6
18b	< 1	< 1	< 1	< 1	< 1	< 1	< 1	< 1	< 1	< 1	< 1	< 1	< 1	< 1	< 1
20a	3.6	3.6	3.6	3.5	3.5	3.7	3.6	3.6	3.4	3.4	3.5	3.5	3.6	3.4	3.3
20b	4.3	3.8	3.8	3.9	3.7	4.5	3.3	3.2	3.1	3.1	3.9	3.6	3.5	3.0	3.2
21	3.3	2.8	2.7	2.2	2.0	3.3	2.8	2.8	2.8	2.6	3.9	3.8	3.6	3.5	3.3
22a	3.0	2.2	1.8	1.5	< 1	2.3	2.3	2.1	1.9	< 1	3.7	3.6	3.7	3.6	< 1
22b	4.6	4.4	4.3	3.8	3.5	3.8	3.7	3.5	3.4	3.2	4.0	4.0	4.0	4.0	3.9
22c	3.0	3.1	3.1	3.0	3.0	3.4	3.0	2.0	1.9	1.5	3.2	3.0	3.3	2.0	< 1
23a	1.0	1.0	< 1	< 1	< 1	3.3	3.3	3.3	3.3	3.5	1.3	1.0	1.0	0.7	< 1
23b	3.0	3.0	3.0	2.5	2.4	3.5	2.3	2.1	< 1	< 1	< 1	< 1	< 1	< 1	< 1
24	4.7	4.0	3.6	3.2	3.1	4.0	3.9	3.8	3.7	3.5	4.8	4.3	4.2	4.3	4.1

< 1 log cfu g^{-1} – lack of viable cells in 1 cm^3 of solution

Examination of isolates ability to survive in bile salts solutions enabled selection of strains for further studies, including their technological properties, possibility to use them in dairy products and feeding trials.

Conclusions

1. Isolated from infants' faeces *Lactobacillus* rods showed diverse susceptibility to chemotherapeutics. All strains were susceptible to rifampicin, nitrofurantoin, chloramphenicol and ampicillin and resistant to nalidixic acid and colistin. Obtained results suggest that chemotherapeutics resistance feature is specific for particular bacterial strains.

2. Tested strains showed high survivability in solutions of pH 2 and 3 which might suggest their ability to survive in strongly acidic environment of the stomach.

3. The strains differed in their survivability in the 1.2-5% of bile salts solutions. The most tolerant strains were: 14, 1, 4a, 4b, 18a, 20a, 20b, 22b, 24.

Translated by Authors

Accepted for print 17.10.2007

References

Arici M., Bilgin B., Sagdic O., Ozdemir C. 2004. *Some characteristics of Lactobacillus isolates from infant feces.* Food Microbiol., 21: 19-24.

Borriello S.P., Hammes W.P., Holzapfel W., Marteau P., Schrezenmeir J., Vaara M., Valtonen V. 2003. *Safety of probiotics that contain lactobacilli or bifidobacteria*. Clin. Infect. Dis., 36: 775-780.

Charteris W.P., Kelly P.M., Morelli L., Collins K. 1998. *Antibiotic susceptibility of potentially probiotic Lactobacillus species*. J. Food Protect., 61(12): 1636-1643.

Erkkilä S., Petäjä E. 2000. *Screening of commercial meat starter cultures at low pH and in the presence of bile salts for potential probiotic use*. Meat Sci., 55: 297-300.

Gomes A.M.P., Malcata F.X. 1999. *Bifidobacterium ssp. and Lactobacillus acidophilus: biological, biochemical, technological and therapeutical properties relevant for use as probiotics.* Trends Food Sci. Tech., 10: 139-157.

Holzapfel W.H, Schillinger U. 2002. *Introduction to pre- and probiotics*. Food Res. Int., 35: 109-116.

Libudzisz Z. 2003. *Ekosystem jelitowy a probiotyki. Bakterie fermentacji mlekowej. Metabolizm, genetyka, wykorzystanie*. Mat. Nauk. Symp., Spała, 76-84.

Maragkoudakisa P.A., Zoumpopouloua G., Miarisa C., Kalantzopoulosa G., Potb B., Tsakalidou E. 2006. *Probiotic potential of Lactobacillus strains isolated from dairy products.* Int. Dairy J. 16: 189-199.

Mattila-Sandholm T., Myllärinen P., Crittenden R., Mogensen G., Fonden R., Saarela M. 2002. *Technological challenges for future probiotic foods*. Int. Dairy J., 12: 173-182.

Modzelewska M., Kornacki K., Kłębukowska L. 2003. *Antimicrobial activity of lactic acid bacteria strains isolated from GI tract of infants against Gram positive and Gram negative bacteria.* Commun. Agr. Appl. Biol. Sci., 68(2): 437-440.

Nguyen T.D.T., Kang J.H., Lee M.S. 2007. *Characterization of Lactobacillus plantarum PH04, a potential probiotic bacterium with cholesterol-lowering effects.* Int. J. Food Microbiol., 113: 358-361.

PARK Y.S., LEE J.Y., KIM Y.S., SHIN D.H. 2002. *Isolation and characterization of lactic acid bacteria from feces of newborn baby and from dongchimi*. J. Agr. Food Chem., 50: 2531-2536.

PENNACCHIA C., ERCOLINI D., BLAIOTTA G., PEPE O., MAURIELLO G., VILLANI F. 2004. *Selection of Lactobacillus strains from fermented sausages for their potential use as probiotics*. Meat Sci., 67: 309-317.

PENNACCHIA C., VAUGHAN E.E., VILLANI F. 2006. *Potential probiotic Lactobacillus strains from fermented sausages: further investigations on their probiotic properties.* Meat Sci., 73: 90-101.

PRASAD J., GILL H., SMART J., GOPAL P. 1998. *Selection and characterization of lactobacillus and Bifidobacterium strains for use as probiotics.* Int. Dairy J., 8: 993-1002.

RÖNKA E., MALINEN E., SAARELA M., RINTA-KOSKI M., AARNIKUNNAS J., PALVA A. 2003. *Probiotic and milk technological properties of Lactobacillus brevis*. Int. J. Food Microbiol., 83: 63-74.

SAARELA M., MOGEMSEN G., FONDÉN R., MÄTTÖ J., MATTILA-SANDHOLM T. 2000. *Probiotic bacteria: safety, functional and technological properties.* J. Biotechnol., 84: 197-215.

SANDERS M.E., KLAENHAMMER T.R. 2001. *Invited review: The scientific basis of Lactobacillus acidophilus NCFM functionality as a probiotic*. J. Dairy Sci., 84: 319-331.

TEMMERMAN R., POT B., HUYS G., SWINGS J. 2003. *Identification and antibiotic susceptibility of bacterial isolates from probiotic products*. Int. J. Food Microbiol., 81: 1-10.

VINDEROLA C.G., REINHEIMER J.A. 2003. *Lactic acid starter and probiotic bacteria: a comparative "in vitro" study of probiotic characteristics and biological barrier resistance*. Food Res. Int., 36: 895-904.

XANTHOPOLOUS V., LITOPOULOU-TZANETAKI E., TZANETAKIS N. 2000. *Characterization of Lactobacillus isolates from infant feaces as dietary adjuncts*. Food Microbiol., 17: 205-215.

ZIEMER C.J., GIBSON G.R. 1998. *An overview of probiotics, prebiotics and synbiotics in the functional food concept: perspectives and future strategies*. Int. Dairy J., 8: 473-479.

POLISH JOURNAL OF NATURAL SCIENCES
Abbrev.: Pol. J. Natur. Sc., Vol 23(2): 374-386, Y. 2008

DOI 10.2478/v10020-008-0029-8

EFFECTS OF WATER STRESS ON LIGHT AND DARK RESPIRATIONS IN TWO MAIZE (*ZEA MAYS* L.) VARIETIES

Nayer Mohammadkhani, Reza Heidari

Department of Biology
Urmia University, Urmia, Iran

K e y w o r d s: light conditions, maize, oxygen uptake, polyethylene glycol 6000, respiration, water stress, *Zea mays*.

A b s t r a c t

Water stress as the one of the important limiting factors in plant growth was applied with polyethylene glycol (PEG) 6000 in solutions of 10, 20, 30 and 40% strengths that achieve water deficit levels of -0.15, -0.49, -1.03 and -1.76 MPa, respectively. After 24 hours treatment, the roots and leaves respirations of two maize (*Zea mays* L.) cultivars – 704 and 301 – were determined in various concentrations of PEG 6000 in light and dark conditions. Respiration rate declined in leaves and roots with increasing PEG concentrations. Decreases of oxygen uptake in roots and leaves of 704 variety were significantly ($p < 0.01$) higher than 301 variety and in light conditions were significantly ($p < 0.01$) higher than in dark. The rate of respiration in the light was lower than in darkness and the decrease of oxygen uptake in water stress in dark conditions was lower than in light.

WPŁYW STRESU WODNEGO NA RESPIRACJĘ DWÓCH ODMIAN KUKURYDZY (*ZEA MAYS* L.), PRZEBIEGAJĄCĄ W ŚWIETLE I W CIEMNOŚCI

Nayer Mohammadkhani, Reza Heidari
Katedra Biologii
Uniwersytet w Urmii, Iran

S ł o w a k l u c z o w e: warunki świetlne, kukurydza, pobór tlenu, glikol polietylenowy 6000, respiracja (oddychanie), stres wodny, *Zea mays*.

Address: Nayer Mohammadkhani,Urmia University, Urmia, Iran, phone: 0098 914 420 2600, e-mail: naier.mohammadkhani@yahoo.com

Abstrakt

Stres wodny jest jednym z najważniejszych czynników ograniczających wzrost roślin. W doświadczeniu zastosowano 10-, 20-, 30- i 40-procentowy roztwór glikolu polietylenowego (PEG) 6000, co pozwoliło osiągnąć warunki niedoboru wody na poziomie odpowiednio -0.15; -0.49; -1,03 i -1.76 MPa. Po upływie 24 godzin określono szybkość oddychania w korzeniach i liściach dwóch odmian kukurydzy (*Zea mays* L.) – 704 i 301 w różnych stężeniach PEG 6000, w świetle i w ciemności. Intensywność oddychania w liściach i korzeniach spadała wraz ze wzrostem stężenia PEG. Spadek poboru tlenu był istotnie ($p < 0,01$) wyższy w korzeniach i liściach odmiany 704 oraz w warunkach dostępu światła. Szybkość respiracji była mniejsza w świetle niż w ciemności, a spadek poboru tlenu w warunkach stresu wodnego niższy w ciemności niż przy dostępie światła.

Introduction

Water stress is one of the important limiting factors in plant growth which has limited the production in 25% of arable lands in the world (LEVITT 1980). The limitation of plant growth imposed by low water availability is mainly due to reductions of plant carbon balance, which is depending on the balance between photosynthesis and respiration (LAMBERS et al. 1998).

The regulation of respiration by drought at the plant physiological level is largely unknown, partly because only a limited number of studies are available and partly because of the apparent contradictions among these studies (FLEXAS et al. 2005). Certainly, the available experimental evidences do not support a clear pattern of respiration in response to drought, different studies showing either increased, unaffected or decreased rates of respiration. Although plant respiration is always retarded by the net loss of relatively large amounts of water, the effects of smaller changes in water content are variable. Respiration rates very widely depending on the species and physiological conditions (WERF VAN DER et al. 1990).

Water stress, particularly combined with light, increases the risk of oxidative stress by increasing the presence of ROS in different cell compartments (SGHERRI et al. 1993, SGHERRI, NAVARI-IZZO 1995, BARTOLI et al. 2004). Perhaps, the strongest direct evidence of light inhibition of respiration in leaves was obtained from in vivo experiments using $^{18}O_2$ (CANVIN et al., 1980). It was demonstrated that rates of $^{18}O_2$ uptake observed in darkness are completely suppressed in light.

The aim of the present study was to undertake a comparative analysis of the effects of water stress on dark and light respirations rates in roots and leaves of two maize cultivars.

Materials and Methods

Plant materials and growth conditions

This study was conducted at biochemistry laboratory, Department of Biology of Urmia University in Iran, during the spring of 2007. Two genotypes of maize (*Zea mays L.*) were used: var. 704 and var. 301 were obtained from the Agricultural Research Center of Iran, Ardebil, Iran. The seeds of both cultivars were germinated in Petri dishes on two layers of filter paper at 25°C in an incubator. After three days, the seedlings transferred to plastic pots (15 cm diameter, 20 cm depth) filled with sand and irrigated with half strength of Hoagland nutrient solution. Six-days seedlings were transferred to hydroponics culture of aerated test tubes containing polyethylene glycol (PEG) 6000 solutions of 10, 20, 30 and 40% strengths to achieve water deficit levels of -0.15, -0.49, -1.03 and -1.76 MPa, respectively (BURLYN et al. 1973, STEUTER et al. 1981, NICHOLAS 1989) as treatments and aerated test tubes containing half strength Hoagland nutrient solution which served as control. Samples were placed in greenhouse during the growth and stress. Light and water stress were applied together. Water stress was applied for 24 hours.

Respiratory measurements

For light respiration, samples were subjected in light during the experiment and the respiration rates were measured. Oxygen uptake of roots and leaves were measured at 25°C using an oxygen meter (WTW model oxi730). Roots and leaves segments (approximately 0.5 g fresh weight) were placed in 4 ml reaction medium (0.25M sucrose, 0.01M tris, 0.01M K_2HPO_4, 0.005M $MgCl_2$, 0.005M EDTA, 0.5mg cm^{-3} BSA) adjusted to pH = 7.2 with HCl and O_2 measured in period of 2 min (JOHN, JAMES 1970).

For dark respiration measurements, leaf and root samples were collected during the light period and stored 4 hours in dark and 20 min in 0.2 mM $CaCl_2$ for membrane stabilization (DELIEU, WALKER 1981, AZCON-BIETO et al. 1994). Samples were placed in the closed electrode cuvette, and depletion of the O_2 concentration in the rapidly stirred solution of the closed cuvette was linear with time. To avoid oxygen-limiting conditions inside the cuvette, all measurements were determined with O_2 concentration above 60% of saturation. Respiration measurements were performed with the oxygen meter (WTW model oxi730). It is well known that the precision of the oxygen electrode techniques for respiration measurements is much higher than techniques based on CO_2 gas-exchange measurements (HURRY et al. 2005).

Statistical analysis

Mean values were taken from measurements of four replications, and "Standard Deviation" of the means was calculated. Differences between means were determined by Univariate Analysis of Variance and Tukey's multiple range tests ($p < 0.01$). Analyses were done using the SPSS software (version 13.0).

Results

Leaf respiration averaged 20.27 μmol O_2 g^{-1} Fw min^{-1} in control plants of 704 var. in light and 24.11 μmol O_2 g^{-1} Fw min^{-1} in dark and in 301 var. 18.35 μmol O_2 g^{-1} Fw min^{-1} in light and 21.86 μmol O_2 g^{-1} Fw min^{-1} in dark conditions. Under severe water stress (water potential -1.76 MPa), leaf respiration of both varieties was lower than control plants. In PEG 40%, leaf respiration of 704 var. was 11.84 μmol O_2 g^{-1} Fw min^{-1} in light and 17.11 μmol O_2 g^{-1} Fw min^{-1} in dark. In 301 var., in severe water stress leaf respiration rate was 11.45 μmol O_2 g^{-1} Fw min^{-1} in light and 17.03 μmol O_2 g^{-1} Fw min^{-1} in darkness (Figure 1).

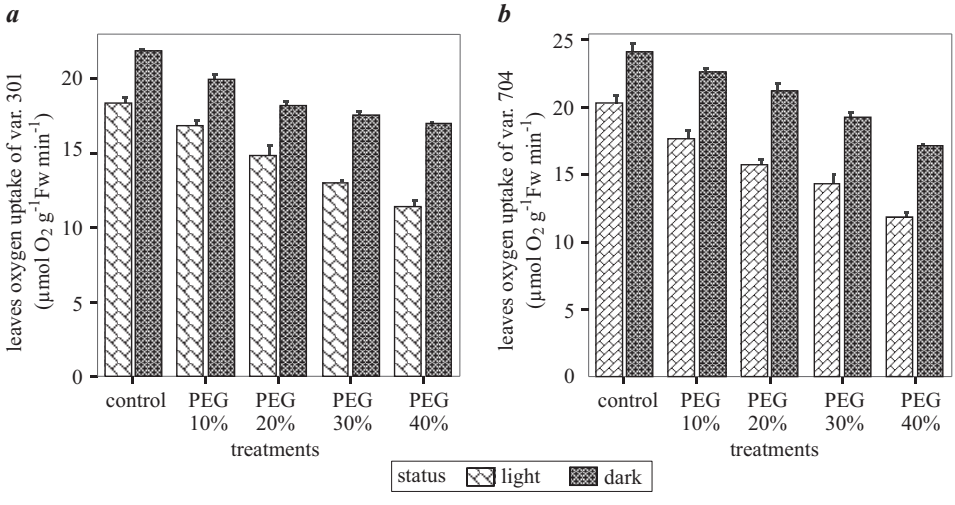


Fig. 1. Effects of different PEG 6000 concentrations on oxygen uptake in leaves of two maize cultivars in light and dark conditions: a – var. 301, b – var. 704. Results are shown as mean ± standard error ($p < 0.01$), obtained from four replications

In control plants of 704 var., roots respiration averaged 18.77 μmol O_2 g^{-1} Fw min^{-1} in light and 20.29 μmol O_2 g^{-1} Fw min^{-1} in dark and in 301 var. was 16.70 μmol O_2 g^{-1} Fw min^{-1} in light and 18.05 μmol O_2 g^{-1} Fw min^{-1} in dark conditions. Under severe water stress, in roots of 704 var., respiration averaged 9.67 μmol O_2 g^{-1} Fw min^{-1} in light and 13.85 μmol O_2 g^{-1} Fw min^{-1} in dark and in roots of 301 var., respiration averaged 10.47 μmol O_2 g^{-1} Fw min^{-1} in light and 14.05 μmol O_2 g^{-1} Fw min^{-1} in dark conditions (Figure 2).

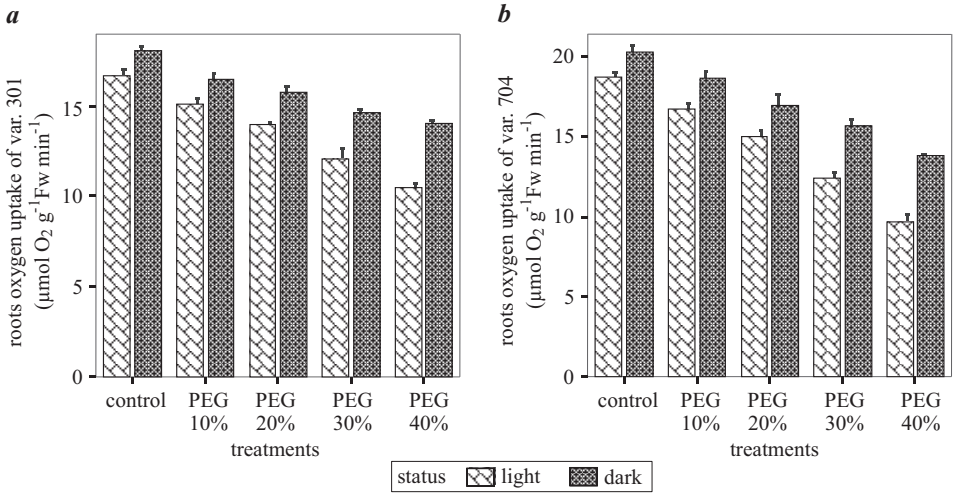


Fig. 2. Effects of different PEG 6000 concentrations on oxygen uptake in roots of two maize cultivars in light and dark conditions: a – var. 301, b – var. 704. Results are shown as mean ± standard error ($p < 0.01$), obtained from four replications

Therefore, comparing light to dark conditions, we observed 16% increase in leaf respiration and 8% increase in root respiration rates of control plants in var. 704 in dark conditions. We observed 31% increase in leaf respiration and 30% increase in root respiration rates in plants subjected to severe water stress in 704 var. in darkness, too.

In 301 var., we observed 16% increase in leaf respiration and 8% increase in root respiration rates in control plants in dark conditions. We observed 30% increase in leaf respiration and 25% increase in root respiration rates in plants treated with PEG 40% in 301 var. in darkness, too.

In severe water stress, respiration rates decreased in light and dark conditions in both roots and leaves. The decreases of respiration rates in roots and leaves of 704 var. were significantly ($p < 0.01$) higher than 301 var. in both light and dark conditions.

Decrease of leaf respiration rates was 42% in 704 var. and 38% in 301 var. as compares to the control plants in light conditions. This decrease was 29% in 704 var. and 22% in 301 var. as compared to the control plants in dark conditions. Decrease of root respiration rates was 48% in 704 var. and 37% in 301 var. as compared to the control plants in light conditions. This decrease was 32% in 704 var. and 22% in 301 var. as compared to the control plants in dark conditions.

The percentage of oxygen consumption decreased gradually with increasing PEG concentrations. The means differences in both varieties were significant at the 0.01 level between all treatments. The decrease of oxygen consumption in 704 var. was higher than 301 var. and in roots was higher than in leaves. In severe water stress, leaves of 704 plants have higher oxygen consumption percentage (4.62% in light conditions and 3.05% in darkness) than 301 plants and roots of 704 plants have higher oxygen consumption percentage (4.77% in light conditions and 3.38% in darkness) than 301 plants.

The decrease of oxygen consumption percentage in light conditions was higher than in darkness. In water potential -1.76 MPa, oxygen consumption percentage of leaves in dark conditions was 15.27% in 704 var. and 13.7% in 301 var. higher than light conditions (Table 1, Table 2, Table 3). In roots, oxygen consumption percentage was 9.77% in 704 var. and 8.38% in 301 var. higher than light conditions (Table 4, Table 5, Table 6).

Table 1

Effects of different PEG 6000 concentrations on oxygen consumption percentage in leaves of two maize cultivars in light and dark conditions*

Treatments	Light		Dark	
	704 var.	301 var.	704 var.	301 var.
	(% control)			
Control	100.0000 ± 0.00000	100.0000 ± 0.00000	100.0000 ± 0.00000	100.0000 ± 0.00000
PEG 10%	79.6000 ± 0.74722	84.4000 ± 0.35824	90.5250 ± 0.68723	92.9000 ± 0.64936
PEG 20%	71.4000 ± 0.65701	77.7000 ± 0.46726	86.0750 ± 0.75429	88.2000 ± 0.46547
PEG 30%	68.0250 ± 0.47675	71.3525 ± 0.50192	82.0250 ± 0.67004	86.1500 ± 0.45917
PEG 40%	62.2750 ± 0.75319	66.9000 ± 0.55076	77.5500 ± 0.47170	80.6000 ± 0.41433

* Results are shown as mean ± standard error ($p < 0.01$), obtained from four replications

Table 2

Multiple Comparisons. Dependent Variable: leaves oxygen consumption of var. 704 (% control)
Tukey HSD

(I) Treatments	(J) Treatments	Mean difference (I-J)	Std. error	Sig.	99% confidence interval	
					lower bound	upper bound
Control	PEG 10%	14.9375*	0.59138	0.000	12.8268	17.0482
	PEG 20%	21.2625*	0.59138	0.000	19.1518	23.3732
	PEG 30%	24.9750*	0.59138	0.000	22.8643	27.0857
	PEG 40%	30.0875*	0.59138	0.000	27.9768	32.1982
PEG 10%	Control	-14.9375*	0.59138	0.000	-17.0482	-12.8268
	PEG 20%	6.3250*	0.59138	0.000	4.2143	8.4357
	PEG 30%	10.0375*	0.59138	0.000	7.9268	12.1482
	PEG 40%	15.1500*	0.59138	0.000	13.0393	17.2607
PEG 20%	Control	-21.2625*	0.59138	0.000	-23.3732	-19.1518
	PEG 10%	-6.3250*	0.59138	0.000	-8.4357	-4.2143
	PEG 30%	3.7125	0.59138	0.000	1.6018	5.8232
	PEG 40%	8.8250*	0.59138	0.000	6.7143	10.9357
PEG 30%	Control	24.9750*	0.59138	0.000	-27.0857	-22.8643
	PEG 10%	-10.0375*	0.59138	0.000	-12.1482	-7.9268
	PEG 20%	-3.7125*	0.59138	0.000	-5.8232	-1.6018
	PEG 40%	5.1125*	0.59138	0.000	3.0018	7.2232
PEG 40%	Control	-30.0875*	0.59138	0.000	-32.1982	-27.9768
	PEG 10%	-15.1500*	0.59138	0.000	-17.2607	-13.0393
	PEG 20%	-8.8250*	0.59138	0.000	-10.9357	-6.7143
	PEG 30%	-5.1125*	0.59138	0.000	-7.2232	-3.0018

Based on observed means

* The mean differences is significant at the 0.01 level

Table 3

Multiple Comparisons. Dependent Variable: leaves oxygen consumption of var. 301 (% control)
Tukey HSD

(I) Treatments	(J) Treatments	Mean difference (I-J)	Std. error	Sig.	99% confidence interval	
					lower bound	upper bound
Control	PEG 10%	11.3500*	0.43849	0.000	9.7849	12.9151
	PEG 20%	17.0500*	0.43849	0.000	15.4849	18.6151
	PEG 30%	21.2488*	0.43849	0.000	19.6837	22.8138
	PEG 40%	26.2500*	0.43849	0.000	24.6849	27.8151
PEG 10%	Control	-11.3500*	0.43849	0.000	-12.9151	-9.7849
	PEG 20%	5.7000*	0.43849	0.000	4.1349	7.2651
	PEG 30%	9.8988*	0.43849	0.000	8.3337	11.4638
	PEG 40%	14.9000*	0.43849	0.000	13.3349	16.4651
PEG 20%	Control	-17.0500*	0.43849	0.000	-18.6151	-15.4849
	PEG 10%	-5.7000*	0.43849	0.000	-7.2651	-4.1349
	PEG 30%	4.1988*	0.43849	0.000	2.6337	5.7638
	PEG 40%	9.2000*	0.43849	0.000	7.6349	10.7651
PEG 30%	Control	-21.2488*	0.43849	0.000	-22.8138	-19.6837
	PEG 10%	-9.8988*	0.43849	0.000	-11.4638	-8.3337
	PEG 20%	-4.1988*	0.43849	0.000	-5.7638	-2.6337
	PEG 40%	5.0013*	0.43849	0.000	3.4362	6.5663
PEG 40%	Control	-26.2500*	0.43849	0.000	-27.8151	-24.6849
	PEG 10%	-14.9000*	0.43849	0.000	-16.4651	-13.3349
	PEG 20%	-9.2000*	0.43849	0.000	-10.7651	-7.6349
	PEG 30%	-5.0013*	0.43849	0.000	-6.5663	-3.4362

Based on observed means
* The mean differences is significant at the 0.01 level

Table 4

Effects of different PEG 6000 concentrations on oxygen consumption percentage in roots of two maize cultivars in light and dark conditions*

Treatments	Light		Dark	
	704 var.	301 var.	704 var.	301 var.
	(% control)			
Control	100.0000 ± 0.00000	100.0000 ± 0.00000	100.0000 ± 0.00000	100.0000 ± 0.00000
PEG 10%	83.4500 ± 0.61981	85.6250 ± 0.30923	85.8000 ± 0.96350	88.3500 ± 0.57373
PEG 20%	75.6750 ± 0.55434	80.2250 ± 0.40697	79.3250 ± 0.57209	83.2750 ± 0.39025
PEG 30%	65.9750 ± 0.68845	72.4750 ± 0.38595	73.0750 ± 0.58077	76.9500 ± 0.50744
PEG 40%	57.4750 ± 0.36827	62.2500 ± 0.47871	67.2500 ± 0.36629	70.6250 ± 0.31721

* Results are shown as mean ± standard error ($p < 0.01$), obtained from four replications

Table 5

Multiple Comparisons. Dependent Variable: roots oxygen consumption of var. 704 (% control)
Tukey HSD

(I) Treatments	(J) Treatments	Mean difference (I-J)	Std. error	Sig.	99% confidence interval	
					lower bound	upper bound
Control	PEG 10%	15.3750*	0.55028	0.000	13.4109	17.3391
	PEG 20%	22.5000*	0.55028	0.000	20.5359	24.4641
	PEG 30%	30.4750*	0.55028	0.000	28.5109	32.4391
	PEG 40%	37.6375*	0.55028	0.000	35.6734	39.6016
PEG 10%	Control	-15.3750*	0.55028	0.000	-17.3391	-13.4109
	PEG 20%	7.1250*	0.55028	0.000	5.1609	9.0891
	PEG 30%	15.1000*	0.55028	0.000	13.1359	17.0641
	PEG 40%	22.2625*	0.55028	0.000	20.2984	24.2266
PEG 20%	Control	-22.5000*	0.55028	0.000	-24.4641	-20.5359
	PEG 10%	-7.1250*	0.55028	0.000	-9.0891	-5.1609
	PEG 30%	7.9750*	0.55028	0.000	6.0109	9.9391
	PEG 40%	15.1375*	0.55028	0.000	13.1734	17.1016
PEG 30%	Control	-30.4750*	0.55028	0.000	-32.4391	-28.5109
	PEG 10%	-15.1000*	0.55028	0.000	-17.0641	-13.1359
	PEG 20%	-7.9750*	0.55028	0.000	-9.9391	-6.0109
	PEG 40%	7.1625*	0.55028	0.000	5.1984	9.1266
PEG 40%	Control	-37.6375*	0.55028	0.000	-39.6016	-35.6734
	PEG 10%	-22.2625*	0.55028	0.000	-24.2266	-20.2984
	PEG 20%	-15.1375*	0.55028	0.000	-17.1016	-13.1734
	PEG 30%	-7.1625*	0.55028	0.000	-9.1266	-5.1984

Based on observed means
* The mean differences is significant at the 0.01 level

Table 6

Multiple Comparisons. Dependent Variable: roots oxygen consumption of var. 301 (% control)
Tukey HSD

(I) Treatments	(J) Treatments	Mean difference (I-J)	Std. error	Sig.	99% confidence interval	
					lower bound	upper bound
Control	PEG 10%	13.0125*	0.38457	0.000	11.6399	14.3851
	PEG 20%	18.2500*	0.38457	0.000	16.8774	19.6226
	PEG 30%	25.2875*	0.38457	0.000	23.9149	26.6601
	PEG 40%	32.0625*	0.38457	0.000	30.6899	33.4351
PEG 10%	Control	-13.0125*	0.38457	0.000	-14.3851	-11.6399
	PEG 20%	5.2375*	0.38457	0.000	3.8649	6.6101
	PEG 30%	12.2750*	0.38457	0.000	10.9024	13.6476
	PEG 40%	19.0500*	0.38457	0.000	17.6774	20.4226
PEG 20%	Control	-18.2500*	0.38457	0.000	-19.6226	-16.8774
	PEG 10%	-5.2375*	0.38457	0.000	-6.6101	-3.8649
	PEG 30%	7.0375*	0.38457	0.000	5.6649	8.4101
	PEG 40%	13.8125*	0.38457	0.000	12.4399	15.1851
PEG 30%	Control	-25.2875*	0.38457	0.000	-26.6601	-23.9149
	PEG 10%	-12.2750*	0.38457	0.000	-13.6476	-10.9024
	PEG 20%	-7.0375*	0.38457	0.000	-8.4101	-5.6649
	PEG 40%	6.7750*	0.38457	0.000	5.4024	8.1476
PEG 40%	Control	-32.0625*	0.38457	0.000	-33.4351	-30.6899
	PEG 10%	-19.0500*	0.38457	0.000	-20.4226	-17.6774
	PEG 20%	-13.8125*	0.38457	0.000	-15.1851	-12.4399
	PEG 30%	-6.7750*	0.38457	0.000	-8.1476	-54024

Based on observed means
* The mean differences is significant at the 0.01 level

Discussion

While several studies described water stress induced decreased respiration rate (GONZALEZ-MELER et al. 1997, GHASHGHAIRE et al. 2001, HAUPT-HERTING et al. 2001), others have shown unaffected rates (LAWLOR 1976, LOBODA 1993) and even stimulation (UPCHURCH et al. 1995, SHEARMANN et al. 1972, ZAGDANSKA 1995). FLEXAS et al. (2005) attributed this controversy to three possible causes: (I) the use of different species, organs and techniques for respiration studies' (II) the presence of complex interactions of respiration rates with other environmental factors and (III) the presence of a threshold of water stress intensity in which a change in the response of respiration to water stress occurs. The first two possible causes were constrained by growing all plants under identical conditions, as studying the effects of water stress on a single plant tissue (i.e. leaves). A large part of the observed differences can be attributed to the effect of growth form.

Our original objective from this study was finding the changes in respiration rates of leaves and roots in light and dark conditions with increasing PEG treatments. We found that with increase of drought stress, respiration rates decreased in both roots and leaves. These results supported previous findings (GONZALEZ-MELER et al. 1997, GHASHGHAIRE et al. 2001, HAUPT--HERTING et al. 2001).

We found that decrease of respiration rates in light conditions were higher than dark conditions in water stress. Although respiration but not photosynthesis continues in the dark, both respiration and photosynthesis are affected by light intensity. The extent of respiration that continues in the light appears to be highly variable. Most studies have reported that the rate of leaf respiration in the light is less than in darkness (KOWALLIK 1982, AZCON-BIETO, OSMOND 1983, BROOKS, FARQUHAR 1985, KROMER 1995, RIBAS-CARBO et al. 2000, PINELLI, LORETO 2003), with the degree of inhibition ranging from 16% to 77% (ATKIN et al. 1997). Obviously, the extent of such inhibition would greatly affect the total daily carbon balance of a given leaf or plant.

Increase of oxygen uptake in dark conditions in leaves was higher than roots in both varieties. In roots of control plants in both varieties, increases of respiration rates in dark conditions were relatively low (only 8%), but in PEG 40% increase of oxygen uptake were significantly ($p < 0.01$) higher than control plants. Therefore, respiration decreased with increasing PEG treatments. But, the decrease in dark conditions was lower than light conditions in leaves and roots of both varieties.

With increase of water stress, respiration rate and oxygen consumption percentage decreased in both roots and leaves. These decreases in light were higher than dark and in 704 plants were higher than 301 plants. Therefore, water stress had a higher effect in 704 plant's respiration than 301 plants and in roots respiration than leaves. The means differences of oxygen uptake and oxygen consumption percentage in both varieties were significant at the 0.01 level between all treatments. These results may suggest that plants of the 301 variety have a better tolerance to water stress as compared to 704 variety.

Acknowledgements

This work supported by Department of Biology of Urmia University, Iran. We greatly acknowledge Dr M. Ilkhanipour for her support in providing facilities.

Accepted for print 30.01.2008

References

ATKIN O.W., WESTBEEK M.H.M., CAMBRIDGE M.L., LAMBERS H., PONS T.L. 1997. *Leaf respiration in light and darkness.* Plant Physiology, 113: 961-965.

AZCON-BIETO J., OSMOND C.B. 1983. *Relationship between photosynthesis and respiration. The effect of carbohydrate status on respiratory rates and the involvement of the alternative pathway in the leaf respiration*. Plant Physiology, 72: 598-603.

AZCON-BIETO J., GONZALEZ-MEHLER M.A., DOHERTY W., DRAKE B.G. 1994. *Accumulation of respiratory O_2* uptake in green tissues of field-grown native species after long-term exposure to elevated atmospheric CO_2. Plant Physiology, 106: 1163-1168.

BARTOLI C.G., GOMEZ F., MARTINEZ D.E., GUIAMET J.J., 2004. *Mitochondria are the main target for oxidative damage in leaves of wheat* (*Triticum aestivum* L.). J. Exp. Bot, 55: 1663-1669.

BOYER J.S. 1982. *Plant productivity and environment*. Science, 218: 443-448.

BROOKS A., FARQUHAR G.D. 1985. *Effect of temprature on the CO_2*/O_2 specificity of ribulose-1, 5-bisphosphate carboxylase/oxygenase and the rate of respiration in the light – estimate from gas-exchange measurements on spinach. Planta, 165: 397-406.

BURLYN E., MIRRILL R. 1973. *The osmotic potential of polyethylene glycol 6000.* Plant Physiol, 51: 914-916.

CANVIN D.T., BERRY J.A., BADGER M.R., FECK H., OSMOND C.B. 1980. *Oxygen exchange in leaves in the light.* Plant Physiology, 66: 302-307.

DELIEU T., WALKER D.A. 1981. *Polarografic measurement of photosynthetic oxygen evolution by leaf discs.* New Phytology, 89: 165-178.

FLEXAS J., GALMES J., RIBAS-CARBO M., MEDRANO H. 2005. *The effects of drought in plant respiration, Advances in photosynthesis and respiration.* [In:] *Advances in photosynthesis and respiration. Plant respiration: from cell to ecosystem. Eds.* Lambers H., Ribas-Carbo M. Springer, Dordrecht, 18(18): 117-194.

GHASHGHAIRE J., DURANCEAU M., BADECK F.W., CORNIC G., ADELINE M.T., DELEENS E. 2001. *$\delta 13$ of CO_2* respired in the dark in relation to of leaf metabolites: comparison between Nicotiana sylvestris and *Helianthus annuus* under drought. Plant Cell and Environment, 24: 505-512.

GONZALEZ-MELER M.A., MATAMALA R., PENUELAS J., 1997, *Effects of prolonged drought stress and nitrogen deficiency on respiratory O_2* uptake of bean and pepper leaves. Photosynthetica, 34: 505-512.

HAUPT-HERTING S., KLUG K., FOCK H.P. 2001. *A new approach to measure gross CO_2* fluxes in leaves. Gross CO_2 assimilation, photo respiration and mitochondrial respiration in the light in tomato under drought stress. Plant Physiology, 126: 388-396.

HURRY V., IGAMBERDIEV A.U., KEERBERG O., PARNIK T., ATKIN O.K., ZARAGOZA-CASTELLS J., GARDESTROM P. 2005. *Respiration in photosynthetic cells: gas exchange components, interactions with photorespiration and the operation of mitochondria in the light.* [In:] Plant respiration: from cell to ecosystem. Eds. Lambers H., Ribas-Carbo M., Springer, Dordrecht, pp. 43-61.

JOHN K., JAMES M. 1970. *The influence of mitochondrial concentration and storage on the respiratory control of isolated plant mitochondria.* Plant Physiol, 45: 382-385.

KOWALLIK W. 1982. *Blue light effects on respiration.* Annual Review of Plant Physiology, 33: 51-72.

KROMER S. 1995. *Respiration during photosynthesis.* Annual Review of Plant Physiology, 46: 45-70.

LAMBERS H., CHAPIN III F.C., PONS T.L. 1998. *Respone of photosynthesis to light*. [In:] *Plant physiological ecology.* Eds. H. Lambers, F.S. Chapin III, T.L. Pons, Springer Verlag, New York, pp. 25-46.

LAWLOR D.W. 1976. *Water stress induced changes in photosynthesis, photo respiration, respiration and CO_2* compensation concentration in wheat. Photosynthetica, 10: 378-387.

LEVITT J. 1980. *Response of plants environmental stress.* Academic Press, New York.

LOBODA T. 1993. *Gas exchange of different spring cereal genotypes under normal and drought conditions.* Photosynthetica, 29: 567-572.

NICHOLAS P. 1989. *Osmotic pressure of aqueous polyethylene glycols.* Plant Physiol., 91: 766-769.

PINELLI P., LORETO F. 2003. *(CO_2)-C^{12} emission from different metabolic pathways measured in illuminated and darkened C_3 and C_4 leaves at low, atmospheric and elevated CO_2 concentration.* Journal of Experimental Botany, 54: 1761-1769.

RIBAS-CARBO M., ROBINSON S.A., GONZALEZ-MELER M.A., LENNON A.M., GILES L., SIEDOW J.N., BERRY J.A. 2000. *Effects of light on respiration and oxygen isotope fractionation in soybean cotyledons.* Plant Cell and Environment, 23: 983-989.

SGHERRI C.L.M., PINZINO C., NAVARI-IZZO F. 1993, *Chemical changes and O_2 production in thylakoid membranes under water stress.* Physiol. Plant, 87: 211-216.

SGHERRI C.L.M., NAVARI-IZZO F. 1995. *Sunflower seedlings subjected to increasing water deficit stress: oxidative stress and defence mechanisms.* Physiol. Plant, 93: 25-30.

SHEARMANN L.L., ESATIN J.D., SULLIVAN C.Y., KINBACHER E.J. 1972. *Carbon dioxide exchange in water-stressed maize sorghum*. Crop. Sci, 12: 406-409.

STEUTER A., MOZAFAR A., GOODIN J. 1981. *Water potential of aqueous polyethylene glycol*. Plant Physiol., 67: 64-67.

UPCHURCH R.P., PETERSON M.L., HAGAN R.M. 1955. *Effect of soil-moisture content on the rate of photosynthesis and respiration in ladino clover* (*Trifolium repens* L.). Plant Physiol., 30: 297-303.

WERFF A. VAN DER, HIROSE T., LAMBERS H. 1990. *Variation in root respiration; causes and consequences for growth.* [In:] *Causes and consequences of variation in growth rate and productivity of higher plants.* The Hague SPB Academic Publishing, pp. 227-240.

ZAGDANSKA B. 1995. *Respiratory energy demand for protein turnover and ion transport in wheat leaves upon water demand*. Physiol. Plant, 95: 428-436.

POLISH JOURNAL OF NATURAL SCIENCES
Abbrev.: Pol. J. Natur. Sc., Vol 23(2): 387-397, Y. 2008

DOI 10.2478/v10020-008-0030-2

RESPIRATORY ACTIVITY OF NEUSTONIC AND PLANKTONIC BACTERIA ISOLATED FROM COASTAL LAKE GARDNO

Piotr Skórczewski, Zbigniew Mudryk

Department of Experimental Biology
Pomeranian Pedagogical University in Słupsk

K e y w o r d s: coastal lake, bacterioneuston, bacterioplankton, respiratory activity.

A b s t r a c t

Respiratory activity of heterotrophic bacterioneuston and bacterioplankton isolated from coastal Lake Gardno was determined. Neustonic and planktonic bacteria oxidized tested organic compounds with various intensity. Casein hydrolyzate was the most actively metabolized respiratory substrate while cellobiose was oxidised least actively. In the presence of all tested substrates, respiratory processes bacterial strains isolated from surface water were more intensive than bacterial strains inhabited subsurface water layer. The level of metabolic activity of bacteria from different parts of the studied lake was shown to differ.

AKTYWNOŚĆ ODDECHOWA NEUSTONOWYCH I PLANKTONOWYCH BAKTERII WYIZOLOWANYCH Z PRZYMORSKIEGO JEZIORA GARDNO

Piotr Skórczewski, Zbigniew Mudryk

Zakład Biologii Eksperymentalnej
Pomorska Akademia Pedagogiczna w Słupsku

S ł o w a k l u c z o w e: jezioro przymorskie, bakterioneuston, bakterioplankton, aktywność oddechowa.

A b s t r a k t

W pracy przedstawiono wyniki badań dotyczące aktywności oddechowej bakterii neustonowych i planktonowych zasiedlających przymorskie estuariowe jezioro Gardno. Wykazano, że z różną aktywnością utleniały one testowane substraty oddechowe. Zarówno bakterioneuston, jak i bak-

Address: Piotr Skórczewski, Pomeranian Pedagogical University, ul. Arciszewskiego 22 B, 76-200 Słupsk, Poland, e-mail: psps@poczta.fm

terioplankton preferował hydrolizat kazeiny, który jest mieszaniną różnych aminokwasów, natomiast najmniej aktywnie utleniana była przez nie cellobioza. Bakterie wyizolowane z powierzchniowych warstw wody charakteryzowały się większą aktywnością oddechową od wyizolowanych z podpowierzchniowych warstw wody. Poziom bakteryjnej aktywności oddechowej w różnych częściach jeziora Gardno był zróżnicowany.

Introduction

Heterotrophic bacteria are important components of water biocenoses as they actively participate in energy flux and metabolic conversions of various organic substrates (BERARD et al. 1995, ANESIO et al. 2003). They can use in respire a wide spectrum of organic compounds as a source of energy, including monomeres and polymeres after their enzymatic hydrolysis (BILLEN, FONTINGY 1987, HOPPE et al. 1998). The rate of respiration substrates by aquatic bacteria depends primarily on their taxonomic structure, physiological diversity and enzymatic activity (MUDRYK 1997, CUNHA et al. 1999, NAGATA et al. 2002). Respiration in bacterial cells is controlled by a multifunctional complex of enzymes determining the flux of electrons and the rate of oxygen diffusion through cell membranes (MARTINEZ, ESTRADA 1992).

The respiratory activity of bacterial microflora is a very good measure of the intensity of energy flow and processes of mineralization of organic matter in water bodies (BIDDANDA et al. 1994, JAHNKE, CRAVEN 1995). However, determination of bacterial respiratory activity in aquatic ecosystems remains difficult, due to unresolved methodical problems. Various methods are currently in use (GRIFFITH, POMEROY 1995, CUNHA et al. 1999, STROTMANN et al. 1999, NAGATA et al. 2002), but they are all subject to measuring errors that do not allow precise and exact determination of the intensity of bacterial respiration. Recently (BEDNARZ, STARZECKA 1998, KONOPKA, ZAKHAROVA 1999), a respirometric method based on a Clark's electrode coupled with a computer system is being increasingly used. This method allows to measure respiratory activity of bacteria in a quick, simple and relatively precise way and therefore it was used in the present study.

The aim of the present study was to gain insight into metabolic behaviour of bacterial microflora in the presence of the substrates most often used in studies of bacterial respiratory and also occurring in natural environments. Respiratory activity of neustonic and planktonic bacteria was measured in order to determine the potential capability of the microflora of estuarine Lake Gardno to decompose organic compounds.

Materials and Methods

Study area. The study was carried out in the coastal estuarine Lake Gardno which is a part of the World Biosphere Reserve – Słowiński National Park (Poland). The lake is very shallow (1.3 m average depth) but covers a large area (2.500 ha). It is supplied by the fresh waters of the River Łupawa while being connected to the Baltic Sea by a 1.3 km channel (Figure 1). Because large quantities of sea water can penetrate into the lake, its waters – or a part of them – acquire seawater properties, with the salinity of 2-5‰. Consistently with the Venetian system, Lake Gardno can be classified as the mixo-oligohaline type (0.5-5.0‰) (DETHIER 1992).

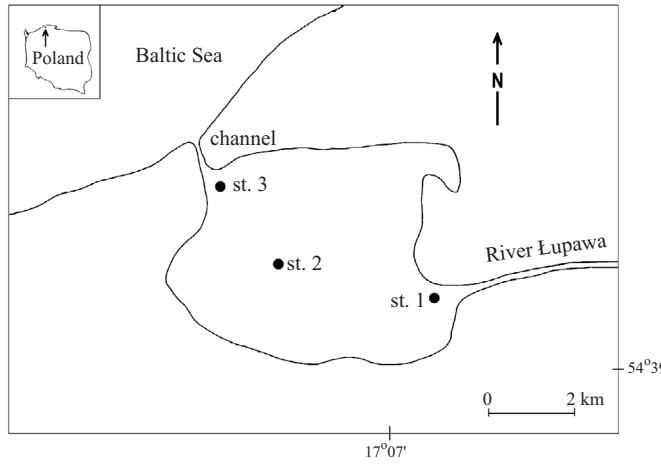


Fig. 1. Lake Gardno, northern Poland, with location of sampling stations

The studied estuary is a polymictic water basin with no thermal or oxygen stratification and with a considerable level of eutrophication. A high trophic level together with high concentration of nutrients (TROJANOWSKI et al. 1991) and penetration of light to the bottom of the lake create perfect conditions for the development of phytoplankton which usually blooms from spring to autumn. The phototrophic community is dominated by the aggregate-forming cyanobacteria species: *Anabena flos-aquae, Aphanizomennon flos-aquae* and *Microcystis aeruginosa* (STRZELECKI, PÓŁTORAK 1971). This shallow and productive lake is characterized by an extensive growth of macrophytes. The emergent macroflora covers 4% of the lake surface and forms a 20-100 m wide offshore belt, a home for many bird species. The main macrophytes are: *Typha angustifolia, Phragmites australis, Scirpus lacustris,* and *Schoenoplectus lacustris.*

Water sampling. Water samples were taken quaterly (in spring, summer, and autumn) at three sites: site 1, near the River Łupawa inflow (freshwater zone); site 2, in the mid-lake (mixed water zone); site 3, close to the outflow of the sea-water (seawater zone) (Figure 1). At each site, three layers of water were sampled. Samples from the film layer (FL, thickness of 90 ± 17 μm) were taken with a 30 x 30 glass plate (HARVEY, BURZELL 1972), while samples from the surface layer (SL, thickness of 240 ± 40 μm) were collected with a 40 x 50 cm Garrett net (24 mesh net of 2.54 cm in square) (GARRETT 1965). Prior to sampling, the glass plate and polyethylene net were rinsed with ethyl alcohol and distilled sterile water. Samples from the subsurface layer (SUB) were taken at the depth of about 10-15 cm. All water samples were placed in sterile glass bottles and stored in an ice-box at a temperature lower than 7°C. The time between sample collection and their analysis usually did not exceed 6-8 h.

Bacteria isolated and measured of respiratory activity. Plate techniques were used in order to isolate neustonic (FL and SL) and planktonic bacteria (SUB). Water samples were vortex mixed, and then serial tenfold dilutions were prepared with sterile buffered water to reach final concentrations ranging from 10^{-1} to 10^{-4}. Diluted samples were inoculated by the spread method in three parallel replicates on iron-peptone agar (IPA) medium (FERRER et al. 1963). Incubation was carried out at 20°C for 10 days. Afterwards, *ca.* 35 bacterial colonies per each water layer were picked out from the whole surface of the plates or from selected sectors and transferred to a semiliquid (5.0 g agar per dm^{-3}) IPA medium. Cultures maintained on this medium after purity control were kept at 4°C and used for the analysis of their respiratory activity.

In order to determine the rate of bacterial respiratory activity, oxygen uptake was measured with Clark's electrode (Rank Brothers Ltd. Model 10) (KONOPKA, ZAKHAROVA 1999). Respiratory activity of 33 bacterial strains from each of the studied water layers was determined. Pure cultures of bacteria were multiplied on IPA agar slants for 48-72 h at 20°C. Subsequently, they were washed off from the slants with phosphate buffer (0.01 M, pH 7.0), centrifuged at 15.000 rpm for 15 min and washed twice with the buffer. The washed bacteria were resuspended in the same buffer and adjusted to the turbidity of 4 in the MacFarland standard. 1 cm^3 of such suspension contained 10^9 bacteria. Casein hydrolyzate (Casamino acids vitamin-free, Difco), glucose, sodium acetate, alanine and cellobiose were used as respiratory substrates. All substrates were in concentration of 10 μM cm^{-3} (STRZELCZYK et al. 1988). Before measurements, the respiratory chamber of Clark's electrode was calibrated with sodium dithionate at the polarizing voltage of 6.0 V. After calibration, 1.5 cm^3 of bacterial suspension and 30 μl of respiratory substrate were put into the respiratory chamber. Changes in voltage on the electrode

were recorded by an analogue recorder XY Line Record TZ 5000 and stored in a computer program BS81x – BS51x Data Recording System Ver. 3.3.05. The number of measurements was set at 30, taken every 6 seconds. During the measurements, the Clark's electrode was connected to a flow stabilizer of temperature ensuring thermal stability in the respiratory chamber. Data corrected for endogenous respiration and oxygen uptake were converted into μl O_2 h^{-1} per 10^9 cells.

Statistical tests (SD,CV, CD) used in this analysis were from VELJI, ALBRIGHT (1986).

Results

The results of the study of respiratory activity of bacteria isolated from Lake Gardno are presented in Table 1. Casein hydrolyzate was the most actively oxidized respiratory substrate (45.0 μl O_2 h^{-1} 10^9 cells), while glucose (28.4 μl O_2 h^{-1} 10^9 cells) and sodium acetate (25.7 μl O_2 h^{-1} 10^9 cells) were utilized less actively. Cellobiose was the least (14.6 μl O_2 h^{-1} 10^9 cells) preferred respiratory substrate.

Table 1

Respiration of estuarine bacteria in the presence of different respiratory substrates (average for all measurements)

Respiratory substrates	Oxygen uptake (μl O_2 h^{-1} 10^9 cells)				
	range	mean	SD	CV (%)	CD
Casein hydrolysate	12.6-164.2	45.03	34.94	77.60	27.12
Glucose	7.1-86.1	28.41	20.40	71.80	14.64
Sodium acetate	7.1-107.1	25.66	20.43	79.63	16.27
Alanine	7.1-57.1	16.70	14.77	88.46	13.07
Cellobiose	2.1-73.5	14.61	15.13	103.57	15.67

Data presented in Figure 2 show differences in the level of respiratory activity of bacteria inhabiting surface (FL and SL) and subsurface (SUB) water layers. All tested substrates were most actively oxidized by neustonic bacteria isolated from the film layer while planktonic bacteria oxidized them with the lowest intensity.

Figure 3 presents data on the level of respiratory activity of bacteria in the seasonal cycle. Generally, the intensity of oxidization of the tested substrates was similar in all seasons; only the intensity of oxidization of cellobiose was higher in spring than in summer and autumn.

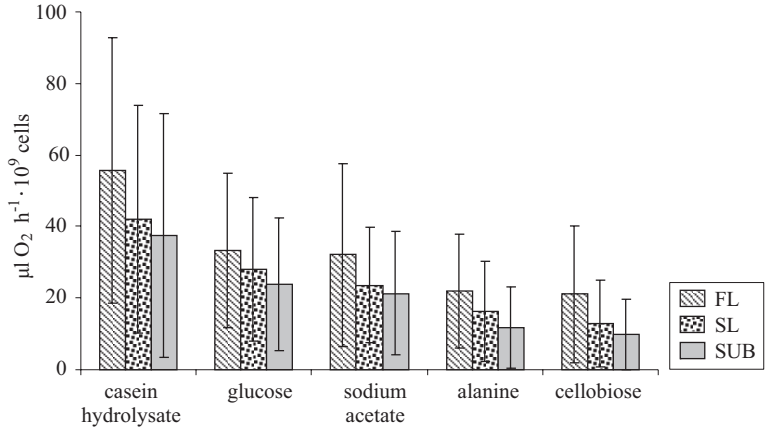


Fig. 2. Respiratory activity of bacteria isolated from surface (FL, SL) and subsurface (SUB) water layers (average value for seasons and stations). Vertical bars represented standard deviation of the mean, $n = 27$

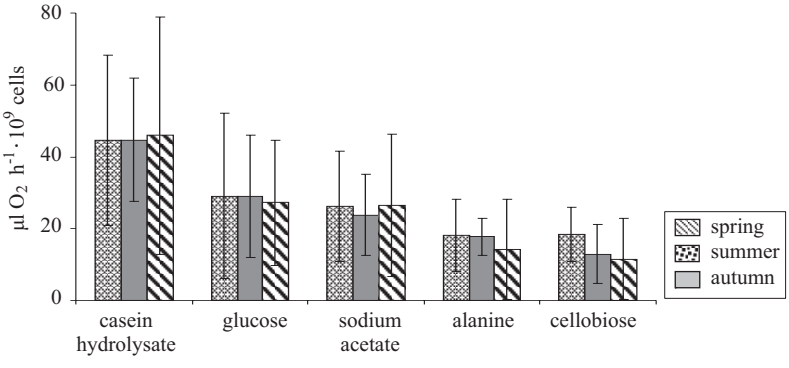


Fig. 3. Seasonal variability oxygen uptake by bacteria isolated from lake Gardno (average for all studied water layers and stations) Vertical bars indicate standard deviation of the mean, spring – $n = 39$, summer – $n = 29$, autumn – n = 31

The intensity of respiration of substrates differed among bacteria inhabiting different parts of the lake (Figure 4). All the tested substrates were oxidized with the highest intensity by bacteria from the seawater zone (site 3), while the lowest rates of oxygen consumption were usually shown by the strains isolated from the mid-lake zone (site 2).

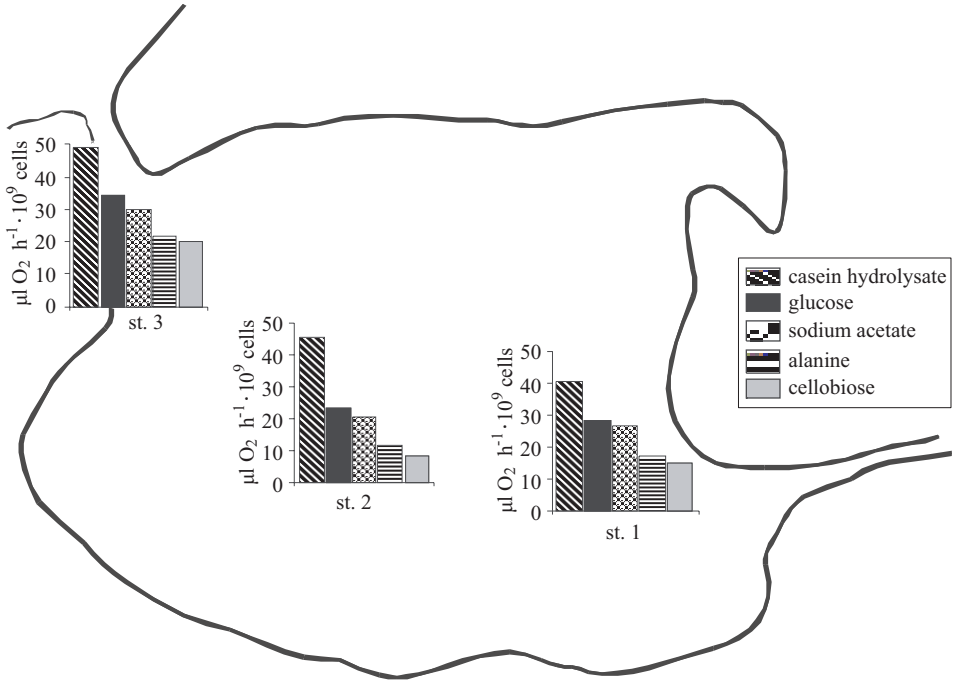


Fig. 4. Percent respiring strains of bacteria in various parts of lake Gardno (data for all water layers and season)

Discussion

Because of a great variety in the quantitative and qualitative composition of organic matter in aquatic ecosystems, many authors (DONDERSKI, STRZELCZYK 1980, STRZELCZYK et al. 1988, MUDRYK 1997) have drawn attention to significant differences in the trophic demands of bacteria and in the level of their respiratory activity. Heterotrophic bacteria isolated from the estuarine Lake Gardno also showed variation in the level of their respiratory activity; the most preferred respiratory substrate was casein hydrolyzate. Active utilization of this amino acid mixture by bacteriocenoses was previously observed in lakes (STRZELCZYK et al. 1988), rivers (OSSOWSKA-CYPRYK 1981), estuaries (MUDRYK 1989, 1997), and coastal marine waters (POMEROY et al. 1994, MUDRYK 1998). According to FUHRMAN, FERGUSON (1986), SIMON (1991), the ability to actively utilize mixtures of amino acids as energy sources is common among aquatic bacterial populations.

The results of several studies (NOVITSKY 1983, RHEINHEIMER 1984, MUDRYK 1997) indicate that glucose can constitute a respiratory substrate actively

assimilated by bacteria. It was also oxidized by bacteria isolated from Lake Gardno, but much less actively then casein hydrolyzate. These data correspond with the results obtained by HOPPE (1977), MUDRYK (1997, 1998) who determined that respiratory activity of brackish water and estuarine bacteria is much higher in relation to amino acid mixtures than to sugars. This can most probably be explained by the fact that sugars are only a source of carbon and energy, while amino acids additionally provide nitrogen.

Studies carried out by FUHRMAN, FERGUSON (1986), MUDRYK (1989), PRIEUR (1989) determined that sodium acetate, due to its simple chemical structure, is actively oxidized by bacteria inhabiting water bodies. Similarly, bacteria isolated from Lake Gardno showed a relatively high level of respiratory activity towards sodium acetate.

In the present study, cellobiose was the least preferred respiratory substrate. Because of its complex chemical structure, oxidation of this carbohydrate requires a considerable input of energy, and hence the level of its utilization by bacteria is low (STRZELCZYK et al. 1988, MUDRYK 1997, GROVER, CHRZANOWSKI 2000).

Many authors (GRIFFITH et al. 1984, BIDDANDA, BENNER 1997, MUDRYK 1998) draw attention to the variability of bacterial respiratory activity in the vertical profile of water basins. In the present study, clear differences in the level of bacterial potential respiratory activity between water layers have also been shown. Generally, it was higher in the surface than in the subsurface layer. Studies using direct microscopic methods with the use of formazan dyes to determine respiring bacteria showed that they were abundant in the surface water layers of salt marshes, and of marine and freshwater ecosystems (HARVEY, YOUNG 1980, HERMANSSON, DAHLBÄCK 1983, MAKI, REMSEN 1989). Probably, high concentrations of organic matter constituting respiratory substrates for bacteria, high level of oxidation and high accumulation of bacteria in surface waters (SÖDERGEN 1993, KNULST et al. 1997) result in a much higher level of respiratory activity of neustonic than of planktonic bacteria.

Different zones of aquatic ecosystems are characterized by different accessibility of respiratory substrates to bacteria (NOVITSKY 1983). Therefore, particular zones of those ecosystems are populated by specific physiological groups of bacteria carrying out respiratory processes with various intensity (GRIFFITHS et al. 1984, VOSJAN et al. 1990, MUDRYK 1997, 1998). Data presented in this paper also show differences in the intensity of assimilation of respiratory substrates by bacteria inhabiting different parts of Lake Gardno. Only few studies were carried out in coastal and estuarine areas (GRIFFITH, POMEROY 1995, MUDRYK 1997, CAFFERY et al. 1998); their results indicate that spatial variations of bacterial respiratory activity reflect variations in the

concentration, composition and degradability of organic matter, as well as changes in the composition of microbial populations. In the seawater zone of Lake Gardno, where large quantities of allochthonous organic matter accumulate due to sewage discharge from the holiday resort of Rowy, respiratory activity was higher than in the less-polluted sampling sites in the mixed and freshwater zones.

The authors realize that the respirometric technique used in this study allows only to measure the potential respiratory activity of bacteria which is never identical to bacterial activity in natural environments (MARTINEZ, ESTRADA 1992). In laboratory conditions it is not possible to generate and monitor short-term changes commonly occurring *in situ*. The results obtained in the present study and their ecological interpretation can however be an important source of information on the potential role of bacteria in the process of oxidation of various organic compounds and therefore in the process of transformation of organic matter in aquatic ecosystems.

Conclusions

The results of the study of respiratory activity of bacterial strains isolated from estuarine Lake Gardno, has led to a series of conclusion.

1. Heterotrophic bacteria showed variation in the level of their respiratory activity; the most preferred respiratory substrate was casein hydrolyzate, while cellobiose was oxidized least actively.

2. All tested substrates were most actively oxidized by neustonic bacteria isolated from the film layer while planktonic bacteria oxidized them with the lowest intensity.

3. The level of metabolic activity of bacteria from different parts of the studied lake was shown to differ, whereas the intensity of oxidization of the tested substrates was similar in all seasons.

Translated by MAŁGORZATA OŻGO

Accepted for print 6.03.2008

References

ANESIO A.M., ABREU P.C., BIDDANDA B. A. 2003. *The role of free attached microorganisms in the decomposition of estuarine macrophyte detritus*. Estuar. Coast. Shelf Sci., 56: 197-201.

BEDNARZ T., STARZECKA A. 1998. *Microbiological processes taking place in Dobczyce reservoir during spring water bloom*. Oceanol. Stud., 2: 13-21.

BERARD A., VOLAT B., MONTUELLE B. 1995. *Bacterial activity and its role in a eutrophic pond*. Arch. Hydrobiol., 134: 499-513.

BIDDANDA B.A., OPSAHI S., BENNER R. 1994. *Plankton respiration and carbon flux through bacterioplankton on the Louisiana shelf.* Limnol. Oceanogr., 39: 1259-1275.

BIDDANDA B.A., BENNER R. 1997. *Major contribution from mesopelagic plankton to heterotrophic metabolism in upper ocean.* Deep-Sea Res., 44: 2069-2085

BILLEN G., FONTIGNY A. 1987. *Dynamics of a phaeocystis – dominated spring bloom in Belgian coastal waters, II Bacterioplankton dynamics.* Mar. Ecol. Prog. Ser., 37: 249-257.

CAFFERY J., CLOREN J.E., GRENZ C. 1998. *Changes in production and respiration during a spring phytoplankton bloom in San Francisco Bay, California, USA: implication for net ecosystem metabolism.* Mar. Ecol. Prog. Ser., 172: 1-12.

CUNHA M.A., ALMEIDA M.A., ALCÃANTRA F. 1999. *Compartments of oxygen consumption in a tidal mesotrophic estuary (Ria de Averio, Portugal).* Acta Oecol., 20: 227-235.

DETHIER M.N. 1992. *Classifying marine and estuarine natural communities: An alternative to the coward in system.* Nat. Areas J., 12: 90- 100.

DONDERSKI W., STRZELCZYK E. 1980. *Metabolic activity of planktonic bacteria in three lakes of different trophy.* Bull. Acad. Pol. Sci., 28: 39-46.

FERRER E.B., STAPERT E.M., SOKALSKI W.T. 1963. *A medium for improved recovery of bacteria from water.* Can. J. Microbiol., 9: 420-422.

FUHRMAN J.A., FERGUSON R.L. 1986. *Nanomolar concentrations and rapid turnover of dissolved free amino acids in sea water: agreement between chemical and microbiological measurements.* Mar. Ecol. Prog. Ser., 33: 237-242.

GARRETT W.D. 1965. *Collection of slick-forming materials from the sea surface.* Limnol. Oceanogr., 10: 602-605.

GRIFFITHS R.P., CALDWELL B.A., MORITA R.Y. 1984. *Observations on microbial percent respiration values in Arctic and subarctic waters and sediments.* Microbial Ecol., 10: 151-164.

GRIFFITH P.C., POMEROY L.R. 1995. *Seasonal and spatial variations in pelagic community respiration on the south-eastern U.S. continental shelf.* Cont. Shelf Res., 15: 815-825.

GROVER J.P., CHRZANOWSKI T.H. 2000. *Seasonal patterns of substrate utilization by bacterioplankton: case studies in four temperate lakes of different latitudes.* Aqua. Microbial Ecol., 23: 41-54.

HERMANSSON M., DAHLBÄCK B. 1983. *Bacterial activity at the air/water interface.* Microbial Ecol., 9: 317-328.

HARVEY G.W., BURZELL L.A. 1972. *A simple microlayer method for small samples.* Limnol. Oceanogr., 17: 156-157.

HARVEY R.W., YOUNG L.Y. 1980. *Enumeration of particle-bound and unattached respiring bacteria in the salt marsh environment.* Appl. Environ. Microbiol., 40: 156-160.

HOPPE H.G. 1977. *Analysis of actively metabolising bacterial populations with the autoradiographic method.* Ecol. Stud., 25: 179-196.

HOPPE H., GIESENHAGEN H., GOCKE K. 1998. *Chaining patterns of bacterial substrate decomposition in a eutrophic gradient.* Aqua. Microbial Ecol., 15: 1-13.

JAHNKE R.A., CRAVEN D.B. 1995. Quantifying the role of heterotrophic bacteria in the carbon cycle: a need for respiration rate measurements. *Limnol. Oceanogr.,* 40: 436-441.

KNULST J.C., BACKLUND P., HESSEN D.O., RIISE G., SÖDERGREN A. 1997. *Response of surface microlayers to artificial acid precipitation in a meso-humic lake in Norway.* Wat. Res., 31: 2177-2186.

KONOPKA A. ZAKHAROVA T. 1999. *Quantification of bacterial lead resistance via activity assays.* J. Microbiol. Meth., 37: 17-22.

MAKI J.S., REMSEN C.C. 1989. *Examination of a freshwater surface microlayers for diel changes in the bacterioneuston.* Hydrobiol., 182: 25-34.

MARTINEZ R., ESTRADA M. 1992. Respiratory electron transport activity of microplankton in the Weddell Sea during early spring: influence of the ice cover and ice edge. *Pol. Biol.,* 12: 275-282.

MUDRYK Z. 1989. *Determination of the metabolic activity of heterotrophic bacteria isolated from estuarine Gardno lake.* Pol. Arch. Hydrobiol., 36: 97-103.

MUDRYK Z. 1997. *Studies on metabolic activity of planktonic bacteria isolated from coastal lake Łebsko.* Pol. J. Environ. Stud., 6: 23-28.

MUDRYK Z. 1998. *Generic composition and metabolic activity of bacteria inhabiting surface seawater layers.* Oceanol. Stud., 3: 57-70.

NAGATA S., MAEKAWA Y., IKEUCHI T., WANG Y., ISHIDA A. 2002. *Effect of compatible solutes on the*

respiratory activity and growth of Escherichia coli K-12 under NaCl stress. J. Biosci. Bioengineer., 94: 384-389.

NOVITSKY J.A. 1983. *Heterotrophic activity throughout a vertical profile of seawater and sediment in Halifax Harbour, Canada.* Appl. Environ. Microbiol., 45: 1753-1760.

OSOWSKA-CYPRYK K. 1981. *Microbiological studies of waters and bottom sediments of Utrata river.* Pol. Arch. Hydrobiol., 28: 357-379.

POMEROY L.R., SHELDON J.E., SHELDON W.M. 1994. *Changes in bacterial numbers and leucine assimilation during estimations of microbial respiratory tares in seawater by the precision Winkler method*. Appl. Environ. Microbiol., 60: 328-332.

PRIEUR D. 1989. *Preliminary study of heterotrophic bacterial communities in water. An Invertebrates From Deep Sea Hydrothermal Vents.* Proc 21st EMBS Gdańsk, 393-401.

RHEINHEIMER G. 1984. *Bacterial ecology of the North and Baltic Seas.* Bot. Mar., 27: 277-299.

SIMON M. 1991. *Isotope dilution of intercellular aminoacids as a tracer of carbon and nitrogen sources of marine planktonic bacteria.* Mar. Ecol. Prog. Ser., 14: 295-301.

SÖDERGREN A. 1993. *Role of aquatic surface microlayer in the dynamics of nutrients and organic compounds in lakes, with implication for their ecotones.* Hydrobiol., 251: 217-225.

STROTMANN U.J., GELDREN A., KUHN A., GENDIG C., KLEIN S. 1999. *Evaluation of a respirometric test method to determine the heterotrophic yield coefficient of activated sludge bacteria*. Chemosphere, 38: 3555-3570.

STRZELCZYK E., STOPIŃSKI M., MYZYK G. 1988. *Studies on metabolic activity of single and mixed cultures of planktonic and benthic bacteria of two lakes of different trophy.* AUNC Limnol. Paper., 16: 3-13.

STRZELECKI J., PÓŁTORAK T. 1971. *Coastal plankton lake Gardno in summer season (in Polish).* Acta Hydrobiol., 13: 269-294.

TROJANOWSKI J., TROJANOWSKA C., KORZENIEWSKI K. 1991. *Trophic state of coastal lakes.* Pol. Arch. Hydrobiol., 38: 23-34.

VELJI M.J., ALBRIGHT J. 1986. *Microscopic enumeration of attached marine bacteria of seawater, marine sediment, faecal matter and kelp blade samples following pyrophosphate and ultrasound treatments.* Can. J. Microbiol., 32: 121-126.

VOSJAN J.H., TIJSSEN S.B., NIEUWLAND G., WETSTEYN F.J. 1990. *Oxygen regime, respiratory activity and biomass of microorganisms and the carbon budget in the Fladen ground area (Northern North Sea) during spring.* Neth. J. Sea Res., 25: 89-99.

POLISH JOURNAL OF NATURAL SCIENCES
Abbrev.: Pol. J. Natur. Sc., Vol 23(2): 398-414, Y. 2008

DOI 10.2478/v10020-008-0031-1

LICHEN BIOTA OF THE GRABIANKA RIVER VALLEY IN THE ELBLĄG UPLAND (WYSOCZYZNA ELBLĄSKA)

Rafał Szymczyk, Anna Zalewska

Department of Botany and Nature Protection
University of Warmia and Mazury in Olsztyn

K e y w o r d s: lichens, river valley, Elbląd Upland (Wysoczyzna Elbląska), northern Poland.

A b s t r a c t

The lichen biota of a forest ravine was studied. Investigations were carried out in the Grabianka river valley situated in the Elbląg Upland (Wysoczyzna Elbląska). The analyzed lichen biota is very interesting and relatively rich. A total of 122 taxa were found in the area, of which 12 are legally protected, 46 are considered endangered in Poland and 13 are indicators of lowland primeval forests. Some other interesting and rare taxa were also recorded, including *Reichlingia leopoldii*, *Mycobilimbia epixanthoides* and *Scoliciosporum pruinosum*. In addition, a number of rare non-lichenized fungi were identified, such as *Tremella cladoniae*, *Chaenothecopsis savonica* or *Mycocalicium subtile*.

BIOTA POROSTÓW DOLINY RZEKI GRABIANKI NA WYSOCZYŹNIE ELBLĄSKIEJ (PÓŁNOCNA POLSKA)

Rafał Szymczyk, Anna Zalewska

Katedra Biologii i Ochrony Przyrody
Uniwersytet Warmińsko-Mazurski w Olsztynie

S ł o w a k l u c z o w e: porosty, doliny rzeczne, Wysoczyzna Elbląska, północna Polska.

A b s t r a k t

Przeprowadzono badania lichenologiczne w górnej części doliny rzeki Grabianki na Wysoczyźnie Elbląskiej. Miały na celu opracowanie składu gatunkowego porostów. Zarejestrowano 122 taksony, odnaleziono 12 gatunków chronionych, 46 zagrożonych w Polsce i 13 gatunków wskaźnikowych lasów naturalnych. Zlokalizowano stanowiska grzybów zlichenizowanych, rzadkich w Polsce, np. *Reichlingia leopoldii*, *Mycobilimbia epixanthoides* i *Scoliciosporum pruinosum*. Podczas analizy zebranego materiału zidentyfikowano dodatkowo 12 gatunków grzybów niezlichenizowanych, np. *Tremella cladoniae*, *Chaenothecopsis savonica* i *Mycocalicium subtile*.

Address: Anna Zalewska, University of Warmia and Mazury, pl. Łódzki 1, 10-727 Olsztyn, Poland, phone: (089) 523 36 98, e-mail: annazale@uwm.edu.pl

Introduction

The Elbląg Upland (Wysoczyzna Elbląska) has been poorly investigated to date with respect to the local lichen biota. The earliest reference data can be found in German reports (OHLERT 1870, LETTAU 1919, NITARDY 1922), but they usually concern single localities of a few species only. Polish lichenological sources, providing fragmentary information on this area, include works of JAKUBOWICZ (1983), SULMA, FAŁTYNOWICZ (1988), FAŁTYNOWICZ, SULMA (1994), as well as atlases (TOBOLEWSKI, KUPCZYK 1974, TOBOLEWSKI 1983, FAŁTYNOWICZ 1999) based on previous reports.

Erosion valleys of small streams and rivers located in the Elbląd Upland seem to be of particular interest from the lichenological perspective. One of them is the Grabianka river valley. Therefore, the aim of this study was to present the species composition of the lichen biota in the upper part of the Grabianka river valley, as well as to make a preliminary analysis of particular habitat groups.

Characteristics of the research area

The Grabianka river valley is situated in the northern part of the Elbląg Upland, in the largest forest complex of this mesoregion. Grabianka has its source in a partly deforested area, south of the village of Pagórki (Figure 1). This is a small river, only 10 km in length, whose catchment area covers 14.3 km^2 (KONIECKA, KOPIEC 2002). In its upper course the river resembles a swift-flowing, high-gradient torrent, with numerous boulders in the bed. Close to the mouth, near the village of Kadyny, the river becomes wide and sluggish, with a sandy bottom.

The study was conducted in the upper part of the Grabianka river valley. The landscape in this part of the Elbląg Upland is almost mountainous, with numerous erosion valleys cutting through high morainal hills. The highest hills, situated at the edge of the investigated part of the Grabianka river valley, reach up to 170 m above sea-level, with relative height difference of 100 m. The height and angle of the majority of slopes in this area range from 60 m to 80 m and from 40^o to 70^o respectively.

The study area is located within the Elbląg-Ostróda climate region of North-Eastern Poland (*Atlas współzależności…* 1986). The climatic conditions of the Elbląg Upland are significantly affected by elevation. Compared to the adjacent regions, this area is characterized by a considerably higher annual range of temperatures, a longer winter, a shorter summer, a longer snow cover duration and a higher annual precipitation total. Elevations, reaching up to 200 m above sea-level, "trap" humid polar-maritime air-masses moving from

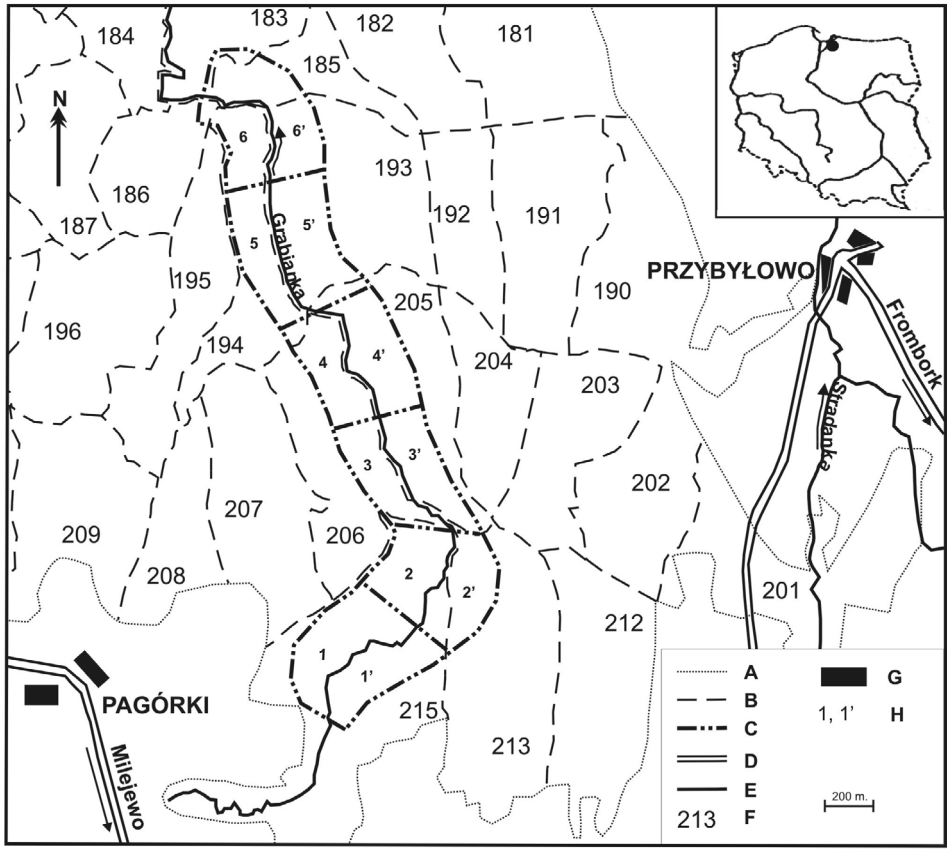


Fig. 1. Location of the research area and division into localities: A – forest border, B – forests section border, C – border of the study area, D – roads, E – rivers, F – numbers of forest sections, G – buildings, H – numbers of localities

northwest. Rainfalls are more frequent and heavier (WOŚ 1999). Rainfall characteristics in the Elbląg Upland are also affected by the vicinity of the Bay of Gdańsk and the Vistula Lagoon. The average annual rainfall total is 700-800 mm, compared to 500-600 mm in adjacent regions.

Both the tops and slopes of the examined valley are covered by beech woods, which can be found in a variety of habitats and forms, from *Luzulo pilosae-Fagetum* – less frequent, typical of acid soils in the lowlands, to locally common *Galio odorati-Fagetum* – typical of fertile soils. Smaller areas are occupied by phytocenoses of the sub-atlantic oak-hornbeam forest *Stellario-Carpinetum* in different ecological forms: poor on the tops of the valley, typical on the slopes and fertile at the base of the slopes. The ash-alder forest

Fraxino-Alnetum can be found at the valley bottom, along the river and at numerous seepages. The submontane ash forest *Carici remotae-Fraxinetum*, very rare in lowlands, grows around water outflow sites on the valley slopes (TOKARZ 1961).

Materials and Methods

According to the map graticule in the Atlas of Lichen Distribution in Poland based on the ATPOL system (CIEŚLIŃSKI, FAŁTYNOWICZ 1993), the research object is situated within square Ad96. Lichenological studies were conducted during the years 2002-2004. A selected fragment of the upper part of the valley was divided into six 0.5 km stretches, numbered 1 to 6. Six localities were established within each stretch: two on the tops, on the left (a) and on the right (a') side of the valley, two on the slopes on both sides (b and b'), and two at the bottom (c and c'). As a result, lichen biota was analyzed at 36 localities. All lichens were identified using standard methods of morphological-anatomic analysis and spot tests, enabling to distinguish between secondary metabolites (PURVIS et al. 1992). Species of the genus *Lepraria* and some other taxa were identified with the use of thin-layer chromatography (ORANGE et al. 2001). Nomenclature follows mostly FAŁTYNOWICZ (2003), except for *Arthonia ruana* A. Massal., *Lecanora albellula* (Nyl.) Th. Fr., *Opegrapha niveoatra* (Borrer) J. R. Laundon, *O. vulgata* (Ach.) Ach. (SANTESSON R. et al. 2004.), and the genera *Coenogonium Ehrenb.* (syn. Dimerella Trev.) (KAUFF, BÜDEL 2005), *Melanelixia* O. Blanco, A. Crespo, Divakar, Essl., D. Hawksw., Lumbsch (BLANCO et al. 2004). The entire collection is stored at the herbarium of the Department of Botany and Nature Protection, University of Warmia and Mazury in Olsztyn (OLS-L).

Results

A total of 110 lichen species and 12 non-lichenized fungi (including 7 lichenicolous and 5 saprophytic fungi), usually described in lichenological works, were identified in the analyzed part of the Grabianka river valley. A list of all recorded taxa, in alphabetical order, is given below. It includes also the types of substrates and numbers of localities for each taxon; if the number of records at a given locality is higher than one, it is given in brackets. Red List threat categories are also given for vanishing lichen species in Poland (CIEŚLIŃSKI et al. 2006): protected species (*Rozporządzenie*... Dz.U. nr 168, poz. 1765, zał. 4) are denoted "P", lichens-indicators of lowland primeval forests

(CZYŻEWSKA, CIEŚLIŃSKI 2003) are denoted "I", while the names of non-lichenized fungi are marked with asterisks (*).

The presented list contains a group of particularly interesting species (24), reported from a few localities in Poland only. The characteristics of their occurrence in the investigated area, followed by some notes on their general distribution patterns, can be found elsewhere (SZYMCZYK 2007).

List of species

Absconditella lignicola Vězda & H. Kilias – bark of *Picea abies, Betula pendula, Fraxinus excelsior*, wood; 7 localities: 2b', 2c', 5a, 5b, 5c', 6b, 6c.

Anisomeridium polypori (Ellis & Everh.) M.E.Barr – bark of *Fraxinus excelsior, Padus avium, Fagus sylvatica, Alnus glutinosa*, wood; 4 localities: 2c (2), 2c' (3), 3c', 5c' (3).

Arthonia didyma Körb. – bark of *Quercus robur*; 1 locality: 1a; EN, I.

A. ruana A. Massal. – bark of *Fraxinus excelsior*; 2 localities: 5c, 5c'; NT.

A. spadicea Leight. – bark of *Alnus glutinosa, Quercus robur, Fagus sylvatica*; 14 localities: 1a, 1b, 1b', 1c (3), 2b' (4), 2b, 2c' (2), 2c, 3b', 3c (2), 4a, 4c (2), 5c', 6c'.

A. vinosa Leight. – bark of *Alnus glutinosa, Quercus robur*; 9 localities: 1c', 2c (2), 2c', 3a, 3b, 3c, 4c, 4c', 6b'; NT, I.

Aspicilia laevata (Ach.) Arnold – stone in river; 1 locality: 3c'; VU.

****Athelia arachnoidea*** (Berk.) Jülich – on *Lecanora conizaeoides, Micarea prasina, Arthonia vinosa*; 10 localities: 1b, 1b', 2b', 3a', 3b, 4a, 4b, 4c', 5b', 5c.

Bacidia fraxinea Lönnr. – bark of *Fraxinus excelsior*; 1 locality: 1b'; DD.

Bacidina arnoldiana (Körb.) V. Wirth & Vězda – bark of *Fagus sylvatica, Carpinus betulus, Alnus glutinosa*; 3 localities: 2b, 2b', 6c' (2); NT.

B. inundata (Fr.) Vězda – stones in river, on tyre in river; 3 localities: 1b', 2c, 3c'.

Baeomyces rufus (Huds.) Rebent. – stones, sandy soil; 5 localities: 2c, 3b', 4a', 5b', 6b' (4).

Biatora efflorescens (Hedl.) Räsänen – bark of *Fagus sylvatica, Quercus robur, Fraxinus excelsior, Carpinus betulus, Betula pendula*; 9 localities: 1a, 1b', 1c, 2b, 3a, 3b', 4b, 5c', 6c; VU.

B. turgidula (Fr.) Nyl. – wood; 1 locality: 2c; VU, I.

Buellia griseovirens (Turner & Borrer ex Sm.) Almb. – bark of *Fagus sylvatica, Fraxinus excelsior, Quercus rubra, Carpinus betulus, Padus avium, Alnus glutinosa, Corylus avellana*; 13 localities: 1a (2), 1b, 2a', 2b (2), 2b', 2c, 2c', 3a, 3a', 3c, 4a' (3), 4c, 5a.

Calicium salicinum Pers. – bark of *Quercus robur, Fraxinus excelsior*, wood; 3 localities: 1a, 2c, 4c' (2); VU.

C. viride Pers. – bark of Fraxinus excelsior; 1 locality: 6c'; VU, I.

Chaenotheca brachypoda (Ach.) Tibell – wood, bark of *Fraxinus excelsior*; 4 localities: 1c', 2c' (2), 3b, 3b'; EN, I.

Ch. brunneola (Ach.) Müll. Arg. – wood; 2 localities: 2c', 4b; EN, I.

Ch. chlorella (Ach.) Müll. Arg. – wood; 2 localities: 2b', 2c'; CR, I.

Ch. chrysocephala (Turner ex Ach.) Th. Fr. – bark of *Alnus glutinosa, Larix decidua, Quercus robur, wood*; 7 locality: 2b, 2b', 3b, 3c, 4a', 4b, 6c'.

Ch. ferruginea (Turner ex Borrer) Mig. – bark of Picea abies, *Alnus glutinosa, Quercus robur, Pinus sylvestris, Betula pendula, wood*; 15 localities: 1a (2), 1b (3), 1b' (2), 1c', 2b (2), 2b' (4), 2c' (3), 3a, 3b', 3c', 4a (2), 4a' (3), 4b' (3), 5b', 6b.

Ch. furfuracea (L.) Tibell – bark of *Alnus glutinosa*, wood; 4 localities: 2b, 2b', 2c, 2c' (3). NT.

Ch. stemonea (Ach.) Müll. Arg. – bark of *Quercus robur*, wood; 3 localities: 2a', 2c', 4b. EN.

Ch. trichialis (Ach.) Th. Fr. – bark of *Carpinus betulus*, wood; 3 localities: 1b, 2a', 2b (2). NT.

Ch. xyloxena Nádv. – wood; 4 localities: 1a, 1b (2), 3a, 3b' (3). VU.

****Chaenothecopsis epithallina*** Tibell – wood; 1 locality: 3b.

****Ch. pusilla*** (Ach.) A. F. W. Schmidt – wood; 2 localities: 1b', 6c'

****Ch. savonica*** (Räsänen) Tibell – wood; 1 locality: 3a

Chrysothrix candelaris (L.) J. R. Laundon – bark of *Fraxinus excelsior, Fagus sylvatica, Quercus robur*; 4 localities: 2c', 5b', 6b', 6c'; CR, P, I.

Cladonia caespiticia (Pers.) Flörke – soil; 2 localities: 4b', 5b'; EN.

C. chlorophaea (Flörke ex Sommerf.) Spreng. – bark of *Alnus glutinosa*, wood; 5 localities: 2a', 3c, 4b, 5c, 5b'.

C. coniocraea (Flörke) Spreng. – bark of *Alnus glutinosa, Fagus sylvatica, Betula pendula, Quercus robur, Picea abies*, wood, soil; 22 localities: 1a, 1a', 1b, 1b' (2), 1c (2), 2b' (3), 2c' (2), 3a, 3a' (2), 3b, 3b', 3c, 4a (2), 4a' (2), 4b, 4b', 4c (2), 5a (3), 6a, 6b' (2), 6c, 6c'.

C. digitata (L.) Hoffm. – bark of *Alnus glutinosa, Betula pendula, Fagus sylvatica, Quercus robur, Picea abies, Pinus sylvestris*, wood, soil; 13 localities: 1a', 1b, 1b', 2b, 2b' (4), 2c, 2c' (2), 4a (2), 4b, 4b', 4c', 5a, 5b', 6c'.

C. fimbriata (L.) Fr. – bark of *Fagus sylvatica, wood, soil*; 6 localities: 1a, 1a', 1b, 3a', 4a', 6b'.

C. furcata (Huds.) Schrad. – soil; 1 locality: 6b'.

C. ochrochlora Flörke – bark of *Alnus glutinosa, Picea abies, Fagus sylvatica, Betula pendula*, wood, soil; 14 localities: 1a (2), 1a', 1b, 1c, 1c' (2), 2a' (2), 2b' (2), 3c, 4b, 4c', 5a, 5c, 5c', 6c'.

C. polydactyla (Flörke) Spreng. – wood; 2 localities: 2c', 4c.

C. rei Schaer. – bark of Alnus glutinosa; 1 locality: 4b'.

C. subulata (L.) Weber ex F. H. Wigg. – soil; 2 localities 3b, 6b'.

****Clypeococcum hypocenomycis*** D. Hawksw. – on *Hypocenomyce scalaris*; 4 localities: 1a, 3b', 4b', 5b'.

Coenogonium pineti (Ach.) Lücking & Lumbsch – bark of *Alnus glutinosa, Picea abies, Fagus sylvatica, Carpinus betulus, Quercus robur, Fraxinus excelsior, Populus tremula, Tilia cordata, Betula pendula*, wood; 24 localities: 1b (3), 1b' (2), 1c, 2b (2), 2b' (8), 2c' (2), 3a', 3b (2), 3b' (2), 3c (4), 3c', 4a, 4a', 4b (2), 4b', 4c, 5a (2), 5a', 5b', 5c', 6b, 6c, 6c' (2).

Collema flaccidum (Ach.) Ach. – stones in river; 2 localities: 4c, 6c; EN.

Evernia prunastri (L.) Ach. – bark of *Fraxinus excelsior, Alnus glutinosa*; 4 localities: 3c' (2), 4c, 4c', 6b; NT, P.

Fellhaneropsis vezdae (Coppins & James) Sérus. & Coppins – bark of *Carpinus betulus*; 1 locality: 2c'; LC, I.

Graphis scripta (L.) Ach. – bark of *Fagus sylvatica, Carpinus betulus, Alnus glutinosa, Fraxinus excelsior, Ulmus glabra, Acer platanoides, Tilia cordata, Padus avium, Corylus avellana*; 29 localities: 1a (2), 1a' (4), 1b (7), 1b' (6), 1c, 1c' (2), 2a' (3), 2b (4), 2b' (7), 2c, 2c' (4), 3a, 3a', 3b (3), 3b' (2), 3c (2), 3c' (4), 4a (2), 4a' (2), 4b (4), 4b' (4), 4c (2), 4c', 5b, 5c, 5c' (2), 6a', 6c, 6c' (2); NT.

Hypocenomyce scalaris (Ach.) M. Choisy – bark of *Picea abies, Quercus robur, Alnus glutinosa Pinus sylvestris, Betula pendula, Fagus sylvatica*, wood; 15 localities: 1a (2), 1a', 1b, 1c', 2b (3), 2b', 3a, 3b' (4), 4a (3), 4a' (3), 4b' (2), 5a (2), 5b' (4), 6b, 6c'.

Hypogymnia physodes (L.) Nyl. – bark of *Fagus sylvatica, Alnus glutinosa, Fraxinus excelsior, Quercus robur, Picea abies, Larix decidua, Betula pendula, Carpinus betulus, Populus tremula*, wood; 32 localities: 1a (3), 1a', 1b (2), 1b' (2), 1c (2), 1c', 2a' (2), 2b (7), 2b', 2c, 2c' (2), 3a (3), 3a' (3), 3b (2), 3c (2), 3c' (8), 4a (2), 4a' (5), 4b, 4b', 4c (3), 4c', 5a (6), 5a', 5b' (3), 5c (3), 5c' (2), 6b, 6c, 6c' (6).

Hypogymnia tubulosa (Schaer.) Hav. – bark of *Fagus sylvatica, Fraxinus excelsior*; 2 localities: 1b', 3a'; NT, P.

Lecanactis abietina (Ach.) Körb. – bark of *Alnus glutinosa*; 1 locality: 4c'; EN, I.

Lecania globulosa (Flörke) van den Boom & Sérus. – bark of *Fraxinus excelsior*; 2 localities: 2c', 4c'; VU.

Lecanora albellula (Nyl.) Th. Fr. – wood; 1 locality: 3b'.

L. argentata (Ach.) Malme – bark of *Fagus sylvatica, Carpinus betulus, Fraxinus excelsior*; 13 localities: 1a, 1b (2), 1b', 2a', 2b', 2c', 3b (3), 3c' (2), 4b (2), 5b' (2), 6a', 6c (2).

L. carpinea (L.) Vain. – bark of *Fagus sylvatica, Carpinus betulus*; 3 localities:3 1b, 2a', 2b'.

L. conizaeoides Nyl. ex Crombie – bark of *Picea abies, Fagus sylvatica, Alnus glutinosa, Pinus sylvestris, Betula pendula, Larix decidua*, wood; 20 localities: 1a, 1a' (2), 1b, 1b' (2), 1c' (2), 2a' (2), 2b (4), 2b' (3), 3a (2), 3a' (4), 3b' (2), 3c (2), 3c', 4a (2), 4a' (4), 4b' (2), 5a (3), 5b' (5), 5c, 6b'.

L. expallens Ach. – bark of *Quercus robur, Fagus sylvatica, Fraxinus excelsior, Alnus glutinosa, Carpinus betulus, Larix decidua, Populus tremula*; 20 localities: 1a (2), 2b (5), 2b', 2c' (2), 3b' (2), 3c (2), 3c' (5), 3b, 4a (3), 4a' (5), 4b (3), 4b' (2), 4c' (2), 5a, 5b' (2), 5c' (2), 6b' (2), 6c, 6c'.

L. glabrata (Ach.) Malme – bark of *Fraxinus excelsior, Carpinus betulus*; 2 localities: 4c', 5b'.

L. polytropa (Ehrh. ex Hoffm.) Rabenh. – stone; 1 locality: 2b.

L. pulicaris (Pers.) Ach. – bark of *Fagus sylvatica, Fraxinus excelsior, Carpinus betulus*; 7 localities: 1a, 1a', 2a', 4a', 5a, 5c', 6c'.

Lecidella elaeochroma (Ach.) M. Choisy – bark of *Fagus sylvatica, Carpinus betulus*; 3 localities: 2a', 4b', 5b'.

Lepraria elobata Tønsberg – bark of *Fagus sylvatica*, wood, stone; 6 localities: 1b', 2b, 3a', 5a', 5b, 5b'.

L. incana (L.) Ach. – bark of *Alnus glutinosa, Fagus sylvatica, Quercus robur, Carpinus betulus, Picea abies*; 15 localities: 1b', 1c (2), 1c', 2a', 2b' (4), 2c' (2), 3a, 3c', 4a (2), 4b (3), 4b', 4c', 5a, 5b, 6c' (2).

L. jackii Tønsberg – bark of *Larix decidu*a, wood; 2 localities: 2b, 4a'.

L. lobificans Nyl. – bark of *Alnus glutinosa, Carpinus betulus, Fagus sylvatica, Tilia cordata, Quercus robur*; 13 localities: 1a, 1b, 1b' (3), 1c (3), 1c', 2c, 2c' (4), 3c, 4a', 4b, 4c, 5b, 5c.

****Lichenoconium erodens*** M. S. Christ. & D. Hawksw. – on *Lecanora conizaeoides*; 5 localities: 2b, 2b', 4a, 4a' (2), 5b'.

****L. lecanorae*** (Jaap) D. Hawksw. – on *Lecanora conizaeoides*; 7 localities: 1a, 1a', 1b', 2a', 2b, 3c, 4b'.

Melanelixia fuliginosa (Fr. ex Duby) O. Blanco, A. Crespo, Divakar, Essl., D. Hawksw. & Lumbsch – bark of *Fagus sylvatica, Carpinus betulus, Fraxinus excelsior, Alnus glutinosa, Quercus robur, Padus avium*; 19 localities: 1a (3), 1a', 1b (2), 1c, 2a' (3), 2b (2), 2b' (2), 2c, 2c' (2), 3b, 4a' (3), 4b (2), 4b', 4c, 5a, 5b, 5b' (2), 6b', 6c'; P.

Micarea botryoides (Nyl.) Coppins – bark of *Picea abies*; 1 locality: 2a'.

M. micrococca (Körb.) Gams ex Coppins – bark of *Carpinus betulus*, wood; 6 localities: 1b', 2c', 3b, 3b', 4c, 5a'.

M. misella (Nyl). Hedl. – wood; 5 localities: 1c' (2), 2b', 2c', 5a.

M. prasina Fr. – bark of *Betula pendula*, wood; 2 localities: 3b, 5c.

****Monodictis epilepraria*** Kukwa & Diederich – on *Lepraria* spp.; 3 localities: 2c', 3b, 6c'.

Mycobilimbia epixanthoides (Nyl.) Vitik., Kuusinen, Lommi & T. Uen ex Haffellner & Turk – bark of *Fagus sylvatica, Fraxinus excelsior;* 2 localities: 5c', 6c'

Mycoblastus fucatus (Stirt.) Zahlbr. – bark of *Fagus sylvatica, Alnus glutinosa, Carpinus betulus, Quercus robur, Tilia cordata*; 15 localities: 1a, 1a' (2), 1b, 1c, 2b' (3), 3a' (2), 3c, 3c', 4a' (4), 4b', 4c', 5a, 5a', 5b, 6c' (2).

****Mycocalicium subtile*** (Pers.) Szatala – wood; 1 locality: 4b'

Ochrolechia androgyna (Hoffm) Arnold – bark of *Fagus sylvatica, Betula pendula*; 3 localities: 1a, 4b', 5a; VU.

Opegrapha atra Pers. – bark of *Fagus sylvatica*; 1 locality: 5b'; EN.

O. viridis (Pers. ex Ach.) Behlen & Deserger – bark of *Carpinus betulus, Fraxinus excelsior, Fagus sylvatica, Quercus robur, Alnus glutinosa, Ulmus glabra*; 15 localities: 1b' (4), 1c, 2b' (4), 2c' (2), 3b' (3), 3c' (3), 4b, 4b', 4c' (2), 5b' (2), 5c', 6a', 6b', 6c (2), 6c'; VU, I.

O. niveoatra (Borrer) J. R. Laundon – bark of *Carpinus betulus, Fraxinus excelsior, Fagus sylvatica, Alnus glutinosa*; 8 localities: 1b', 1c', 2b', 3b, 3c' (3), 4b', 5c', 6c': VU.

O. vulgata (Ach.) Ach. – bark of *Fagus sylvatica, Carpinus betulus, Quercus robur, Fraxinus excelsior, Tilia cordata*; 7 localities: 1b', 3b (2), 3c', 4b (2), 4b', 4c, 6b': VU.

Parmelia submontana Nádv. ex Hale – bark of *Fagus sylvatica*; 1 locality: 2a'; VU, P.

P. sulcata Taylor – bark of *Fagus sylvatica, Quercus robur, Alnus glutinosa*; 6 localities: 1a (3), 2a', 4a, 4a' (2), 4b', 4c.

Parmeliopsis ambigua (Wulfen) Nyl. – bark of *Fagus sylvatica, Carpinus betulus*; 7 localities:1a (2), 2a', 2b, 3a' (2), 3b', 4a (2), 4a' (2); P.

Peltigera polydactylon (Neck.) Hoffm. – bark of *Fagus sylvatica, stone*; 2 localities: 1b, 5b; EN, P.

P. praetextata (Flörke ex Sommerf.) Zopf – bark of *Fraxinus excelsior*, wood; 5 localities: 2c, 3b, 3c', 5c, 6c'; VU, P.

Pertusaria amara (Ach.) Nyl. – bark of *Fagus sylvatica, Carpinus betulus, Fraxinus excelsior, Quercus robur, Alnus glutinosa*; 19 localities: 1a (2), 1b (3), 2a', 2b (2), 2b', 2c' (2), 3a', 3b, 3c' (3), 4a, 4a' (4), 4b, 4b', 4c, 5a (3), 5b', 6a', 6b', 6c' (2).

P. coccodes (Ach.) Nyl. – bark of *Fagus sylvatica, Carpinus betulus, Quercus robur*; 8 localities: 1a (2), 3b, 4a (2), 4a' (2), 4b, 5a (2), 6c; NT.

P. flavida (DC.) J. R. Laundon – bark of *Fagus sylvatica*; 1 locality: 4b; EN, W.

P. hemisphaerica (Flörke) Erichsen – bark of *Fagus sylvatica, Quercus robur*; 4 localities: 3a, 4a', 4b, 4b'; VU, I.

P. leioplaca DC. – bark of *Carpinus betulus, Fagus sylvatica, Fraxinus excelsior*; 8 localities: 1b', 2a', 2b' (3), 3b', 4a', 4c', 5b', 6c'; NT.

P. pertusa (Weigel) Tuck. – bark of *Fagus sylvatica, Carpinus betulus, Quercus robur*; 10 localities: 2a, 2a', 2b', 3b, 4a, 4a' (2), 4b (3), 4b' (3), 5a, 6a'; VU.

Phlyctis argena (Spreng.) Flot. – bark of *Fagus sylvatica*, *Fraxinus excelsior*, *Quercus robur*, *Carpinus betulus*, *Picea abies*, *Padus avium*' 18 localities: 1a, 2b (2), 2b', 2c, 2c', 3a, 3b, 3b', 3c', 4a' (2), 4b, 4c', 5a, 5b, 5b', 5c' (3), 6b', 6c' (2).

Placynthiella dasaea (Stirt.) Trnsberg – bark of *Fagus sylvatica*, *Populus tremula*, wood; 9 localities: 1b', 2b', 3a', 3b', 4b', 5b, 5b' (2), 5c, 6c'.

P. icmalea (Ach.) Coppins & P. James – bark of *Betula pendula*, *Alnus glutinosa*, *Fagus sylvatica*, *Pinus sylvestris*, *Picea abies*, wood; 17 localities: 1a (2), 1b, 1b', 1c, 2b, 2b', 2c', 3a' (2), 3b' (2), 4a (2), 4b (2), 4b', 4c, 5a (3), 5b, 5b', 5c.

P. oligotropha (J. R. Laundon) Cooppins & P. James – soil; 2 localities: 1b, 6b'.

Platismatia glauca W. L. Culb. & C. F. Culb – bark of *Fagus sylvatica*, *Alnus glutinosa*, *Fraxinus excelsior*; 5 localities: 1b', 2b', 3c', 5c, 6b'; P.

Porpidia crustulata (Ach.) Hertel & Knoph – stone; 1 localities: 2c.

P. soredizodes (Lamy ex Nyl.) J. R. Laundon – stone; 7 localities: 1b, 1c', 4a', 4b, 4b', 4c, 5b'.

Pseudevernia furfuracea (L.) Zopf – bark of *Fagus sylvatica*, *Fraxinus excelsior*; 3 localities: 1b', 3c' (2), 5b'; P.

Pseudosagedia aenea (Wallr.) Hafellner & Kalb. – bark of *Fagus sylvatica*, *Carpinus betulus*, *Alnus glutinosa*, *Fraxinus excelsior*, *Ulmus glabra*, *Acer platanoides*, *Picea abies*; 18 localities: 1a, 1b (3), 1b' (3), 1c', 2b, 2b' (5), 2c', 3a' (2), 3b' (2), 3c, 3c' (2), 4a' (2), 4b' (2), 5b (2), 5b' (5), 5c' (2), 6c (2).

P. chlorotica (Ach.) Hafellner & Kalb – stone; 6 localities: 1b' (2), 1c, 2c, 4b, 4b' (2), 5c.

****Pycnidiella resine*** (Fr. ex Fr.) Höhnel – *Larix decidua* resin; 1 locality: 2b.

Pyrenula nitida (Weigel) Ach. – bark of *Fagus sylvatica*, *Carpinus betulus*, *Fraxinus excelsior*; 14 localities: 1b' (4), 2b', 2c', 3b (4), 3b' (3), 3c', 4b (5), 4b' (5), 4c (2), 4c', 5b', 5c', 6a', 6c'; VU.

P. nitidella (Flörke ex Schaer.) Müll. Arg. – bark of *Fraxinus excelsior*; 1 locality: 4c'; EN, I.

Ramalina farinacea (L.) Ach. – bark of *Fagus sylvatica*, *Quercus robur*, *Fraxinus excelsior*; 4 localities: 2a', 2c', 4a', 4b'; VU, P.

Reichlingia leopoldii Diederich & Scheid. – bark of *Alnus glutinosa*; 1 locality: 3c.

Ropalospora viridis (Tønsberg) Tønsberg – bark of *Fagus sylvatica*, *Alnus glutinosa*, *Carpinus betulus*, *Quercus robur*, wood; 8 localities: 1a' (2), 2b', 3a', 3c, 4b, 4c (2), 5b', 5c.

Scoliciosporum pruinosum (P. James) Vězda – bark of *Alnus glutinosa*; 1 locality: 3b'; DD.

****Taeniolella punctata*** M. S. Christ. & D. Hawksw. – on *Graphis scripta*; 4 localities: 1b, 3c', 3b, 6c'.

Thelocarpon intermediellum Nyl. – wood; 1 locality: 1a'; VU.

T. lichenicola (Fuckel) Poelt & Hafellner – bark of *Picea abies*; 1 locality: 2b'.

Trapelia coarctata (Sm.) M. Choisy – stone; 2 localities: 3b, 6c.

T. placodioides Coppins & P. James – stone; 3 localities: 2c, 3c', 5b'.

Trapeliopsis flexuosa (Fr.) Coppins & P. James – wood; 1 locality: 3a'.

T. granulosa (Hoffm.) Lumbsch – bark of *Pinus sylvestris*, wood, soil; 8 localities: 1a, 2c', 3b', 4a, 4a' (2), 5a (2), 5b' (3), 6b.

T. pseudogranulosa Coppins & P. James – wood; 2 localities: 4b', 6c'.

****Tremella cladoniae*** Diederich & M. S. Christ. – on *Cladonia* sp.; 1 locality: 4c.

Usnea filipendula Stirt. – bark of *Fraxinus excelsior*, *Alnus glutinosa*; 4 localities: 3c, 3c', 4c', 6c' VU, P.

Verrucaria dolosa Hepp – stone; 2 localities: 3c, 4c'.

V. hydrela Ach. – stone in river; 1 locality: 5c'; VU.

V. muralis Ach. – concrete; 1 locality: 5c'.

V. praetermissa (Trevis.) Anzi – stone in river; 3 localities: 2c, 4c, 5c'; NT

Analysis of lichen biota

In the examined part of the Grabianka river valley particular taxa were encountered at 1 to 36 localities. The largest group (28) was formed by species present at one or two localities, while the smallest one comprised common lichens, found at all (36) localities. Lichens with a wide ecological scale, such as *Hypogymnia physodes* and *Parmelia sulcata*, reached the highest frequency in the study area. It is interesting that the group of locally most frequent species included also *Graphis scripta* (common), *Opegrapha viridis* and *Pyrenula nitida* (frequent), considered to be threatened in Poland (CIEŚLIŃSKI et al. 2006). These are hygrophilous and skiophilous species, for which the phytoclimatic conditions of the Grabianka river valley are optimal.

Epiphytic lichens

Epiphytes constitute the most important ecological group in the analyzed biota, and their domination is directly determined by a wide diversity

of available habitats and substrates. Representatives of this group were found on 15 phorophytes. Eighty corticolous species identified in the valley accounted for 81% of total lichen biota. The richest epiphytic lichen biota was reported from *Fagus sylvatica*, *Alnus glutinosa*, *Fraxinus exelsior*, *Quercus* spp. and *Carpinus betulus*.

The beech *Fagus sylvatica* is a key stand-forming species in forest communities of the Elbląg Upland (TOKARZ 1961). Fifty-five lichen species were recorded on this phorophyte. The most frequent of them were *Graphis scripta*, *Hypogymnia physodes*, *Melanelixia subaurifera*, *Pseudosagedia aenea*, *Pyrenula nitida*, *Pertusaria amara* and *P. pertusa*. The following interesting forest lichens, listed as endangered on the Polish Red List (CIEŚLIŃSKI et al. 2006), were found on *Fagus sylvatica*: *Opegrapha atra*, *O. niveoatra*, *O. viridis*, *O. vulgata*, *Parmelia submontana*, *Peltigera polydactylon*, *Pertusaria flavida* and *P. hemisphaerica*.

The hornbeam *Carpinus betulus* has a mesotrophic and slightly acidic bark, which remains smooth or slightly cracked and generally does not peel (BARKMAN 1958). Thirty lichen species were observed on hornbeams. Of particular note is *Fellhaneropsis vezdae*, known from single localities in Poland only. The most common taxa encountered on this phorophyte were *Graphis scripta*, *Pyrenula nitida*, *Opegrapha viridis*, *Lecanora argentata* and *Pseudosagedia aenea*.

Apart from *Fagus sylvatica* and *Carpinus betulus,* abundant epiphytic lichen biota was recorded on the black alder and on the European ash. These two phorophytes are the main components of ash-alder communities at the bottom of the Grabianka river valley. Forty lichen species were collected from *Alnus glutinosa*. Many of them are particularly interesting taxa, very rare or poorly known in Poland, e.g. *Scoliciosporum pruinosum*, *Reichlingia leopoldii* or *Mycobilimbia epixanthoides*. Attention should be also paid to lichens associated with lowland primeval forests, such as *Arthonia vinosa*, *Lecanactis abietina* and *Opegrapha viridis* (CZYŻEWSKA, CIEŚLIŃSKI 2003), as well as to lichens growing on black alders in specific microhabitats, e.g. *Chaenotheca furfuracea* – found only at the root collar, or *Anisomeridium polypori* – collected from submerged roots. The sub-neutrophilic and eutrophic bark of *Fraxinus excelsior* provides the most favorable conditions for the development of rich lichen vegetation (BARKMAN 1958, BATES, BROWN 1981). Although ash trees are scarce in the analyzed part of the Grabianka river valley, as many as 37 lichen species were reported from this phorophyte. The most interesting among them are *Arthonia ruana*, *Bacidia fraxinea*, *Calicium salicinum*, *C. viride*, *Chaenotheca brachypoda*, *Pyrenula nitidella* and *Usnea filipendula*, all threatened in Poland.

The current lichen biota of common oaks (*Quercus robur*, *Q. petraea*) in the Grabianka river valley comprises 35 taxa. A large group is formed by members of the genera *Calicium* and *Chaenotheca,* which occupy deep grooves in strongly cracked bark of old trees. *Arthonia didyma* – an indicator of lowland primeval forests (CZYŻEWSKA, CIEŚLIŃSKI 2003) which is on the verge of dying out in Poland (CIEŚLIŃSKI et al. 2006), was found exclusively on oak trees.

Epiphytes were also observed on not abundant specimens of *Picea abies*, *Betula pendula*, *Tilia cordata*, *Padus avium* and *Acer platanoides*, which constituted admixture components of the examined forest communities. A total of 50 lichen species were reported from the above phorophytes. The spruce had the richest lichen biota (21), and the most common taxa encountered on this phorophyte were *Lecanora conizaeoides* and *Chaenotheca ferruginea*.

Epixylic lichens

A distinguishing feature of the Grabianka river valley is natural accumulation of dead wood, providing favorable conditions for the growth of epixylic lichens. In the analyzed part of the valley the occurrence of epixyles was studied on three types of substrates, i.e. dead standing barkless trunks, stumps and lying logs. Those forms of dead wood were analyzed separately due to, among others, a different degree of wood decomposition and moisture content, which significantly affect the species diversity of lichens.

The epixylic lichen biota in the investigated area comprises 40 taxa, which accounts for 37% of all recorded species. The majority of lichens growing on dead wood were also found on tree bark. The group of taxa living also on the ground, predominantly members of the genera *Cladonia* and *Placynthiella,* was smaller. Only 10 lichens were recorded exclusively on wood. Many of them are endangered in Poland or belong to the group of indicators of primeval forests. These are: *Chaenotheca brunneola* (EN, I), *Ch. chlorella* (CR, I), *Ch. trichialis* (NT), *Ch. xyloxena* (VU), *Cladonia polydactyla*, *Lecanora albellula*, *Biatora turgidula* (VU, W), *Micarea misella*, *Thelocarpon intermediellum* (VU), *Trapeliopsis flexuosa* and *T. pseudogranulosa*.

Both the composition of lichen species and their frequency on particular types of dead wood, determined in the present study, are consistent with the findings of other authors (MÜHLE, LE BLANC 1975, DANIËLS 1983, CIEŚLIŃSKI 1985, LAAKA 1995, CHLEBICKI et al. 1996, KOLANKO, MATWIEJUK 1999). Species formerly assigned to the order *Caliciales* (*Calicium*, *Chaenotheca*, *Chaenothecopsis*, *Mycocalicium*) were observed on standing trunks. These taxa show a distinct preference for exposed, dry, hard wood tissue. Humus-loving species of the genera *Cladonia*, *Trapeliopsis* and *Placynthiella* have a relatively high qualitative contribution on soft, rotting wood of stumps and lying logs.

The highest number of species (34) was identified on lying logs. The most frequent taxa on this type of substrate were *Absconditella lignicola*, *Cladonia* spp., *Micarea misella*, *M. prasina*, *Peltigera praetextata*, *Placynthiella dasaea* and *P. icmalea*. The number of lichens growing on stumps was lower (19). This was most probably related to considerably lower availability of such substrates within the study area. The list of the most frequent lichen species found on stumps is similar to that in the previous group. The fewest (17), but most interesting, lichens (17) were found on standing barkless trunks. Eight species of the genus *Chaenotheca*, threatened in Poland, were reported from the study area. Seven of them occurred on this type of substrate: *Chaenotheca brachypoda*, *Ch. bruneola*, *Ch. chlorella*, *Ch. furfuracea*, *Ch. stemonea*, *Ch. trichialis* and *Ch. xyloxena* (CIEŚLIŃSKI et al. 2006).

Lichens of other habitats

Epilithic and epigeic lichens were identified at a few localities in the investigated part of the Grabianka river valley. The small number of epilithes (17) resulted from low frequency of certain types of substrates. In the area under analysis almost all epilithic lichen species were observed on non-calcareous substrates, i.e. boulders in the river bed or small stones on the valley slopes. The relatively most frequent saxicolous lichens were *Pseudosagedia chlorotica* and *Porpidia soredizodes*. Artificial saxicolus substrates containing calcium carbonate are encountered sporadically in the Grabianka river valley. Two species fairly frequent in Poland, *Verrucaria dolosa* and *V. muralis,* were found on a concrete culvert.

Interesting species which occur primarily in the mountains were noted at a few or single localities: *Aspicilia laevata*, *Bacidina inundata*, *Collema flaccidum*, *Verrucaria hydrela* and *V. praetermissa*. *Collema flaccidum* is rare in Poland and has been reported from some scattered localities in the northern part of the Polish Lowland only (FAŁTYNOWICZ 1999). This lichen has been previously recorded in the Grabianka river valley, near the village of Kadyny (KOOPE 1939**,** FAŁTYNOWICZ 1999). Another two sites with its thalli were found in the analyzed part of the valley. The epilithic species *Bacidina inundata* was observed on an untypical substrate, namely a rubber tire lying on the river bed, where it formed large thalli with fruiting bodies.

The relatively small number of epigeic lichens (13 taxa) recorded in the study area is a consequence of low local frequency of their favorite habitats. In shaded deciduous forest communities, on fertile fresh or moist substrates, lichens have to compete for light and space with vascular plants and bryophytes, so their occurrence is practically limited to road embankments. Terricolous lichens recorded most frequently in the research area were

Baeomyces rufus, *Cladonia coniocraea* and *Trapeliopsis granulosa* (often with fruiting bodies). Of particular note is *Cladonia caespiticia* – a species known in Poland from a few localities only, more frequent in Western Pomerania (FAŁTYNOWICZ 1992), rare in North-Eastern Poland (CIEŚLIŃSKI 2003), previously reported from the Borecka Forest (ZALEWSKA 1998). In the Grabianka river valley it was found at two localities, on acid soil in a beech wood.

Conclusions

A total of 110 lichen species were identified in the upper part of the Grabianka river valley. This is a high number for such a small area (100 ha), which confirms a substantial diversity of habitats available to the examined group of organisms. Many of the recorded lichens are particularly interesting, rare, vanishing or poorly investigated species known for their unique habitat requirements.

A distinguishing feature of the study area is the occurrence of 12 protected species and a high contribution (46) of Red List taxa (CIEŚLIŃSKI et al. 2006), characterized by high sensitivity to human pressure and vanishing from numerous localities. Another advantage of the analyzed lichen biota is the presence of 13 species-indicators of lowland natural forests (CZYŻEWSKA, CIEŚLIŃSKI 2003), which testifies to the good condition of local forest communities.

It may be concluded that the investigated part of the Grabianka river valley can be considered a refuge for rare and vanishing in Poland. Comparable results were reported by other authors who studied lichen biota in the valleys of small rivers in the region of Pomerania, e.g. Reknica (RUTKOWSKI 1993), Drwęca (RUTKOWSKI, SŁOWIK 1999), Zagórska Struga (FAŁTYNOWICZ et al. 2000) and Radunia (FAŁTYNOWICZ, KRÓLAK 2001).

Acknowledgements

The authors are grateful to dr Martin Kukwa, dr Paweł Czarnota and dr Jurga Motiejunaite for revising of some indentificatins. We are also indebted to an anonymous reviewer for valuable suggestions on the manuscript.

Rafał Szymczyk received financial support for this project from the European Social Fund and the Budget within the framework of the Integrated Operational Program for Regional Development 2004-2006.

Translated by ALEKSANDRA POPRAWSKA

Accepted for print 5.11.2007

References

Atlas współzależności parametrów meteorologicznych i geograficznych w Polsce. IV. Klimat północno-wschodniej Polski. 1986. Red. M. Stopa-Boryczka. Wyd. UW, Warszawa, 1-509.

BARKMAN J.J. 1958. *Phytosociology and ecology of cryptogamic epiphytes*. – Van Gorcum & Comp., Assen, 1-628 (resissued 1969).

BATES J.W., BROWN D.H. 1981. *Epiphyte differentiation between Quercus petraea and Fraxinus excelsior trees in a maritime area of South West England*. Vegetatio, 48: 61-70.

BLANCO O., CRESPO A., DIVAKARx P.K., ESSLINGER T.L., HAWKSWORTH D.L., LUMBSCH H.T. 2004. *Melanelixia* and *Melanohalea*, *two new genera segregated from Melanelia* (*Parmeliaceae*) *based on molecular and morphological data*. – Mycol. Res., 108(8): 873-884.

CHLEBICKI A., ŻARNOWIEC J., CIEŚLIŃSKI S., KLAMA H., BUJAKIEWICZ A., ZAŁUSKI T. 1996. *Epixylites, lignicolous fungi and their links with different kinds of wood*. [In:] *Cryptogamous plants in the forest communties of Białowieża National Park. Functional groups analysis and general synthesis* (*Project CRYPTO* 3). *Phytocoenosis 8* (*N.S.*). Eds. J.B. Faliński, W. Mułenko. Archivum Geobotanicum, 6: 75-109.

CIEŚLIŃSKI S. 1985. *Transformations on the flora of the epiphytic and epixylic lichens in the territory of the Holy Cross National Park*. Roczn. Świętokrzyski, 12: 125-142.

CIEŚLIŃSKI S. 2003. *Distribution Atlas of the Lichens* (*Lichenes*) *of North-Eastern Poland*. Phytocoenosis 15 (N.S.), Supplementum Cartographiae. Geobotanicae, 15: 1-426.

CIEŚLIŃSKI S., CZYŻEWSKA K., FABISZEWSKI J. 2006. *Red list of the lichens in Poland*. [In:] *Red list of plants and fungi in Poland*. Eds. Z. Mirek, K. Zarzycki, W. Wojewoda, Z. Szeląg, W. Szafer. Institute of Botany of the Polish Academy of Sciences, Kraków, pp. 72-89.

CIEŚLIŃSKI S., FAŁTYNOWICZ W. 1993. *Note from editors*. [In:] *Atlas of the geographical distribution of lichens in Poland*. Part I. Eds. S. Cieśliński, W. Fałtynowicz. W. Szafer Institute of Botany of the Polish Academy of Sciences, Kraków, 7-9.

CZYŻEWSKA K., CIEŚLIŃSKI S. 2003. *Lichens – indicators of lowland old-growth forest in Poland*. Monogr. Bot., 91: 223-239.

DANIËLS F.J.A. 1983. *Lichen communities on stumps of Pinus sylvestris L. in the Netherlands*. Phytocoenologia, 11(3): 431-444.

FAŁTYNOWICZ W. 1992. *The lichens of Western Pomerania. An ecogoeographical study*. Polish Bot. Stud., 4: 1-183.

FAŁTYNOWICZ W. 1999. *Collema flaccidum* (Ach.) Ach. [In:] *Atlas of the geographical distribution of lichens in Poland*. Eds. S. Cieśliński, W. Fałtynowicz, 2: 13-17. W. Szafer Institute of Botany, Polish Academy of Science, Kraków.

FAŁTYNOWICZ W. 2003. *The lichens, lichenicolous and allied fungi of Poland. Annotated checklist*. W. Szafer Institute of Botany, Polish Academy of Science, Kraków: 1-435.

FAŁTYNOWICZ W., KRÓLAK D. 2001. *Lichens of the nature reserve 'Jar Rzeki Raduni' in the Kaszubskie Lake District* (*northern Poland*). – Acta Bot. Cassub., 2: 133-141.

FAŁTYNOWICZ W., SULMA T. 1994. *Distributional data to epiphytic taxa of the Pertusariaceae* (*lichenized Ascomycotina*) *in Poland*. – Fragm. Flor. Geobot. Polonica, 39(1): 291-296.

FAŁTYNOWICZ W., MARCINKOWSKA E., RUTKOWSKI P. 2000. *Lichens of the nature reserve 'Dolina Zagórskiej Strugi' near Rumia in Kaszubskie Lake District*. – Acta Bot. Cassub., 1: 119-126.

JAKUBOWICZ Z. 1983. *Porosty Wzniesienia Elbląskiego,* Zakład Taksonomii Roślin UAM, Poznań (praca magisterska).

KAUFF F., BÜDEL B. 2005. *Ascoma ontogeny and apothecial anatomy in the Gyalectaceae* (*Ostropales, Ascomycota*) *support the re-establishment of the Coenogoniaceae*. Bryologist, 108(2): 272-281.

KOLANKO K., MATWIEJUK A. 1999. *Epixylic lichens of the Knyszyńska Forest and its vicinity*. – Fragm. Flor. Geobot. Polonica, 6: 235-243.

KONIECKA H., KOPIEC J. 2002. *Monitoring wód Zalewu Wiślanego*. [In:] *Raport o stanie środowiska województwa warmińsko-mazurskiego w roku 2001*. Red. S. Rózański. Biblioteka Monitoringu Środowiska, Olsztyn, 100-120.

KOPPE F. 1939. *Ein kleiner Beitrag zur Flechtenflora von Danzig*. Bericht des Ber. Westpr. Bot.-Zool. Ver., 61: 65-66.

LAAKA S. 1995. *Epixylic lichens on conifer logs in four natural forests in Finland*. Graphis Scripta, 7: 25-31.

LETTAU G. 1919. *Nachträge zur Lichenenflora von Ost- und Westpreussen*. Schrift. Kgl. Phys.-ökon Ges. Königsberg, 60: 5-21.

MÜHLE H., BLANC F. le. 1975. *Bryophyte end lichen succession on decaying logs*. I. *Analyses along evaporational gradient in eastern Canada*. Journal of the Hattori Botanical Laboratory, 38: 1-33.

NITARDY E. 1922. *Neu kryptogamen des Elbinger Kreises*. Ber. Westpr. Bot.-Zool. Ver., 44: 6-7.

OHLERT A. 1870**.** *Zusamenstellung der Lichenen der Provinz Preussen*. Schrift. Kgl. Phys.-ökon Ges. Königsberg, 11: 1-51.

ORANGE A., JAMES P.W., WHITE F.J. 2001. *Microchemical methods of the identification of lichens*. British Lichen Society London, pp. 1-101.

PURVIS O.W., COPPINS B.J., HAWKSWORTH D.L., JAMES P.W., MOORE D.M. 1992. *The lichen flora of Great Britain and Ireland*. Natural History Museum i British Lichen Society, London, pp. 1-710.

Rozporządzenie Ministra Środowiska z dnia 9 lipca 2004 r. w sprawie gatunków dziko występujących grzybów objętych ochroną, Dz.U. nr 168, poz. 1765.

RUTKOWSKI P. 1993. *The lichens of the 'Jar Rzeki Reknicy' reserve in the Kaszuby lake district*. Parki Nar. Rez. Przyr., 12(4): 29-40.

RUTKOWSKI P., SŁOWIK J. 1999. *Lichen flora of the Drwęca valley near Nowe Miasto Lubawskie*. Fragm. Flor. Geobot. Polonica, 6: 211-217.

SANTESSON R., MOBERG R., NORDIN A., TØNBERG T., VITIKAINEN O. 2004. *Lichen-forming and lichenicolous fungi of Fennoscandia*. Museum of Evolution, Uppsala University.

SULMA T., FAŁTYNOWICZ W. 1988. *Materiały do rozmieszczenia porostów z rodziny Parmeliaceae w Polsce*. Acta Mycol., 23(1): 107-123.

SZYMCZYK R. 2007. *Rare and interesting lichen and lichenicolous fungi collected in Wysoczyzna Elbląska Hills* (*North Poland*). Fragm. Flor. Geobot. Polonica, 14(1): 167-173.

TOBOLEWSKI Z. 1983. *Lichens* (*Lichenes*). [In:] *Atlas of Geographical Distribution of Spore-Plants in Poland*. VIII. Eds. J. Szwejkowski, T. Wojterski. PWN, Warszawa-Poznań, ss. 1-25.

TOBOLEWSKI Z., KUPCZYK B. 1974. *Lichens (Lichenes).* [In:] *Atlas of Geographical Distribution of Spore-Plants in Poland*. II. Eds. J. Szwejkowski, T. Wojterski, PWN, Warszawa-Poznań, pp. 1-25.

TOKARZ H. 1961. *Zespoły leśne Wysoczyzny Elbląskiej*. Acta Biol. Med., 5(7): 120-245.

WOŚ A. 1999. *Klimat Polski*. PWN, Warszawa, 1-299.

ZALEWSKA A. 1998. *Remarks about some lichen species in Borecka Primeval Forest* (*North-East Poland*). Botanica Lithuanica, 4(2): 157-168.

POLISH JOURNAL OF NATURAL SCIENCES
Abbrev.: Pol. J. Natur. Sc., Vol 23(2): 415-427, Y. 2008

DOI 10.2478/v10020-008-0032-0

IMPACT OF SOLAR RADIATION, INCLUDING UV, ON THE ACTIVITY OF INTRA- AND EXTRACELLULAR BACTERIAL ENZYMES FROM THE SURFACE MICROLAYER

Maciej Walczak

Chair of Environmental Microbiology and Biotechnology
Nicolaus Copernicus University in Toruń

K e y w o r d s: solar radiation, UV radiation, activity of enzymes, surface microlayer.

A b s t r a c t

The present study examined the impact of solar radiation, including UVB, on the activity of intra- and extracellular bacterial enzymes from the surface microlayer (SM). Following results of the study, it was concluded that solar radiation has a significant impact on the activity of extracellular hydrolases in the SM water. In contrast, no clear changes in activity of these enzymes were observed in subsurface water (SW). The activity of intracellular enzymes (cellular dehydrogenases) of bacteria inhabiting the SM and SW underwent no significant changes in the presence of solar radiation. Furthermore, the study demonstrated also substantial changes in the numbers of bacteria occurring in the SM water depending on the intensity of solar radiation.

WPŁYW PROMIENIOWANIA SŁONECZNEGO I ZAWARTEGO W NIM UV NA AKTYWNOŚĆ ENZYMÓW WEWNĄTRZ- I ZEWNĄTRZKOMÓRKOWYCH BAKTERII MIKROWARSTWY POWIERZCHNIOWEJ

Maciej Walczak

Zakład Mikrobiologii Środowiskowej i Biotechnologii
Uniwersytet Mikołaja Kopernika w Toruniu

S ł o w a k l u c z o w e: promieniowanie słoneczne, promieniowanie UV, aktywność enzymów, mikrowarstwa powierzchniowa.

Address: Maciej Walczak, Nicolaus Copernicus University, ul. Gagarina 9, 87-100 Toruń, phone: (056) 611 44 33, e-mail: walczak@biol.uni.torun.pl

Abstrakt

W prezentowanej pracy badano wpływ promieniowania słonecznego wraz z UVB na aktywność wewnątrz- i zewnątrzkomórkowych enzymów bakterii mikrowarstwy powierzchniowej (MP). Stwierdzono, że promieniowanie słoneczne wpływa w sposób istotny na aktywność zewnątrzkomórkowych hydrolaz w wodzie MP. W wodzie podpowierzchniowej natomiast (WPP) nie obserwowano wyraźnych zmian aktywności tych enzymów. Z kolei aktywność enzymów wewnątrzkomórkowych (dehydrogenaz komórkowych) bakterii zasiedlających MP i WPP nie ulegała istotnym zmianom pod wpływem promieniowania słonecznego. W toku prowadzonych badań stwierdzono także bardzo wyraźne zmiany liczebności bakterii występujących w wodzie MP w zależności od natężenia promieniowania słonecznego.

Introduction

A water body, be it an inland reservoir or an ocean, does not constitute a uniform environment, and can be divided into a variety zones, both vertically and horizontally. Vertically, the outer layer consists of a surface microlayer. The surface microlayer covers l of the earth's surface and contains an infinitely small volume of the earth's total water mass. According to MACINTYRE et al. (1974), this layer is the most important millimeter in the ocean.

This layer constitutes a particular chemical and physical environment, which differs substantially from the subsurface water. The surface microlayer is formed by adhesion forces, which are a result of intermolecular attraction and surface tension on the interface of two media: air and water. This leads to the accumulation of organic and inorganic compounds within the layer. The surface microlayer often contains an elevated number of bacteria, called bacterioneuston. The bacterioneuston both consume and produce organic substances contributing to the development of the surface microlayer. Due to the fact that bacterioneuston inhabits the surface microlayer, its members are exposed to stressful ecological factors to a greater degree than organisms inhabiting the water column. Potential harmful factors, such as intense solar radiation, temperature, changes in salinity, and the presence of toxic substances or heavy metals, play an important role in the dynamics of growth and survival. All these factors are selective and affect the microbiological composition of this environment.

Light reaching a water surface may penetrate the water column down to a depth of several dozen meters. However, the highest light intensity occurs within the upper ca. dozen centimeters (LAMPERT, SOMER 2001). The quantity of absorbed and diffused solar radiation varies and depends on the concentration and type of organic matter present in the water. At high concentrations of dissolved organic matter, which contains a considerable fraction of humic substances, the harmful UVB radiation penetrates only the upper several

centimeters of the water (HESSEN et al. 1997). According to ZAITSEV (1971), the upper 10 cm of the water column absorbs ca. 75% of the UV radiation at λ = 254 nm. Considering the entire range of solar radiation reaching the air-water interface, medium wave UV radiation, i.e. UVB = 290-320 nm and UVA = 320-400 nm, is of the highest biological importance due to its harmful effects (COCKELL 2000). Radiation in this wavelength range causes DNA damage (lethal effect) or inhibits the growth of organisms by inhibiting enzyme synthesis, reducing active transport and inducing mutations, all of which are sublethal effects (COCKELL 2000). Therefore, it is not surprising that insolation is one of the primary factors affecting the number and activity of the bacterioneuston. It is known that intense solar radiation limits the numbers of all microorganisms in a water body, although the amount of harmful UV radiation reaching deeper water layers is significantly reduced as a result of absorption and diffusion.

Despite numerous studies reporting potentially unfavorable impacts of light on bacterioneuston, many empirical studies exist which fail to demonstrate differences in neuston activity with and without solar exposure (HERMANSSON, DAHLBÄCK 1983) and also report an insignificant impact of UV and visible light on total bacterioneuston activity (GARABETIAN 1991, WILIAMS et al. 1986). On the other hand, there are numerous studies that demonstrate that solar radiation, in particular UVB radiation, is detrimental to the production of bacterial biomass and exoenzyme activity (KAISER, HERNDL 1997, HERDL et al. 1993). It is also noteworthy that photooxidation of DOM and POM, which results in the release of considerable quantities of easily assimilable organic matter to the environment and may increase bacterioplankton activity (JORGENSE et al. 1998, HERNDL et al. 1997), occurs under the influence of UV.

This study examined the impact of solar radiation on the activity of cellular dehydrogenases and extracellular hydrolytic enzymes. The activity of cellular dehydrogenases participating in electron transport within the respiratory chain constitutes a measure of the metabolic activity of a cell. Therefore, by measuring dehydrogenase activity it is possible to obtain an unequivocal answer as to whether UV inhibits metabolic activity of bacterioneuston or whether low molecular weight matter released by UV contributes to an increase in metabolic activity of bacterial cells. Through analysis of hydrolase activity, it is possible to determine whether and to what degree UV radiation affects the enzymatic distribution of organic matter contained in a water body.

Materials and Methods

Object of the study

The surveys were carried out in the Jeziorak Mały lake. This lake is located within the city of Iława and is part of the Pojezierze Iławskie lake district. The surface area of the lake equals 26 ha, with a maximal depth of 6.4 m. The lake has no inlets or outlets, but is connected to Jeziorak lake by a narrow and shallow (1.5 m) strait in its northern section.

Sample collection

Water samples used for analyses were collected in spring (May), summer (July) and autumn (October) of 2005. Surface microlayer water was collected by a Garrett technique (1965) using: 1) a Plexiglass plate, which collects a 150 μm layer of the water, and 2) a Garrett mesh with a pore diameter of 200 μm, which collects a 200 μm layer of the water.

Subsurface water was collected from a depth of 20 cm using a sterile, glass pipette, using an automatic pump Pippet-boy (De Ville Biotechnology). Water was collected every 3 hours over a 24-hour period.

Samples were poured into sterile, glass containers, from which 10 cm^3 subsamples were obtained for analysis.

Microbiological analyzes

The samples were analyzed for total number of bacteria (TNB), number of metabolically active bacteria – TNAB (with an active electron transport system), activity of cellular dehydrogenases, and extracellular hydrolases. In all of the above analyses, the sample incubation was carried out for 2 hours in lacustrine water (*in situ*) with appropriate reagents. Visible light and UVB intensities were measured simultaneously with collection of samples for microbiological analyses (sensors, Slandi and Solar Light Co.).

Total number of bacteria (TNB) was determined by a direct enumeration method on membrane filters (Millipore) with a pore diameter of 0.22 μm. Samples were stained with acridine orange (Zimmermann 1977), and visualized on an epifluorescent microscope (Carl Zeiss Jena).

Number of metabolically active bacteria (TNAB) was determined following the ZIMMERMANN et al. method (1978).

Analysis of cellular dehydrogenase activity

The applied method utilizes measurements of the quantity of triphenyl formazan (TF) produced from a colorless INT substrate. The activity of dehydrogenases manifests itself in the process of substrate dehydrogenation. The color intensity is proportional to the activity of cellular dehydrogenases.

In order to conduct the test, 10 ml water samples containing 1 ml of 0.2% solution of INT (*2-p-iodophenyl-3-p-nitrophenyl-5-phenyl tetrazolium chloride)* were incubated for 2 hours in situ and then preserved with 1 cm^3 of 40% formalin.

In the laboratory, 5 cm^3 subsamples then were transferred to test tubes with fitted stoppers, and 10 ml of *n-butyl* alcohol was added to each subsample, and the solution was properly mixed. Samples were placed in a 90°C water bath for 5 min. 5 cm^3 samples of butyl extract then were centrifuged for 5 min at 6000 rpm. The supernatant was removed and spectrophotometric measurements of free tri-formazan were made in relation to a standard curve at a wavelength of 490 nm. Obtained results were standardized to dehydrogenase activity of a single bacterial cell.

Activity of hydrolytic enzymes (HA)

In order to determine HA, 10 ml water samples were transferred to small vials, and 1 cm^3 of fluorescein diacetate (FDA) solution in acetone (1000 $\mu g\ cm^{-3}$) was added. After the incubation period, the reaction was interrupted and the samples were preserved in 1 cm^3 of 40% formalin. FDA is a nonspecific substrate for all hydrolytic enzymes. Acetate and fluorescein are produced through decomposition of colorless FDA. The concentration of fluorescein was measured on a fluorometer HITACH F-2500 in relation to a standard curve. The obtained value of total hydrolytic enzyme activity in each water sample was adjusted to a single metabolically active cell.

Results

Results for TNB, TNAB are presented in Table 1, and solar radiation in Table 2. Average values of TNB and TNAB in a specific month are always greater in the SM than in the SW (average 1). However, it is clear that the differences between SM and SW are greater in May and July, during months with intense solar radiation (TNB May: SM – 45.53, SW – $25.55 \cdot 10^6$ cells cm^{-3}; July: SM – 25.59, SW – $15.42 \cdot 10^6$ cells cm^{-3}). This pattern is even clearer

if average values for darkness (absence of radiation) are compared (average 3). In the absence of radiation, average values of both TNB and TNAB in May and July are several times greater in the SM than in the SW. In contrast, in October when the solar radiation is much less intense and "aggressive", mean values (average 3) of TNB and TNAB in the SM and SW water are very similar (TNB in SM – 4.82, in SW – 3.72 · 10^6 cells cm^{-3}; TNAB in SM – 1.07, in SW 0.89 · 10^6 cells cm^{-3}).

Table 1

Total number of bacteria (TNB) and number of metabolically active bacteria (TNAB) in the water samples

Sampling time	May		July		October	
	SM	SW	SM	SW	SM	SW
6:00 a.m.	22.94* 4.24**	25.73 4.69	9.97 5.61	25.43 24.56	**6.32** **1.11**	**2.83** **0.28**
9:00 a.m.	22.52 6.93	24.43 9.97	13.22 4.86	12.97 2.87	8.23 0.42	9.31 0.55
12:00 a.m.	23.44 8.98	29.02 8.88	17.52 10.10	3.49 0.87	6.88 0.28	8.64 0.83
3:00 p.m.	26.43 6.08	42.98 19.65	17.33 8.11	4.74 2.87	5.91 3.46	6.98 1.39
6:00 p.m.	**54.93** **15.52**	**25.93** **15.79**	**nd** **nd**	**nd** **nd**	**5.24** **1.11**	**3.16** **0.28**
9:00 p.m.	**45.23** **12.54**	**22.93** **14.96**	**45.00** **15.84**	**33.66** **17.83**	**4.99** **1.53**	**3.32** **1.66**
12:00 p.m.	**77.24** **22.27**	**20.44** **3.80**	**20.20** **13.28**	**23.69** **12.84**	**3.16** **0.97**	**3.82** **0.28**
3:00 a.m.	**91.56** **16.61**	**12.97** **3.24**	**55.86** **36.78**	**3.99** **0.87**	**4.41** **0.63**	**5.49** **1.94**
Average 1	45.53 11.64	25.55 10.12	25.59 13.51	15.42 8.96	5.64 1.19	5.44 0.90
Average 2	30.06 8.35	29.62 11.79	14.51 7.17	11.65 7.79	7.01 1.39	8.31 0.92
Average 3	**71.34** **17.14**	**18.78** **7.33**	**40.35** **21.96**	**20.45** **10.52**	**4.82** **1.07**	**3.72** **0.89**

SM – surface microlayer; SW subsurface water; * – TNB · 10^6 cm^{-3}; ** – TNAB · 10^6 cm^{-3}; **00** – bold type; Average 1 – general average for the entire sample series, Average 2 – average for the period of solar radiation; Average 3 – average for the period in the absence of solar radiation; nd – no data

When comparing average values of TNB and TNAB in specific months during the period of intense solar radiation (average 2), no significant differences were observed in bacterial abundance within the SM and SW. However, in May and July, average TNAB is higher in the SW than in the SM.

Table 2

The intense of solar light and UVB radiation in surface microlayer and subsurface water

Sampling time	May				July				October			
	light (klx)		UVB ($\mu W\ cm^{-2}$)		light (klx)		UVB ($\mu W\ cm^{-2}$)		light (klx)		UVB ($\mu W\ cm^{-2}$)	
	SM	SW	SM	SW	SM	SW	SM	SW	SM	SW	SM	SW
6:00 a.m.	40.0	9.0	5.88	0.29	14.0	6.0	1.50	0.33	4.0	1.0	0.34	0.07
9:00 a.m.	82.0	46.0	11.92	1.48	30.0	22.0	3.37	1.22	14.0	5.0	1.19	0.38
12:00 a.m.	125.0	60.0	18.11	1.93	40.0	28.0	6.00	1.55	20.0	8.0	1.71	0.6
13:00 p.m.	76.0	6.0	11.0	0.19	38.0	26.0	4.56	1.32	22.0	10.0	1.88	0.76
6:00 p.m.	10.0	8.0	1.42	0.25	15.0	8.0	1.68	0.44	0.1	0.0	0.00	0.00
9:00 p.m.	0.04	0.0	0.00	0.00	0.8	0.1	0.09	0.00	0.0	0.0	0.00	0.00
12:00 p.m.	0.0	0.0	0.00	0.00	0.0	0.0	0.00	0.00	0.0	0.0	0.00	0.00
3:00 a.m.	0.32	0.01	0.05	0.00	0.0	0.0	0.00	0.00	0.0	0.0	0.00	0.00

The above results demonstrating the impact of changes in solar radiation intensity on TNB and TNAB in the SM and SW indicate that radiation is an important factor reducing the number of bacteria in the SM.

Figure 1 and Figure 2 present results of the analysis of the impact of solar radiation intensity on bacterial cellular dehydrogenase activity in the SM and SW. Results demonstrated no significant effect of solar intensity on the activity of these enzymes. The activity of bacterial cellular dehydrogenases in the SM did not differ statistically in the presence or absence of solar radiation in any examined season. In the presence of solar radiation and in the SM water, the average activity of these enzymes equaled: May – 0.0309, July – 0.00645, October – $0.127 \cdot 10^{-6}$ μmol TF/cell. In the absence of solar radiation, the activity equaled: 0.111, 0.0043 and $0.2287 \cdot 10^{-6}$ μmol TF/cell, respectively. In contrast, the activity of these bacterial enzymes in the SW was more variable. A much higher activity of dehydrogenases in the presence of solar radiation than in its absence was observed in October. Due to the fact that similar, but statistically insignificant, trends were observed in the SM, it could be concluded that dehydrogenase activity was affected by slight, but important increases in water temperature during the day.

Figure 3 and Figure 4 present results of research on the activity of extracellular hydrolytic enzymes in the SM and SW. Based on the presented data, the activity of extracellular hydrolases in the SM was found to be greatly affected by changes in solar radiation. Activity of these enzymes was significantly greater in darkness during the months characterized by intense solar radiation (May and July). In contrast, in October, when the solar radiation is

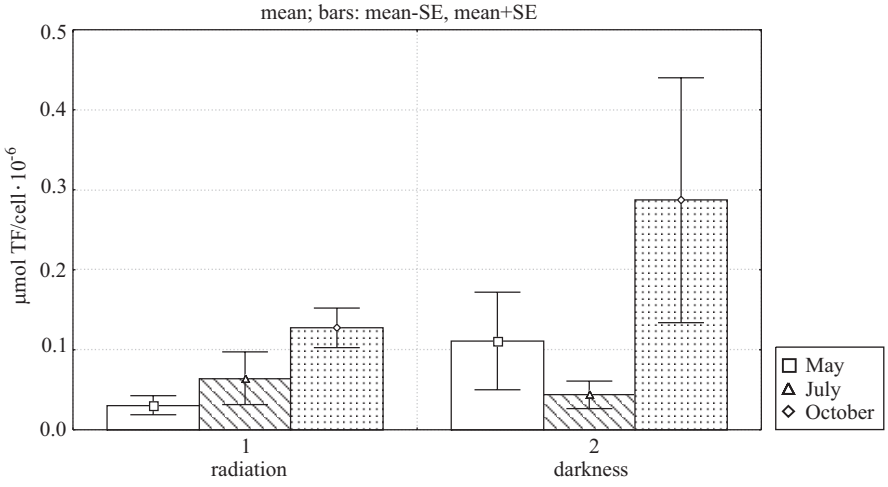


Fig. 1. Impact of solar radiation intensity on bacterial cellular dehydrogenase activity in the SM

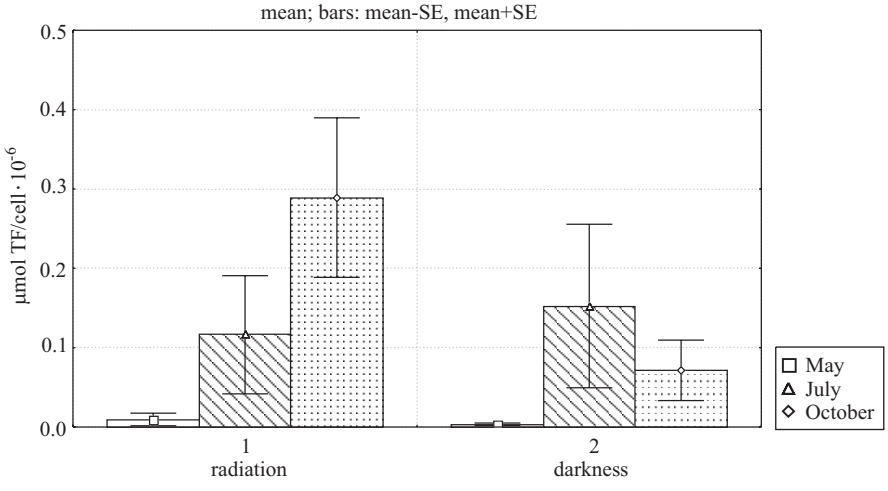


Fig. 2. Impact of solar radiation intensity on bacterial cellular dehydrogenase activity in the SW

weaker, the activity of extracellular hydrolases was almost identical in the presence and absence of radiation. Additionally, in October the average activity of these enzymes per metabolically active bacterial cell was the highest observed in the entire research period. In May and July, no differences in hydrolase activity were observed in samples collected in the presence and absence of solar radiation in the SW. In October samples, a higher activity of these enzymes in the SW was observed in the presence of radiation.

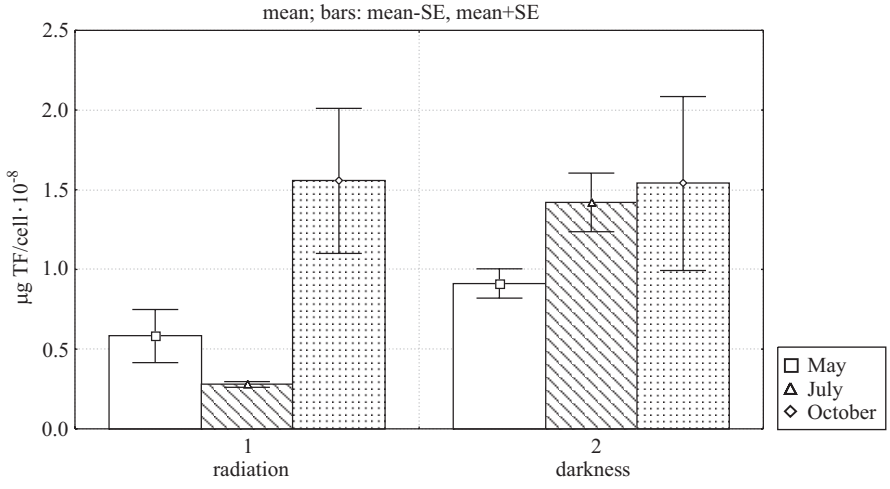


Fig. 3. Impact of solar radiation intensity on bacterial extracellular hydrolytic enzymes activity in the SM

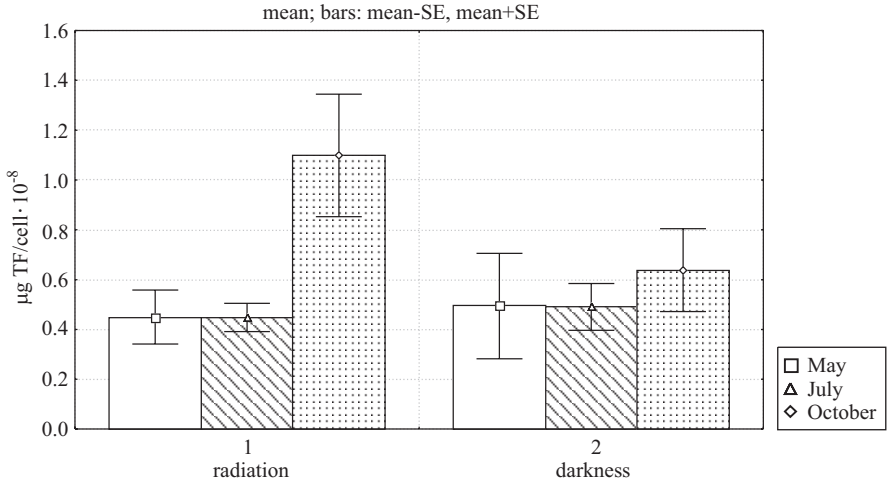


Fig. 4. Impact of solar radiation intensity on bacterial extracellular hydrolytic enzymes activity in the SW

Discussion

Solar radiation reaching a water body is one of the factors controlling the development of life in this environment. Solar radiation increases water temperature, changes gas solubility, and enables photosynthesis to occur.

Solar radiation also contains harmful UV radiation, which typically has a negative impact on organisms. On the other hand, UV radiation has a decisive impact on the photolytic distribution of organic matter, which considerably increases the quantity of easily accessible matter available to heterotrophic organisms. The process of photolytic degradation of organic matter is the second most important (after enzymatic catalysis) mechanism for decomposition of organic matter. Frequently, UV radiation initiates the decomposition of matter that is extremely resistant to enzymatic decomposition.

The highest levels of solar radiation, including UV, that reach a water body are concentrated in surface layers. The most insolated water layer in aquatic systems is certainly the surface microlayer. The intensity of solar radiation reaching this layer is identical to the quantity of radiation reaching terrestrial surfaces. The amount of radiation penetrating the surface microlayer affects organisms living within that water layer as well as decomposition and matter circulation processes.

This observation was confirmed by the results of this study, which examined the impact of solar radiation on bacteria inhabiting the surface microlayer. The conducted research demonstrated that radiation affects both the total number of bacteria and the number of bacteria with an active electron transport system in the SM water (Table 1). The abundances of bacteria (TNB and TNAB) decreased as a result of exposure to solar radiation. CHRÓST, FAUST (1999) obtained similar results. In the present study, increased numbers of bacteria were observed in morning hours, that is, following the night; whereas, in afternoon hours, after a full day of exposure to solar radiation, the number of bacteria in water samples decreased. These fluctuations were very distinct and significant, yet remain difficult to explain given the impossiblility of unambiguously determining if reduced numbers of bacteria in the SM water in the presence of intense solar radiation were an effect of bacterial migration into deeper water layers or due to decreased cell division rates. The information provided in the literature is also inconsistent. Some researchers have suggested that solar radiation, including UV, has no significant impact on the number of bacteria in the SM or that such an impact is very limited (DALBÄCK 1983, DENWARD et al. 1999, SKÓRCZEWSKI, MUDRYK 2003). Numerous researchers (KAISER, HERNDL 1997, HERNDL et al. 1997, DAVIDSON 1998, CHRÓST, FAUST 1999) obtained similar results, signifying that DNA synthesis in bacterial cells was inhibited. CHRÓST, FAUST (1999) obtained similar results. According to the cited studies, solar radiation with UV hindered incorporation of 3H – thymidyne from 15 to 50% in comparison to the control (no radiation).

The present study includes results of research on changes in the activity of cellular dehydrogenases in the presence of solar radiation. The analysis demonstrated that solar radiation had no significant impact on cellular

dehydrogenase activity of bacteria from both the SM and SW (Figure 1 and Figure 2). Unfortunately, no prior results of studies analyzing the effect of solar radiation on activity of these enzymes have been found in the available literature, which prevents broader comparison of the presented results. The observed lack of impact of solar radiation on cellular dehydrogenases can be explained by the fact that these are intracellular enzymes. Therefore, they are protected by cellular membranes or carotenoid pigments inside the cell. Furthermore, it is difficult to unambiguously confirm that UV did not limit the activity of these enzymes. The measured activity of cellular dehydrogenases can be the result of two types of UV effects. Firstly, UV may directly inhibit the activity of dehydrogenases. Secondly, photooxidation of organic matter in water occurs under the influence of UV radiation (HÄDER et al. 1998). This phenomenon considerably increases the quantity of nutritional substances easily accessible to bacteria, contributing to an increase in the activity of dehydrogenases participating in the oxidation of organic compounds. Thus, it seems that the observed lack of differences in the activity of these enzymes under the influence of solar radiation constitutes a combined result of both direct and indirect effects of UV radiation.

In contrast to the activity of cellular dehydrogenases, a strong relationship was observed between the activity of extracellular hydrolases in the SM and solar radiation. A significant impact of solar radiation on hydrolase activity occurred in the SM water samples in all three studied seasons (spring, summer, and autumn). Enzyme activity was lower in the presence of solar radiation, especially in May and July. BOAVIDA, WETZEL (1998) report similar results indicating a negative impact of solar radiation on hydrolytic enzyme activity. These authors observed that phosphatase activity was considerably reduced in the presence of solar radiation, particularly in water devoid of humic substances, which provides protection against UV (HÄDER et al. 1998).

The activity of β-glucosidase and urease also was inhibited by several dozen percent in the presence of strong solar radiation (JORGENSEN et al. 1998). All of these enzymes (urease, phosphatase, and glucosidase) are categorized as extracellular hydrolases. Therefore, they are secreted to the external environment, where there is no protection against the harmful effect of UV. Furthermore, it was also observed that extracellular enzymes unbound to organic matter are inhibited to a much higher degree (50-60%). In contrast, inhibition of enzymes bound with organic matter equaled only 30% (HERNDL et al. 1993). This result appears to confirm the fact that enzymes unbound to organic matter undergo complete degradation under the influence of UV. But, according to SCULLY et al. (2003), reactive forms of oxygen also play an important role in the decomposition of organic matter under the influence of UV. These compounds are produced in water in the presence of UV and organic matter

and may indirectly inhibit the activity of extracellular enzymes. Furthermore, inhibition of enzyme activity in the SM water may also be a result of high temperature. This inference applies only to summer months (May–August). During this period, in the presence of strong insolation, the water surface (0.5 cm) frequently reached a temperature of over 30°C, which may have an inhibiting effect on numerous enzymes (WALCZAK, DONDERSKI 2003)

In sum, the conducted research confirmed and expanded earlier reports regarding the importance of solar radiation and UV on the activity of bacterial enzymes in aquatic environments. However, it should be noted that the effect of solar radiation, including UV, is not limited to simple and direct impacts on bacterial cells. Radiation affects the environment through a wide range of indirect means, e.g. organic matter photooxidation or the impact on phyto- and zooplankton, which also has a considerable impact on the activity of aquatic bacteria.

Conclusions

The study examined the impact of solar radiation, including UV, on the activity of bacterial enzymes in the SM. The conducted research demonstrated that solar radiation has an essential impact on extracellular enzyme activity in this water layer. Activity of hydrolytic enzymes in the SM water was always lower in the presence of solar radiation. No clear impact of solar radiation on the activity of cellular dehydrogenases was observed. However, changes in the activity of these enzymes may occur, but are difficult to capture due to the occurrence of many different types of effects related to solar radiation.

Translated by GRZEGORZ DZIADURSKI

Accepted for print 22.11.2007

References

BOAVIDA M.J., WETZEL R.G. 1998. *Inhibition of phosphatase activity by dissolved humic substances and hydrolytic reactivation by natural ultraviolet light*. Freshwater Biology, 40: 285-293.

CHRÓST R.J., FAUST M.A. 1999. *Consequences of solar radiation on bacterial secondary production and growth rates in subtropical coastal water (Atlantic Coral Reef off Belize, Central America)*. Aquatic Microbiol. Ecol., 20**:** 39-48.

COCKELL C.S. 2000. *Ultraviolet radiation and the photobiology of earth's early oceans*. Origins of Life and Evol. of the Biosph., 30**:** 467-499.

DAHLBÄCK B. 1983. *Microbial accumulation of interfaces. Marine micro – organisms and interfaces*. Univ. of Göteborg, 3: 12-15.

DAVIDSON A.T. 1998. *The impact of UVB radiation on marine plankton*. Mutation Research., 422: 119-129

DENWART C.M.T., EDLING H., TRANVIK L.J. 1999. *Effects of solar radiation on bacterial and fungal density on aquatic plant detritus*. Freshwater Biology, 41: 575-582.

GARABETIAN F. 1991. *14*C-glucose ptake and 14C-CO2 production in surface microlayer and surface--water samples: influence of UV and visible radiation. Mar. Ecol. Prog. Ser., 77: 21-26.

GARRETT W.P. 1965. *Collection of slick-forming materials from the sea surface*. Limnol. Oceanogr. 10: 602-605

HÄDER D.P., KUMAR H.D., SMITH R.C., WORREST R.C. 1998. *Effects on aquatic ecosystems*. J. Photochem. Photobiol., 46: 53-68.

HESSEN D.O., GJESSING E.T., KNULST J., FJELD E. 1997. *TOC fluctuations in humic lake as related to catchment acidification, season and climate*. Biogeochemistry, 36: 139-151.

HERMANSSON M., DAHLBÄCK B. 1983. *Bacterial activity at the air/water interface*. Microbiol. Ecol., 9: 317-328.

HERNDL G.J., BRUGGER A., HAGER S., KAISER E., OBERNOSTERER I., REITNER B., SLEZAK D. 1997. *Role of utraviolet-B radiation on bacterioplankton and availability of dissolved organic matter*. Vegetatio, 128: 43-51.

HERNDL G.J., MULLER-NIKLAS G., FRICK J. 1993. *Major role of ultraviolet-B in controlling bacterioplankton growth in the surface layer of the ocean*. Nature, 361: 717-719.

JORGENSEN N.O.G., TRANVIK L., EDLING H., GRANELI W., LIDELL M. 1998. *Effects of sunlight on occurrence and bacterial turnover of specific carbon and nitrogen compounds in lake water*. FEMS Microbiology Ecol., 25: 217-227.

KAISER E., HERNDL G.J. 1997. *Rapid recovery of marine bacterioplankton activity after inhibition by UV radiation in coastal waters*. Appl. Environ. Microbiol., 63: 4026-4031.

LAMPERT W., SOMMER U. 2001. *Ekologia wód śródlądowych*. PWN, Warszawa.

MACINTYRE W.G., SMITH C.L., MUNDAY J.C., GIBSON V.M., LAKE J.L., WINDSOR J.G., DUPUY J.L., HARRISON W., OBSERHOLZER J.D. 1974. *Investigation of surface films – Chesapeake Bay entrance*. US Environ. Prot. Agency Ser. EPA-670/2-73-099

SCULLY N.M., COOPER W.J., TRANVIK L.J. 2003. *Photochemical effects on microbial activity in natural waters: the interaction of reactive oxygen species and dissolved organic matter*. FEMS, Microbiol. Ecol., 43: 353-357.

SKÓRCZEWSKI P., MUDRYK Z. 2003. *Dynamics of daily changes to the number and production of estuarine bacterioneuston and bacterioplankton*. AUMC, Limnological Papers, XXIII: 55-63.

WALCZAK M., DONDERSKI W. 2003. *Changes in the number of neustonic bacteria over 24 hours*. AUNC, Limnological Papers, XXIII, 65-79.

WILLIAMS P.M., CARLUCCI A.F., HENRICHS S.M., VLEET E.S. VAN, HORRIGAN S.G., REID F.M.H., ROBERTSON K.J. 1986. *Chemical and microbiological studies of sea-surface films in the southern Gulf of California and off the west coast of Baja California*. Mar. Chem., 19: 17-98.

ZAITSEV Y.P. 1971. *Marine Neustonology*. Israel Prog. Scientific Tranl. Jerusalem

ZIMMERMANN R. 1977. *Estimation of bacterial number and biomass by epifluorescence microscopy and scaninig electron microscopy.* [In:] *Microbial ecology of a brackish water environment*. Ed. G. Rheinheimer. Springer-Verlag, New York, p. 103-110.

ZIMMERMANN R., ITURRIAGA R., BECKER-BIRCK J. 1978. *Simultaneous determination of the total number of aquatic bacteria and the number thereof involved in respiration*. Appl. Environ. Microbiol., 12: 926-935.

POLISH JOURNAL OF NATURAL SCIENCES
Abbrev.: Pol. J. Natur. Sc., Vol 23(2): 428-439, Y. 2008

DOI 10.2478/v10020-008-0033-z

PSYCHROPHILIC AND PSYCHROTROPHIC BACTERIA IN THE WATER OF LAKE HAŃCZA

Anna Gotkowska-Płachta

Department of Environmental Microbiology
University of Warmia and Mazury in Olsztyn

K e y w o r d s: psychrophilic bacteria, psychrotrophic bacteria, Lake Hańcza.

A b s t r a c t

The study presents the results of quantitative and qualitative analyses of heterotrophic bacteria (psychrophilic and psychrotrophic) in the water of Lake Hańcza – the deepest lake in Poland. Research was carried out from May to October 2000, in monthly intervals, at a research site located at the deepest point of the lake (108.5 m). Bacteria were incubated at 4, 10 and 25°C. Statistically significant differences were demonstrated between mean numbers of the bacteria examined depending on the temperature of incubation and period of water sample collection. The lowest numbers of the bacteria examined were recorded at an incubation temp. of 4°C, whereas the highest were at an incubation temp. of 25°C. In particular experimental months, on average, a higher count of aerobic psychrotrophic bacteria was observed in August, whereas that of psychrophilic bacteria was found in October. Amongst the identified microorganisms, *Flavobacterium, Alcaligenes* and *Micrococcus* genera were found to predominate, whereas the predominants of the family *Enterobacteriaceae* appeared to be *Serratia marcescens*, *S. rubidaea*, and *Enterobacter aerogenes*.

BAKTERIE PSYCHROFILNE I PSYCHROTROFOWE W WODZIE JEZIORA HAŃCZA

Anna Gotkowska-Płachta
Katedra Mikrobiologii Środowiskowej
Uniwersytet Warmińsko-Mazurski w Olsztynie

K e y w o r d s: bakterie psychrofilne, bakterie psychrotrofowe, jezioro Hańcza.

A b s t r a k t

W pracy przedstawiono wyniki badań ilościowych i jakościowych bakterii heterotroficznych (psychrofilnych i psychrotrofowych) w wodzie najgłębszego w Polsce jeziora Hańcza. Badania prowadzono w 2000 r., od maja do października, w odstępach jednomiesięcznych na stanowisku

Address: Anna Gotkowska-Płachta, University of Warmia and Mazury, ul. Romana Prawocheńskiego 1, 10-957 Olsztyn-Kortowo, Poland, phone: (089) 523 37 52 aniagp@uwm.edu.pl

usytuowanym w najgłębszym (108,5 m) miejscu jeziora. Bakterie inkubowano w trzech temperaturach – 4, 10 i 25°C. Stwierdzono statystycznie istotne różnice między średnimi liczebnościami badanych drobnoustrojów w zależności od temperatury inkubacji i daty poboru próbek wody. Najmniejszą liczbę bakterii odnotowano w temperaturze inkubacji 4°C, a najwyższą w 25°C. Więcej tlenowych bakterii psychrotrofowych stwierdzono w sierpniu, a bakterii psychrofilnych – w październiku. Wśród zidentyfikowanych drobnoustrojów dominowały bakterie z rodzaju *Flavobacterium, Alcaligenes* oraz *Micrococcus*. W obrębie rodziny *Enterobacteriaceae* najliczniej występowały: *Serratia marcescens, S. rubidaea* i *Enterobacter aerogenes*.

Introduction

Morphological and physiological properties of psychrophilic and psychrotrophic bacteria have been the subject of a severale studies (KAMIŃSKI, FERRONI 1980a,b, MORITA 1975, RUSSELL 1997, 2000, SCHERER, NEUHAUS 2002, ŚWIĘCICKA et al. 1997). The authores data refer chiefly to their occurrence, biochemical activity and functions in sea waters, arctic environment, bottom deposits, and food products. In contrast, there are scarce data on this type of microflora in pure and deep freshwater lakes where the main life-inhibiting factors are the low levels of nutrients and oxygen. "Cryophilic" bacteria adapted to life at low temperatures are usually heterotrophs rather than autotrophs (DELILLE, PERRET 1989). According to (CHO, AZAM 1990) and (COLE et al. 1988), heterotrophs are capable of assimilating soluble organic matter and adding it into own biomass, thus becoming an important source of food for organisms at higher levels of the food chain. In addition, these microorganisms synthesize various ectoenzymes that efficiently hydrolyze numerous substrates at low temperatures, thus playing a key role in the process of matter and energy circulation, synthesis of vitamins and other growth factors, consequently limiting the level of life in habitats with low trophy (FELLER 2003, HERBERT 1986, MARGESIN, SCHINNER 1993 1994, RUSELL 2000, DONDERSKI, BURKOWSKA 2001). In the reported study, an attempt was made to determine the quantitative and qualitative composition of psychrophilic and psychrotrophic bacteria in the water of Lake Hańcza – the deepest (108.5 m) and one of the cleanest lakes in Poland.

Material and Methods

Study area. Lake Hańcza (Figure 1) is located in the highest, north--eastern part of the East-Suwalski Lake District (CHOIŃSKI (1995). It is the deepest (108.5 m of depth) gutter reservoir in Poland and in the central part of European Depression (Cydzik et al. 1982). Waters of the Czarna Hańcza

River, as well as those of several small activate streams flow into the lake, especially during periods of heavy rainfalls. Due to its extraordinary natural, geographical, geological, and limnological value, the lake has been under reservation protection since 1963, and since 1976 it has been incorporated into the Suwalski Landscape Park. Detailed data on the lake are provided in Table 1 as well manuscripts by GOTKOWSKA-PŁACHTA et al. (2003, 2005).

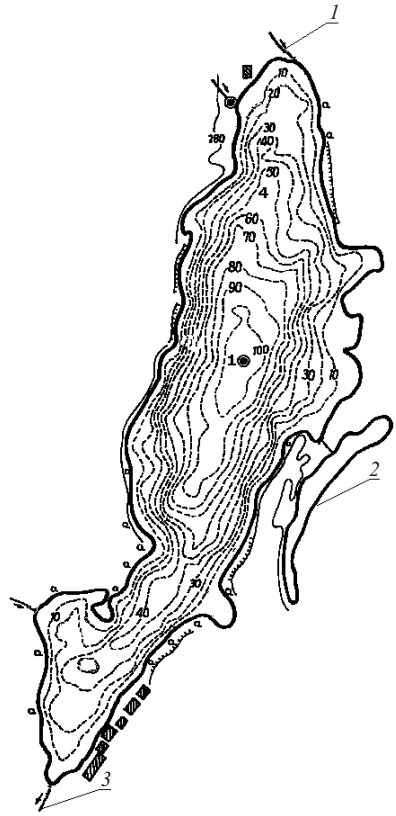


Fig. 1. Location sketch of the Hańcza Lake (1 – water sampling site): *1* – Czarna Hancza River (inflow), *2* – Boczniel Lake, *3* – Czarna Hancza River (outflow)

Sample collection. Water samples were collected in 1-month intervals from May to October 2000. The research site selected for analyses was located at the deepest point (108.5) of Lake Hańcza. Samples of water from the surface layer of the lake (0.3 m) were collected directly into sterile glass vessels with a volume of 300 cm^3, whereas those from a depth of 1, 2, 5, 10, 30, 50, 70, 90 and 108 m were collected with a Ruttner apparatus to similar glass vessels.

Table 1
Some morphometric data on Lake Hańcza, according to the morphometric card, by the Institute of Inland Fisheries, Ruhee-Stangenberg (1934) EŁ-5/54-71/66

Parameter	Value
Altitude a.s.l.	229.0
Latitude	54° 16'
Longitute	22° 49'
Basin	Czarna Hańcza, Niemen, Bałtyk
Water surface area, ha	311.4
Maximum depth, m	108.5
Mean depth, m	38.7
Volume, 10^3 m^3	120364.1
Maximum length, m	4525
Maximum width, m	1175
Effective length, m	4050
Effective width, m	1175
Total coastline, m	11750
Total basin surface, km^2	39.7

Next, all samples were immediately placed in thermo bags with inserts, enabling them to be cooled down 4-6°C, transported to the laboratory and subjected to microbiological analyses.

Microbiological analyses involved determinations of the number and qualitative composition of heterotrophic bacteria on nutritive agar with tryptone, glucose and yeast extract (TGY) at 1:8 dilution and at temperatures of 4, 10 and 25°C. Assays were carried out in three parallel replications for each sample of water. To determine the qualitative composition of heterotrophic bacteria grown on the TGY culture medium (1:8) at incubation temperatures of 4, 10 and 25°C, 30-40 colonies differing in terms of morphology were deinoculated at random from each culture into respective agar slants with the same medium and incubated under the same temperature conditions. The pure cultures obtained were identified by means of a BERGEY'S key (1994). Systematic classification of the strains was additionally determined based on API 20 E and API 20 NE tests (bioMerieux).

Statistical analysis. In order to obtain information concerning potential differences between bacteria numbers at various temperature of incubation and in various periods of study, use was made of a one-way analysis of variance (ANOVA), whereas the homogeneity of variance was determined with the Levene test. Once the test was significant, the KRUSKAL-WALLIS test was also used (STANISZ 1998). Results of microbiological analyses were also processed statistically with Spearman's correlation rank method, in which changes in the numbers of heterotrophic bacteria were compared in respect of different temperatures of their incubation. In this paper, only general statistical correla-

tions were provided and presented graphically below; detailed results of analyses are available at the author's.

Results and Discussion

In the water of Lake Hańcza (the central, deepest – 108.5 m – pelagic zone) statistically significant differences were demonstrated between mean numbers of bacteria examined depending on the temperature of incubation and period of water sample collection (Figure 2). The lowest counts of heterotrophic bacteria

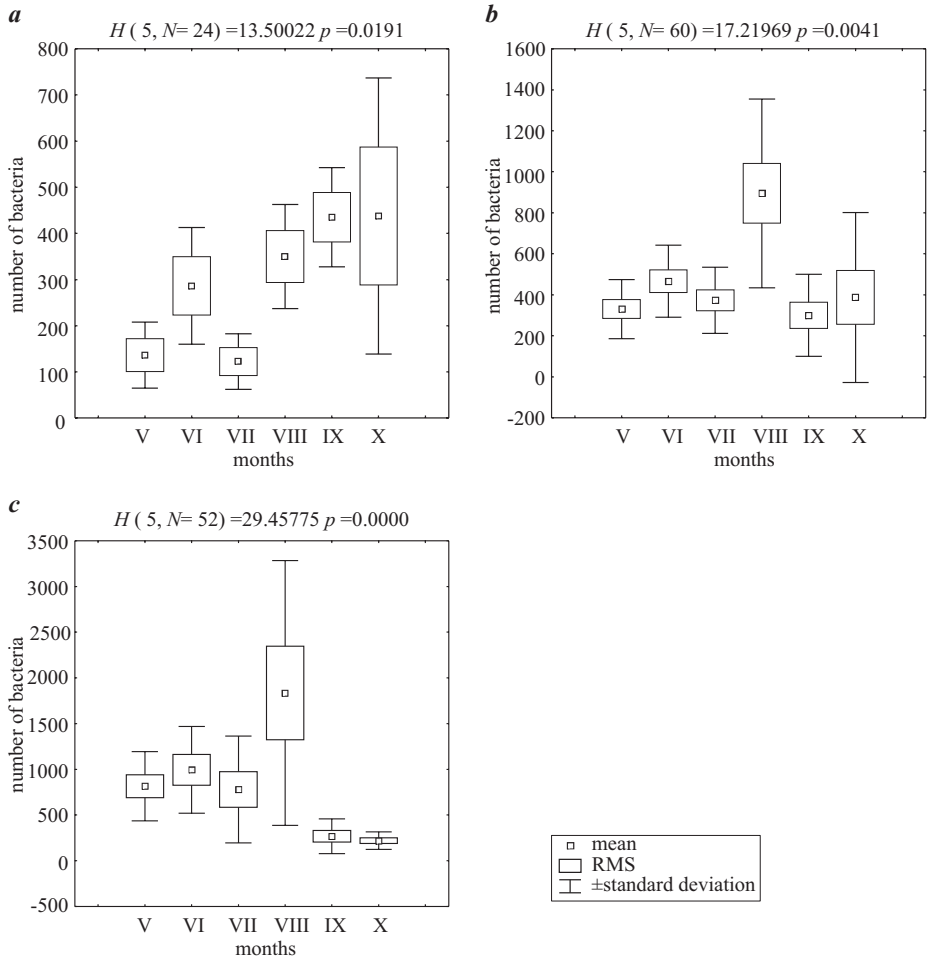


Fig. 2. Averages of numbers (± standard deviation and ± random mean square-RMS) heterotrophic bacteria (cfu cm^{-3}) at: *a* – 4°C, *b* – 10°C and *c* – 25°C in the water of Hańcza Lake. Independent variable (assembling): months. ANOVA test of Kruskala-Wallisa ranges

Table 2

The number (cfu cm^{-3}) of heterotrophic bacteria on TGY (1:8) medium at 25, 10, and 4°C in the Hańcza Lake water (site 1) in 2000 year

Depth. (m)	Months											
	V		VI		VII		VIII		IX		X	
	Temperature of water (°C)	25/10/4°C	Temperature of water (°C)	25/10/4°C	Temperature of water (°C)	25/10/4°C	Temperature of water (°C)	25/10/4°C	Temperature of water (°C)	25/10/4°C	Temperature of water (°C)	25/10/4°C
0.3	15.8	1085/520	18.2	790/320	19.5	555/460	20.5	2125/1250	13.6	690/320	10.2	230/100
1	14.1	1590/380	18.2	610/525	19.4	335/275	20.4	1875/1100	13.7	135/115	10.2	280/55
2	12.7	680/200	18.2	2060/580	19.4	2010/400	20.4	1170/530	13.7	145/55	10.3	240/110
5	9.8	950/485	17.9	900/720	19.4	250/245	20.4	1195/420	13.6	160/125	10.3	130/40
10	6.2	1020/530	7.0	915/450	19.1	425/150	20.3	2510/680	11.0	425/90	10.3	235/35
30	4.2	560/290	4.3	1200/430	11.2	960/300	9.5	135/575	4.2	145/320	4.2	155/95
50	4.0	490/350/240	4.1	930/700/450	7.2	1380/690/100	6.4	4885/1900/450	4.1	250/500/300	4.2	225/755/330
70	4.2	460/200/125	4.1	-/325/320	5.4	365/550/150	6.0	785/1200/400	4.1	120/480/500	4.1	80/1000/450
90	4.2	-/180/100	4.1	-/460/200	5.4	-/400/50	6.0	-/700/190	4.1	-/640/540	4.1	-/950/840
108	4.2	500/170/80	4.1	550/150/174	4.9	740/264/189	4.9	-/590/360	4.2	350/355/400	4.1	410/730/130

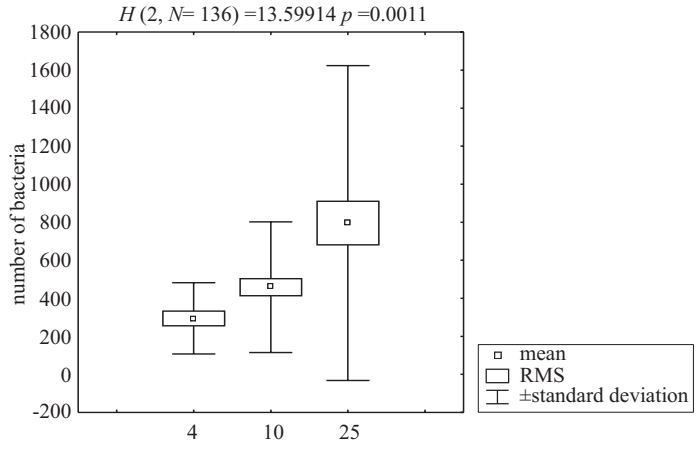


Fig. 3. Averages of numbers (± standard deviation and ± random mean square-RMS) heterotrophic bacteria (cfu cm^{-3}) at 4, 10, and 25°C in the water of Hańcza Lake. Independent variable (assembling): temperature. ANOVA test of Kruskala-Wallisa ranges

were recorded at an incubation temperature of 4°C, (50-840 cfu cm^{-3}), higher ones at a temp. of 10°C (35-1900 cfu cm^{-3}), and the highest ones at a temp. of 25°C (80-4885 cfu cm^{-3}), (Table 2, Figure 3). In particular experimental months, on average, their higher numbers were reported in August (incubation temp. of 25 and 10°C) or in October (incubation temp. 4°C) (Figure 2). No statistically significant differences in the mean numbers of bacteria were observed for particular depths in water of Hańcza Lake, although higher counts of microorganisms were recorded at a depth of 50 m (incubation temp. of 25 and 10°C) and at 90 m (incubation temp. of 4°C) (Table 2). Usually, however, their vertical stratification in the pelagic zone was microzonal and resulted, probably, from the "focal" distribution of organic and mineral matter in the aquifer. According to a number of authors (SZKLAR-MCAULEY, FERRONI 1986, FERRONI, KAMIŃSKI 1980, KAMIŃSKI, FERRONI 1980, GOTKOWSKA-PŁACHTA et al. 2003), the growth of "cryophilic" heterotrophic bacteria is determined by a variety of physicochemical factors, the level of biogenic substances and environment they inhabit. One of the more important factors limiting their growth is temperature (HOYOUX et al. 2004). LOBOVA et al. (2004) demonstrated the distinct influence of this factor on the growth and distribution of heterotrophic bacteria in water of Lake Shira located in the south of the Caucasian Republic. They observed an increase in the number of heterotrophic bacteria incubated at a temp. of 5°C in deeper parts of the aquifer where the temperature of the water accounted for 2.8°C on average, and that of bacteria determined at a temp. of 25°C – in the warm epilimnion of the lake (at a depth of 0.5 m) at water temperatures ranging from 18 to 22°C. FERRONI and

KAMIŃSKI (1980b) as well as SZKLAR-MCAULEY, FERRONI (1986) also emphasized the impact of incubation temperature on the growth of heterotrophic bacteria. In samples of interstitial water collected from the small, shallow, eutrophic Lake Betel located in Canada, bacteria incubated at a temp. of 18°C were the most abundant, whereas the number of psychrotrophic bacteria incubated at a temp. of 2°C was observed to increase along with a decrease in water temperature below 10°C. In the water of Lake Hańcza, a statistically significant correlation was found between water temperature and the number of heterotrophic bacteria determined at a temp. 25°C (Table 3). They occurred in high numbers in August when the temperature of Lake Hańcza water was the highest (4.9-20.5°C). In turn, in October at lower water temperature (4.1-10.2°C), higher counts were observed for bacteria determined at a temp. of 4°C (Table 2). A statistically significant, positive correlation was also found between the number of bacteria incubated at temp. of 25 and 10°C as well as between the number of bacteria incubated at temp. of 10 and 4°C. In contrast, no significant correlations were demonstrated between counts of microorganisms growing at temp. of 4 and 25°C. Thus, bacteria growing at a temp. of 4°C were classified as psychrophilic forms, whereas those growing at a temp. of 25°C – as psychrotrophic bacteria. The division of the "cryophilic" bacteria into psychrophilic and psychrotrophic ones is still a subject of discussion (ŚWIĘCICKA 1997). Practically, those microorganisms can be isolated from almost each site on earth, which can be linked with their ability to adapt to variable environmental conditions (FELLER 2003, HOYOUX et al. 2004, ŚWIĘCICKA 1997). MORITA (1975) postulated the name "psychrotrophs" for microorganisms whose maximum growth proceeds at temperatures higher than 20°C, and the name "psychrophilic" for those that grow at temperatures of ca. 15-20°C. Although this division has not been adopted, it is more and more often claimed that the psychrophilic bacteria develop mainly in habitats with fixed low temperatures, e.g. polar areas, the bottoms of deep seas, oceans

Table 3

Statistical evaluation with the method of Spearman correlation ranks, between numbers of heterotrophic bacteria incubated at temp. of 4, 10 and 25°C obtained over the entire experimental period and temperature of the water of Lake Hańcza

Parameter	Spearman's coefficient of correlation (BD eliminated in couple)		
Heterotrophic bacteria incubated at:	Temperature of Lake Hańcza water	heterotrophic bacteria incubated at:	
		25°C	10°C
4°C	-0.381443	-0.215500	0.560854*
10°C	-0.062042	0.494321*	1.000000
25°C	0.392326*	1.000000	0.494321

* Important correlations ($\alpha < 0.05$) marked

Table 4

Percentage of heterotrophic bacteria isolated from water of Lake Hańcza (site 1) at 4, 10 and 25°C in 2000 r.

Genus	Site 1																	
	Temperature of incubation																	
	4°C						10°C						25°C					
	V	VI	VII	VIII	IX	X	V	VI	VII	VIII	IX	X	V	VI	VII	VIII	IX	X
Aeromonas sp.	14.5	13.2	18.6	14.2	5.8	8.6	13.0	6.7	11.3	16.5	14.5	6.7	6.8	10.0	10.3	5.7	12.9	12.1
Acinetobacter sp.	0	3.5	0	7.1	0	5.7	0	0	5.0	7.5	0	0.5	0	0	8.9	12.0	5.2	2.0
Alcaligenes sp.	10.8	5.3	16.8	12.9	2.9	12.6	10.6	15.7	8.0	6.8	14.5	14.5	27.5	8.4	6.8	5.7	9.1	12.6
Bacillus sp.	4.4	1	3.2	8.2	8	1.7	3.0	4.8	2.0	5.0	5.0	4.4	0.6	10.5	20	5.7	5.3	3.3
Bacillus mycoides	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	0	0
Brevundimonas sp.	3.3	0	0	0	0	0	1.6	0	0	0	0	0	2.1	0	0	0	0	2.5
Brevundimonas vesicularis.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2.8
Chromobacterium violaceum.	9.2	0	4.1	0	0	0	0.9	0	0	0	0	0	0.4	0	0	0	0	5.0
Corynebacterium sp.	0	0	0	0	3.3	0	1.4	0	1.0	2.2	2.7	0	2.1	4.4	0	2.3	0	2.0
Flavobacterium sp.	20.2	28.8	20.7	12.1	24.2	23.0	15.2	20.5	17.8	14.0	25.0	25.0	25.4	18.1	13.7	18.3	22.8	19.1
Lactobacillus sp.	0	0	2.0	0	0	0	0	0	0	0	0	0	0	0	3.2	0	0	0
Micrococcus sp.	6.6	5.1	20.0	15.5	12.2	20.4	20.0	20.6	15.0	15.0	15.1	25.5	13.1	25.3	3.8	5.1	8.8	18.9
Pseudomonas sp.	3.7	8.7	0	7.1	14.4	6.6	3.6	0	5.2	4.1	6.7	10.5	7.7	9.0	1.8	4.5	9.1	3.5
Sarcina sp.	0	2.6	0	0	4.1	0	0	0	2.0	3.0	0	0	3.3	0.9	0	0.7	4.1	0
Streptococcus sp.	0	3.0	6.5	0	5.0	5.0	17.1	0	2.5	5.0	0	4.0	0	6.6	0	0	4.1	8.8
Vibrio sp.	14.7	17.5	2.0	13.6	3.3	10.6	3.0	10.2	18.2	10.7	8.4	4.5	3.6	4.6	24.3	27.9	11.7	1.4
Xanthomonas sp.	7.3	4.4	0	0	0	0	1.5	3.6	0	4.1	0	0.9	4.7	0	1.4	2.9	0.9	1.7
Enterobacteriaceae	5.3	5.2	5.1	8.6	12.3	4.8	7.6	15.5	10.5	4.1	3.1	3.0	2.0	1.6	3.3	4.5	3.3	2.8
Nonidentified	0	1.7	1.0	0.7	4.5	1.0	1.5	2.4	1.5	2.0	5.0	0.5	0.7	0.6	2.5	1.7	2.7	1.5
Genus	Bacteria from *Enterobacteriaceae* family																	
Citrobacter diversus	0	0	0	4.2	16.3	0	10	0.6	9.1	12.5	0	13.2	9.1	8.2	20.8	2.4	12	0
Citrobacter freundii	0	0	0	0	2.3	0	4	1	0	7.5	0	0	3.6	5.5	20.8	2.8	7.5	0
Enterobacter aerogenes	0	8.3	40	16.7	20.9	11.9	6	5.8	16.7	0	15	11.3	18.2	6.8	9.2	10.3	0	0
Escherichia coli	0	0	0	4.2	0	0	0	9.8	0	2.7	0	0	14.5	4.1	0	5.2	7.5	0
Ewingella americana	0	0	0	0	7	0	8	0	0	0	0	10	9.1	0	7.5	17.2	0	10.7
Providencia stuartii	31	0	0	8.3	4.6	0	8	0	0	0	0	0	0	6.9	0	0	5	0
Serratia marcescens	34.5	50	40	8.3	25.6	47.6	56	41.3	50	13.9	25	37.8	36.4	27.4	41.7	51.7	25	71.4
Serratia rubidaea	34.5	0	20	8.3	7	40.5	2	39	0	27.8	20	9.4	9.1	13.7	0	3.5	25	17.9
Yersinia enterocolitica	0	0	0	25	9.3	0	2	2.5	7.6	29.4	0	8.9	0	0	0	0	5.5	0
Plesiomonas shigelloides	0	0	0	16.7	7	0	0	0	0	0	15	0	0	13.7	0	0	12.5	0
Chryseomonas luteola	0	16.7	0	0	0	0	0	0	0	0.6	25	9.4	0	0	0	0	0	0
Morganella morganii	0	25	0	8.3	0	0	4	0	16.6	5.6	0	0	0	13.7	0	6.9	0	0

or lakes. In the water of Lake Hańcza, over the entire experimental period, a constant low temperature (4.0-7.2°C) occurred at a depth below 50 m, at which samples were collected for determination of psychrophilic bacteria.

Amongst the heterotrophic bacteria determined at different incubation temperatures in the water of Lake Hańcza, 17 taxonomic groups were isolated (Table 4). They were predominated by Gram-negative bacteria of the genus *Flavobacterium* (up to 28.8% at a temp. of 4°C) and *Alcaligenes* (up to 27.5% at a temp. of 25°C) as well as Gram-positive cocci of the genus *Micrococcus* (up to 25.5% at a temp. of 10°C). Usually, these bacteria occurred in higher numbers in the spring (May, June) and autumn (September, October). Amongst the *Enterobacteriaceae* family bacteria detected in the water of Lake Hańcza, more abundant were species: *Serratia marcescens* (over 50% at each of temperatures examined) as well as *Serratia rubidaea* and *Enterobacter aerogenes* (up to 40.5% at a temp. of 4°C), (Table 4). Other species than in the pelagic zone predominated in the microflora of bottom deposits of Lake Hańcza, i.e. *Pseudomonas* and *Acinetobacter* genera among Gram-negative bacteria and spore-forming bacteria of the genus *Bacillus* among the Gram-positive ones (GOTKOWSKA-PŁACHTA et al. 2003). Also, in deposits of the mesotrophic Lake Jasne and those of Lake Gardno, these bacteria were the most abundant (DONDERSKI 1983, MUDRYK, DONDERSKI 1993). These results were quite different than findings reported for the oligotrophic Lake Czarne (DONDERSKI 1983) which was predominated by bacteria of the group *Flavobacterium – Cytophaga*. According to LOBOVA et al. (2004), the deepest central part of Lake Shira was predominated by the strains: *Pseudomonas, Acinetobacter, Flavobacterium* and *Halococcus,* whereas bacteria of the genus *Bacillus* were determined as allochthonous microflora and were isolated in the highest numbers from samples of water collected at sites exposed to anthropogenic effects. In the water of Lake Hańcza, higher counts of these bacteria were observed only in the summer and autumn (Table 4), which may be linked with their penetration into the aquifer with surface runoffs from pastures and arable fields surrounding the lake which, in those periods, are utilized most intensively for agricultural purposes.

Conclusion

1. Statistically significant differences were demonstrated between the mean numbers of bacteria examined depending on incubation temperature and period of water sampling, and a significant, positive correlation was found between water temperature and the number of aerobic heterotrophic bacteria determined at a temp. of 25°C.

2. The lowest numbers of aerobic heterotrophic bacteria were reported at an incubation temperature of 4°C, whereas the highest ones – at a temp. of 25°C. In particular experimental months, on average, higher counts of aerobic heterotrophic bacteria were recorded in August (incubation temp. of 25 and 10°C) or in October (incubation temp. of 4°C).

3. Amongst the heterotrophic bacteria determined at various incubation temperatures in water of Lake Hańcza, 17 taxonomic groups were isolated. The incubation temperature had no significant effect on the qualitative composition of bacteria examined.

4. Bacteria detected in water of Lake Hańcza were predominated by Gram--negative rods of the genera *Flavobacterium* and *Alcaligenes* and by Gram--positive cocci of the genus *Micrococcus*. Amongst bacteria of the family *Enterobacteriaceae* identified in the water of Lake Hańcza, the species *Serratia marcescens*, *S. rubidaea*, and *Enterobacter aerogenes* appeared to be more abundant.

Translated by JOLANTA IDŹKOWSKA

Accepted for print 8.11.2007

References

CHO B.C., AZAM F. 1990. *Biogeochemical significance of bacterial biomass in oceans euphotic zone*. Mar. Ecol. Progr. Ser., 63: 253-259.

CHOIŃSKI A. 1995. *Zarys limnologii fizycznej Polski*. Wyd. Nauk. UAM, Poznań.

COLE J.J., FINDLAY S., PACE M. 1988. *Bacterial production in fresh and seawater ecosystems*: Across system overview. Mar. Ecol. Prog. Ser., 43: 1-10.

CYDZIK D., KUDELSKA D., SOSZKA H. 1982. *Atlas stanu czystości jezior Polski badanych w latach 1974-1978*, t. II, Warszawa, IKŚ, ss. 390-393.

DELILLE D., PERRET E. 1989. *Influence of temperature on the growth potential of southern polar marine bacteria*. Microbiol. Ecol., 18: 117-123.

DONDERSKI W. 1983. *Tlenowe bakterie heterotroficzne jezior o różnej trofii*. Wyd. UMK Toruń, ss. 1-105.

DONDERSKI W., BURKOWSKA A. 2001. *The influence of humic substances on the development of heterotrophic bacteria from various physiological groups*. Słupskie prace przyrodnicze, 1: 27-36.

FELLER G. 2003. *Molecular adaptations to cold in psychrophilic enzymes*. CMLS Cell. Mol. Life. Sci., 60: 648-662.

FERRONI G.D., KAMIŃSKI J.S. 1980. *Psychrophiles, psychrotrophs, and mesophiles in an environment which experiences seasonal temperature fluctuations*. Can. J. Microbiol., 26: 1184-1191.

GOTKOWSKA-PŁACHTA A., KORZENIEWSKA E., NIEWOLAK S. 2005. *Pollution degree and sanitary state indicator bacteria as the indicators of the purity of Lake Hańcza waters*. Arch. Ochr. Środ., Protection, 2: 53-68.

GOTKOWSKA-PŁACHTA A., NIEWOLAK S., KORZENIEWSKA E. 2003. *Bacteria in Lake Hańcza bottom sediments*. AUNC, Prace Limnologiczne, XXIII, 110: 191-195.

HERBERT R.A. 1986. *The ecology and physiology of psychrophilic microorganisms*. [In:] Herbert R.A., Codd G.A. *Microbes in extreme environments*. Eds. Herbert R.A., Codd G.A., Academic Press, New York, pp. 1-23.

HOYOUX A., BLAISE V., COLLINS T., D'AMICO S., GRATIA E., HUSTON A.L., MARX J-C., SONAN G., ZENG Y. 2004. *Extreme catalysts from low-temperature environments*. J. Biosci. Bioeng., 98(5): 317-330.

KAMIŃSKI J.S., FERRONI G.D. 1980. *Growth of psychrophilic and mesophilic, aquatic bacterial isolates at the environmental temperature*. Wat. Res., 14: 815-820.

LOBOVA T.I., LISTOVA L.V. POPOVA L., YU. 2003. *Distribution of heterotrophic bacteria in Lake Shira*. Microbiology., 73(1): 89-93.

MARGESIN R., SCHINNER F. 1993. *Psychrophilic and psychrotrophic proteolytic microorganisms from environmental habitats*. Ag. Biotech. News anf Information, 5(4): 153N-157N.

MARGESIN R., SCHINNER F. 1994. *Psychrotrophic proteolytic bacteria of dairy orgin*. Bbiodegradation abstracts., 8: 1-6.

MORITA R.Y. 1975. *Psychrophilic bacteria*. Bacteriol. Rev., 39: 144-167.

MUDRYK Z., DONDERSKI W. 1993. *Generic composition and nutrional requirements of heterotrophic bacteria in the estuarine Gardno lake*. Acta Univ. N. Copernici. Limnol. Papers, 18: 35-44.

RUSSELL N.J. 2000. *Toward a molecular understanding of cold activity of enzymes from psychrophiles*. Extremophiles, 4: 83-90.

RUSSELL N.J. 1997. *Psychrophilic bacteria-molecular adaptations of membrane lipids*. Comp. Biochem. Physiol., 118A: 489-493.

SCHERER S., NEUHAUS K. 2002. *Life at low temperatures*. *The prokaryotes. An Evolving Electronic Resource for the Microbiological Community*, Ed. M. Dworkin et al., 3rd edition, release 3.9, April 1, 2001, Springer-Verlag, New York, <http://link.springer-ny.com/link/service/books/10125/>.

STANISZ A. 1998. *Przystępny kurs statystyki w oparciu o program STATISTICA PL na przykładach z medycyny*. StatSoft Poland Sp. z o.o., Kraków, 263-292.

SZKLAR-MCAULEY I.A., FERRONI G.,D. 1986. *Psychrophiles, psychrotrophs, and glucose mineralization in a lake with seasonal temperature fluctuations*. J. Gen. Appl. Microbiol., 32: 383-391.

ŚWIĘCICKA I., BUCZEK J., HAUSCHILD T. 1997. *Psychrofile i psychrotrofy*. Post. Mikrobiol., 36: 53-70.

The Williams and Wilkins Company. 1994. *Bergey's Manual of Determinative Bacteriology*, USA.

POLISH JOURNAL OF NATURAL SCIENCES
Abbrev.: Pol. J. Natur. Sc., Vol 23(2): 440-449, Y. 2008

DOI 10.2478/v10020-008-0034-y

ASSESSMENT OF THE TROPHIC STATE OF A RESTORED URBAN LAKE BASED ON ZOOPLANKTON COMMUNITY STRUCTURE AND ZOOPLANKTON-RELATED INDICES

Ewa Paturej

Department of Applied Ecology
University of Warmia and Mazury in Olsztyn

K e y w o r d s: zooplankton, restoration, diversity, zooplankton-related trophic state indices.

A b s t r a c t

The analysis of zooplankton community structure in Lake Długie was based on the materials collected during the years 2000-2004 in a monthly cycle. Zooplankton was studied following lake restoration by various methods as well as in the year when no restoration was carried out. It was found that the number of zooplankton species, their abundance and biomass were greatly variable and depended on lake trophy and abiotic environmental factors. The restoration of Lake Długie resulted in gradual changes in the zooplankton community structure, including a wider species diversity and a decrease in the densities of indicator species of high trophy levels.

OCENA TROFII REKULTYWOWANEGO JEZIORA ŚRÓDMIEJSKIEGO NA PODSTAWIE STRUKTURY ZESPOŁU I INDEKSÓW ZOOPLANKTONOWYCH

Ewa Paturej

Katedra Ekologii Stosowanej
Uniwersytet Warmińsko-Mazurski w Olsztynie

S ł o w a k l u c z o w e: zooplankton, rekultywacja, różnorodność, zooplanktonowe wskaźniki trofii.

A b s t r a k t

Analizę struktury zespołu zooplanktonu Jeziora Długiego w Olsztynie oparto na materiałach zebranych w latach 2000-2004 w cyklu miesięcznym. Badano zooplankton jeziora rekultywowanego różnymi metodami, a także w roku bez rekultywacji. Wyniki badań pozwoliły stwierdzić,

Address: Ewa Paturej, University of Warmia and Mazury, ul. Oczapowskiego 5, 10-957 Olsztyn, Poland, phone: (089) 523 51 89, e-mail: epaturej@uwm.edu.pl

że rejestrowana liczba gatunków oraz liczebność i biomasa zwierząt planktonowych były zróżnicowane i zależne od trofii jeziora i czynników abiotycznych środowiska. Po przeprowadzonej rekultywacji Jeziora Długiego następowała powolna przebudowa struktury zooplanktonu na korzyść większego bogactwa gatunkowego oraz zmniejszenia zagęszczenia gatunków wskaźnikowych trofii wód.

Introduction

Industrialization and urbanization pose a serious threat to the natural environment, including increased degradation of aquatic ecosystems. The degree of lake eutrophication is affected by catchment area management. Urban lakes are particularly exposed to danger. An example may be Lake Długie in Olsztyn, which has been receiving domestic sewage and rainwater since the 1970s. The inflow of nitrogen and phosphorus compounds stopped primary production for some time, which contributed to the domination of destruction processes over production processes (PATUREJ, BOWSZYS 2005). It is common knowledge that one of the most effective ways to reduce trophy levels in a lake is to control nutrient inputs from the catchment area. This is also one of the very first protective measures preceding the decision on lake restoration. Lake Długie was also offered such a chance for renovation. The discharge of sanitary sewage into the lake was reduced in 1973 and then stopped in 1976. According to GROCHOWSKA, GAWROŃSKA (2004) and GAWROŃSKA et al. (2005), cutting down on the pollution was not sufficient for the natural renovation of the lake, but this measure must have been taken prior to the planned restoration. The first unsuccessful attempt to restore Lake Długie involved the supply of clean water from the nearby Lake Ukiel. The restoration project implemented in 1987 included artificial aeration of water, combined with thermal destratification which lasted until the year 2000. Long-term artificial aeration improved environmental conditions in the lake. The amount of biogenic substances in water decreased substantially, but their concentrations were still too high to limit primary production. The observed decrease in the rate of phosphorus reduction and its high concentration in water indicated that the trophy levels in Lake Długie could be no longer successfully reduced by this method. Thus, a restoration method involving phosphorus precipitation followed by its binding in the sediments (PAX) was employed in 2001, for the first time in Poland.

Among planktonic animals zooplankton are good bioindicators of physical and chemical conditions in the aquatic environment, while the abundance of biogenic compounds induces the development of forms typical of eutrophic waters (HILLBRICHT-ILKOWSKA 1977, KARABIN 1985 a,b, HALL et al. 2002). Therefore, the aim of the present study was to verify the hypothesis that

changes observed in the communities of Rotifera and Crustacea may provide a basis for the classification of water masses of a lake with respect to the trophic state.

Materials and Methods

The study was performed on the biological materials collected during the years 2000-2004, in a monthly cycle (April-November) in Lake Długie (20°27,8'N; 53°47,2'E; 102,8 m above sea level), located in the western part of the city of Olsztyn, in the basin of the Łyna River and the Pregoła River. The following restoration measures were taken over the experimental period: 2000 – aeration of water masses, 2001-2003 – application of PAX (an aqueous solution of polyaluminum chloride) three times, i.e. in the spring of 2001, in the fall of 2002 and in the fall of 2003, at a dose of 20 tons, 2004 – a control year (no restoration procedures).

The catchment area of Lake Długie is relatively small – 0.54 km^2, of which approximately 40% is woodland and approximately 60% is urbanized land and wasteland. The lake basin can be divided into three parts. The central part is the largest (13.5 ha) and the deepest (17.3 m), and accumulates 67% of the total water mass. This part shows typical features of bradimictic circulation, has a poorly developed shoreline and is surrounded by built-up areas in the west and by partly afforested areas in the east. In the northern part of the lake there is a large bay, covering an area of 11 ha, 5 m deep, containing 30% of the total water mass. This bay has features characteristic of shallow lakes, where water mixing is limited. It's shoreline is well-developed and the shores are almost entirely covered with forests. The smallest (2.5 ha) and the shallowest (3.5 m) part of the lake is its southern bay (3% of the total water mass). Long-term sewage discharge resulted in the shallowing of this bay, so today it shows the features of shallow and polymictic water bodies. It is surrounded by urbanized areas. Lake Długie is considered to be hydrologically closed (LOSSOW et al. 1979).

Samples for biological and chemical examinations were taken at three sampling sites situated in different parts of the lake. In the central part of the lake the cross section of the water column was divided into three vertical subsections: 0-5 m, 5-10 m and 10-15 m. Samples were taken with a 5-liter Ruttner sampler dragged from surface to bottom. 30 liters of water were collected at each sampling site. Water temperature, Secchi disk visibility (SDV) and dissolved oxygen content were measured by standard methods (Standard Methods 1976) developed at the Department of Environmental Engineering and Protection, University of Warmia and Mazury in Olsztyn. A total of 208 samples were collected over the experimental period.

The abundance of planktonic organisms (N, individ. dm^{-3}) was determined using the Hensen formula (STARMACH 1955), while the biomass of rotifers and crustaceans (B, mg dm^{-3}) was estimated by an indirect method (BOTTRELL et al. 1976, EJSMONT-KARABIN 1998). Zooplankton-related trophic state indices and the lake trophic state index (TSI_{SD}) follow KARABIN (1985a) and CARLSON (1977), respectively. In order to determine the structure and diversity of zooplankton communities, species abundance (number of species in the sample) and species diversity (Shannon-Weaver index; H_N, H_B) were analyzed (KREBS 1996).

Differences in the abundance and biomass of zooplankton (Rotifera, Crustacea), number of species and values of the Shannon index between particular stages of the study (artificial aeration, use of PAX, period after the completion of lake restoration) were determined by ANOVA and Kruskal--Wallis test, following the finding of the logarithm $\log_{10}(x+1)$. The Kendall's coefficient of rank correlation was applied to compare the numbers and biomass of Rotifera and Crustacea as well as species abundance and the physiochemical parameters of water (temperature, dissolved oxygen content, Secchi disk visibility).

Results

Over the 2000 to 2004 period the zooplankton community of Lake Długie was characterized by differentiated qualitative composition, which resulted from a different trophic state and the measures taken to restore the lake. In the year 2000, following artificial aeration and water mass destratification, 33 zooplankton species were identified, including 9 indicators of lake trophy. During the years 2001 to 2003, when phosphorus inactivation with the aluminum coagulant PAX was carried out, 64 zooplankton species were recorded, including 15 trophy indicators. In 2004, after lake restoration, 24 and 8 species were identified, respectively. High-trophy indicators, whose domination and proportion in the plankton increases along with increasing trophy levels, are referred to as the so called ecological group II. Among Rotifera they were represented by *Anuraeopsis fissa*, *Brachionus angularis*, *B. diversicornis*, *Filinia longiseta*, *Keratella cochlearis* f. *tecta*, *K. quadrata*, *Pompholyx sulcata*, *Proales* sp., *Trichocerca cylindrica* and *T. pusilla*, while among Crustacea by *Bosmina longirostris*, *Chydorus sphaericus*, *Diaphanosoma brachyurum*, *Mesocyclops leuckarti* and *M. oithonoides*. More indicatory species were identified among rotifers than among crustaceans (5 to 10 versus 3 to 5).

Species abundance and biological diversity of zooplankton in Lake Długie

varied widely and were found to be statistically significant ($p < 0.05$) both during the restoration period and afterwards (Figure 1). The highest number of zooplankton species was recorded towards the end of lake restoration (2003), which corresponded to high values of the indices of species abundance and biomass (H_N, H_B) and an improvement in environmental conditions (Table 1). In the other years the values of these indices were lower due to the domination of single species in the planktonic fauna. The winter of 2004 was extremely severe and a thick ice cover contributed to oxygen deficiency in the bottom--layer of the lake, while in the summer of 2004 excess eutrophicated water from Lake Ukiel was discharged into Lake Długie. Blue-green algal blooms, which negatively affect zooplankton populations acting as natural filters, were also observed.

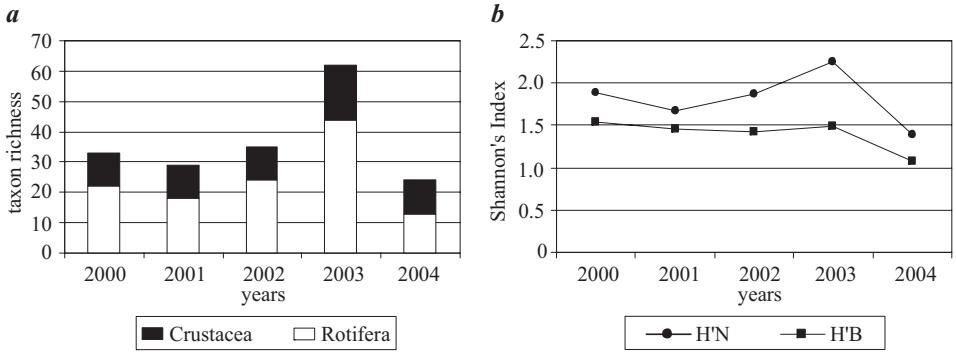


Fig. 1. Taxon richness (a) and Shannon's Index (b) based on zoopalankton abundance (H'N) and biomas (H'B) in Lake Długie

Table 1

Physicochemical parameters of water in the Lake Długie in years 2000-2004

Parameters \ Year	2000	2001	2002	2003	2004
Temperature (°C)	14.7 (5.6-21.6)	14.0 (6.1-22.9)	15.0 (5.3-21.6)	12.1 (2.1-23.1)	11.8 (3.4-23.5)
Dissolved oxygen (mg O_2 dm^{-3})	8.4 (4.3-16.4)	8.9 (0.9-13.3)	9.3 (7.7-13.9)	9.6 (7.4-11.2)	8.4 (5.0-9.4)
Secchi Disk Visibility (SDV, m)	0.8 (0.5-1.1)	1.2 (0.8-1.7)	1.4 (0.8-2.0)	2.0 (1.8-2.6)	1.9 (1.0-2.9)

The abundance and biomass of Rotifera ($p < 0.01$, $p < 0.01$) and Crustacea ($p < 0.01$, $p < 0.001$) in Lake Długie were at a high level at the first stage of restoration (2000-2001), showing statistically significant differences.

In subsequent years (2002-2003) the use of PAX enabled to eliminate pond species from the planktonic fauna, which was reflected by zooplankton densities and biomass (Figure 2). There were highly significant statistical relationships between the effect of temperature on the numbers and biomass of Crustacea and zooplankton species abundance ($p < 0.001$) over the period of PAX application, as well as significant relationships with the abundance and biomass of Rotifera ($p < 0.05$), (Table 2). Non-significant, negative correlations were recorded with respect to dissolved oxygen content. This parameter was found to have a significant effect on the biomass of Rotifera only. A positive, non-significant effect was noted in the case of water transparency.

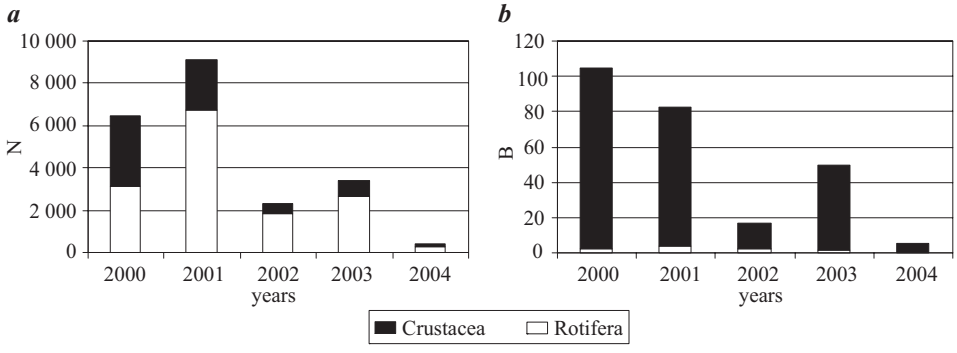


Fig. 2. Average density (N, ind. dm^{-3}) and biomas (B, mg dm^{-3}) of Rotifera and Crustacea in Lake Długie

Table 2

Kendall's coefficients of rank correlation between zooplankton and physico-chemical parameters of water in the Lake Długie in years 2000-2004

Pameters	T			O_2			SDV		
	aeration	PAX	without	aeration	PAX	without	aeration	PAX	without
R_N	0.143	0.331*	0.400	-0.048	-0.070	0.400	-0.524	0.125	-0.200
R_B	0.048	0.285*	0.359	0.238	0.292*	0.359	-0.429	0.014	-0.598
C_N	0.714*	0.470**	0.400	-0429	-0.056	0.000	0.048	-0.014	-0.600
C_B	0.619*	0.460**	0.200	-0.333	-0.042	-0.600	-0.048	0.093	0.000
T_R	0.293	0.339**	0.527	0.000	-0.092	-0.738	0.098	0.226	0.105

Significant correlations in ranking of all sites are indicated by: * $p < 0.05$, ** $p < 0.001$. T – temperature, O_2 – dissolved oxygen, SDV – Secchi disk visibility; aeration – term of artificial aeration, PAX – implementation of PAX, without – term after recultivation; R_N – abundance of Rotifera, R_B – biomass of Rotifera, C_N – abundance of Crustacea, C_B – biomass of Crustacea, T_R – taxon richness

The analysis of the structural characteristics of zooplankton as bioindicators of lake eutrophication (Table 3) revealed that the years 2000 to 2003 were similar in terms of the degree of water mass eutrophication (eutrophy with

symptoms of meso-eutrophy on the one hand and polytrophy on the other), whereas in 2004 the cleanliness state of the lake improved considerably (meso-eutrophy/mesotrophy). In 2000 an eutrophic-polytrophic character of water in Lake Długie was confirmed by both the trophic state index based on Secchi disk visibility (TSI_{SD}) and the proportion of the *tecta* form in the population of *Keratella cochlearis* – one of the most common trophy indicators. The abundance of rotifers increased with increasing trophy levels and decreased as the environmental conditions in the lake improved. With respect to the other indices, based on the structural characteristics of crustaceans, in particular on the contribution of Cyclopidae to the total biomass of Crustacea and on the ratio between the Cyclopidae biomass and the Cladocera biomass, Lake Długie was classified as strongly eutrophic in 2000-2002. At the end of the restoration process (2003) water quality improved, which was reflected in higher transparency values and a higher dissolved oxygen content (Table 1).

Table 3

Zooplankton-based indices of the trophic state of Lake Długie in years 2000-2004: TSISD (CARLSON 1977), proportion of ecological group II in the biomass of the indicator rotifer community (%, group II – Rotifera), proportion of *tecta* in the abundance of *Keratella cochlearis* (%, TECTA), abundance of rotifers (indiv. dm^{-3}, Rotifera N), proportion of ecological group II in the biomass of the indicator crustacean community (%, group II – Crustacea), proportion of Cyclopidae in the biomass of Crustacea (%, Cyclopidae in B Crust.), ratio between Cyclopidae biomass and Cladocera biomass (mg dm^{-3}, B_{CY}:B_{CL}) and ranges of values for trophic types of lakes (KARABIN 1985a)

Year	TSI_{SD}	II group–Rotifera	TECTA	Rotifera N	II group–Crustacea	Cyclopidae in B Crust.	B_{CY}:B_{CL}
Restoration							
2000	e/p	e	me/e/p	me/e	me/m	e	e
2001	e	me	m/e	m/e/p	m	e	e
2002	e/me	m/me/e	me/e	me/e	m	e	e
2003	me	me	me/m	e/me	m	e/me/m	me/m
After restoration							
2004	me	me	m	m	m	me/m	me/m
Trophic types	Ranges of values						
Mesotrophy (m)	<45	<10	0–5	<400	<25	<15	<0.2
Meso-eutrophy (me)	45-55	10-90	5-20	<400	25-60	15-30	0.2-0.8
Eutrophy (e)	55-65	>90	20-60	400-2000	>60	>30	>0.8
Polytrophy (p)	>65	>90	>60	>2000	–	–	–

Discussion

Many species of rotifers and crustaceans show high tolerance for changing environmental conditions and thus may serve as indicators of the trophic state of lakes (LITYŃSKI 1925, RADWAN 1976, KARABIN 1985a). However, the results

of faunal analysis alone are insufficient to determine the correlation between the trophic state of a lake and zooplankton community structure. From the perspective of bioindication it is necessary to find relationships between the trophic state of a lake and the number of zooplankton structural characteristics on the basis of numerical data.

It was found that in Lake Długie progressing eutrophication was accompanied by a constant increase in the abundance and biomass of rotifers and crustaceans. Zooplankton densities were dominated by Rotifera (75% of total zooplankton), and zooplankton biomass – by Crustacea (93%). However, the highest density of rotifers, observed in 2001 (6708 individ. dm^{-3}), was much lower than that recorded by WIDUTO (1979) in the seventies when domestic sewage was discharged into the lake (15602 individ. dm^{-3}). According to some authors, the densities of rotifers are high in eutrophicated urban lakes, and many of them are indicators of the trophic state of water. Examples of such lakes are: Lake Zamkowe (PATALAS 1956), lakes located in the town of Szczytno (BITTEL 1974), Lake Głębokie in Szczecin (SZLAUER 1996), coastal lakes (PATUREJ 2005). This opinion was confirmed by studies conducted on Lake Długie. The community of planktonic rotifers was characterized by greater species abundance than the community of crustaceans. A similar relationship was observed by CERBIN, KOKOCIŃSKI (1998), who examined zooplankton populations in Lake Kaliszańskie Duże, by BIELAŃSKA-GRAJNER, PILARCZYK (1996) in Lake Rybnik as well as by RADWAN et al. (2002), who analyzed water ecosystems in Polesie.

The decrease in the abundance of rotifers and crustaceans as well as the increase in the number of species and diversity of zooplankton, observed at the final stage of restoration, suggest that the environmental conditions in the lake improved (Table 1). The change in the trophic state of the lake was reflected in a higher N/P ratio, which increased from the values typical of domestic sewage (4-6) to those observed in mesotrophic lakes (> 25) (GAWROŃSKA et al. 2005). The species composition of zooplankton communities changed as well: there appeared taxa typical of low-trophy waters, e.g. *Daphnia cristata*, while species characteristic of ponds and small water bodies, such as *Filinia brachiata*, *Brachionus urceolaris, Pedalia mira,* were gradually eliminated. Zooplankton-related trophic state indices based on the populations of rotifers and crustaceans also indicated an improvement in water quality (Table 3). PATUREJ, BOWSZYS (2005) demonstrated that the structure of zooplankton communities in restored bodies of water differs from that in lakes free from human interference. The reason for those differences is not only temperature-sensitivity of organisms, but also a whole variety of ecological factors (including, among others, oxygen conditions, calcium content, the pH of water, the presence of blue-greens). Many authors (KARABIN 1985 a,b, RADWAN 1976,

HILLBRICHT-ILKOWSKA 1977, SZLAUER 1996, CERBIN, KOKOCIŃSKI 1998) share the opinion that trophic relations in lakes are the main factor determining the abundance, biomass and species structure of zooplankton.

Conclusions

According to the zooplankton-based indices, the trophic state of the restored Lake Długie can be considered satisfactory, especially taking into account the fact that in the 1970s this lake was classified as saprotrophic. At the completion of the experiment the cleanliness state of the lake improved, which enabled to classify Lake Długie into the transitory phase between meso-eutrophy and mesotrophy. The stability of this improvement will depend on the pollutant load introduced into the lake. Currently the most serious threat is posed by the discharge of excess water from Lake Ukiel into Lake Długie, and by the use of bait by anglers. It is necessary to establish and implement rational fisheries management principles in the lake, including the so called biomanipulation which promotes the development of predatory fish and limits the development of cyprinoid fish.

Translated by ALEKSANDRA POPRAWSKA

Accepted for print 3.09.2007

References

BIELAŃSKA-GRAJNER I., PILARCZYK K. 1996. *Zooplankton of heated water of Rybnik reservoir in the years 1987-1988*. Acta. Biol. Silesiana, 28: 170-186.

BITTEL L. 1974. *Pelagiczny plankton zwierzęcy jezior szczycieńskich*. Zesz. Nauk. ART. Olszt., 3: 81-9.

BOTTRELL H.H., DUNCAN A., GLIWICZ Z.M., GRYGIEREK E., HERZIG A., HILLBRICHT-ILKOWSKA A., KURASAWA H., LARSSON P., WĘGLEŃSKA T. 1976. *A review of some problems in zooplankton production studies*. Norw. J. Zool., 24: 419-456.

CARLSON R.E. 1977. *A trophic state index for lakes*. Limnol. Oceanogr., 22: 361-369.

CEBRIN S., KOKOCIŃSKI M. 1998. *Zbiorowiska planktonu roślinnego i zwierzęcego Jeziora Kaliszańskiego Dużego – ocena stanu biologicznego i istniejących zagrożeń*. Bad. Fizjogr. Pol. Zach., 47: 245-256.

EJSMONT-KARABIN J. 1998. *Empirical equations for biomass calculation of planktonic rotifers*. Pol. Arch. Hydrobiol., 45(4): 513-522.

GAWROŃSKA H., LOSSOW K., GROCHOWSKA J. 2005. *Rekultywacja Jeziora Długiego w Olsztynie.* Wyd. „Edycja", Olsztyn: 5-52.

GROCHOWSKA J., GAWROŃSKA H. 2004. *Restoration effectiveness of a degraded lake using multi-year artificial aeration.* Pol. J. Environm. Stud., 13(6): 671-681.

HALL J., CATHERINE J.B., CAROLYN W. 2002. *Environmental gradients and zooplankton distribution in a shallow, tidal lake*. Arch. Feur Hydrobiol., 154(3): 485-497.

HILLBRICHT-ILKOWSKA A. 1977. *Trophic relations and energy flow in pelagic plankton*. Pol. Ecol. Stud., 3(1): 3-98.

KARABIN A. 1985a. *Pelagic zooplankton (Rotatoria+Crustacea) variation in the process of lake eutrophication*. I. *Structural and quantitative features*. Ekol. Pol., 33(4): 567-616.

KARABIN A. 1985b. *Pelagic zooplankton (Rotatoria+Crustacea) variation in the process of lake eutrophication*. II. *Modifying effect of biotic agents*. Ekol. Pol., 33(4): 617-644

KREBS Ch.J. 1996. *Ekologia*. Wyd. Nauk., PWN, Warszawa, pp. 735.

LITYŃSKI A. 1925. Próba klasyfikacji biologicznej jezior suwalszczyzny na zasadzie składu zooplanktonu. Spraw. Stacji Hydrob. Wigry, 1(4): 37-56.

LOSSOW K., DROZD H., MIENTKI Cz. 1979. *Termika i układy tlenowe w Jeziorze Długim w Olsztynie*. Zesz. Nauk. ART Olszt., 9: 3-15.

PATALAS K. 1956. Sensowe zmiany w zespole skorupiaków pelagicznych w Jeziorze Zamkowym na tle stosunków termiczno-tlenowych. Pol. Arch. Hydrobiol., 3(16): 203-251.

PATUREJ E. 2005. *Zooplankton przymorskich jezior Pobrzeża Bałtyckiego*. Wyd. UWM-Olsztyn, Rozpr. i Monogr., 110: 1-129.

PATUREJ E., BOWSZYS M. 2005. *Changes in the zooplankton of the restored Lake Długie*. Pol. J. Natur. Sc., 19(2): 321-334.

RADWAN S. 1976. *Planktonic rotifers as indicators of lake trophy*. Ann. UMCS, Sect. C, 31: 227-235.

RADWAN S., MIECZAN T., PŁASKA W., WOJCIECHOWSKA W., SENDER J., JASZCZENKO P. 2002. *Ekosystemy wodne Polesia – aktualny stan oraz kierunki zmian*. Acta. Agroph., 66: 89-120.

Standard Methods for the Examination of Water and Wastewater. 1976, New York.

STARMACH K. 1955. *Metody badań planktonu*, PWRiL, Warszawa, pp. 133

SZLAUER B. 1996. *Zooplankton Jeziora Głębokiego w Szczecinie jako wskaźnik troffi tego zbiornika*. Zesz. Nauk. AR Szczec., 171(22): 19-34.

WIDUTO J. 1979. *Zooplankton Jeziora Długiego w Olsztynie, zanieczyszczonego ściekami gospodarczo-bytowymi*. Zesz. Nauk. ART Olszt., 9: 93-102.

POLISH JOURNAL OF NATURAL SCIENCES
Abbrev.: Pol. J. Natur. Sc., Vol 23(2): 450-460, Y. 2008

DOI 10.2478/v10020-008-0035-x

TREATMENT OF MODEL PULP AND PAPER WASTEWATER BY ELECTROCOAGULATION

Beata Załęska-Chróst, Lech Smoczyński, Regina Wardzyńska

Chair of Chemistry
University of Warmia and Mazury in Olsztyn

K e y w o r d s: electrocoagulation, model pulp and paper wastewater, recirculation system.

A b s t r a c t

Model wastewater whose composition resembled that of pulp and paper wastewater was treated by electrocoagulation under laboratory conditions. The process was carried out in an electrolytic tank with aluminum electrodes. Following electrocoagulation, sedimentation and decantation, COD, turbidity, suspended solids and color were determined in a solution above the sludge layer. The parameters of COD removal efficiency were also calculated. It was found that electrocoagulation is an effective method of wastewater treatment, offering a viable alternative to chemical coagulation. The efficiency of pollutant removal is highly dependent on the initial load of crude sewage.

OCZYSZCZANIE MODELOWYCH ŚCIEKÓW CELULOZOWO-PAPIERNICZYCH METODĄ ELEKTROKOAGULACJI

Beata Załęska-Chróst, Lech Smoczyński, Regina Wardzyńska

Katedra Chemii
Uniwerytet Warmińsko-Mazurski w Olsztynie

S ł o w a k l u c z o w e: elektrokoagulacja, modelowe ścieki celulozowo-papiernicze, system recyrkulacyjny.

A b s t r a k t

W warunkach laboratoryjnych ścieki poddawano elektrokoagulacji. Do badań użyto ścieków modelowych, składem zbliżonych do ścieków celulozowo-papierniczych. Proces prowadzono w elektrolizerze z elektrodami glinowymi. Po elektrokoagulacji, sedymentacji i dekantacji w roztworze nad osadem mierzono: ChZT, mętność, zawiesiny i barwę. Obliczono wskaźniki charakteryzujące usuwanie ChZT z elektrokoagulowanych ścieków. Stwierdzono, że elektrokoagulacja jest skuteczną metodą oczyszczania ścieków i pozostaje alternatywą koagulacji chemicznej. Wynik oczyszczania w dużym stopniu zależy od obciążenia ścieków surowych.

Address: Beata Załęska-Chróst, University of Warmia and Mazury, Plac Łódzki 4, 10-957 Olsztyn, Poland, phone (089) 523 37 38, e-mail: bezetka@uwm.edu.pl

Introduction

Wastewater treatment is one of the elements of complex environmental protection, primarily of ground and underground water purity protection. The basic purpose of this process is to change the composition and properties of wastewater prior to discharge, so as to assure that it will not disturb the natural ecological balance in the receiving water body, and that such a water body will further provide clean water for domestic, commercial and industrial uses (TAŁAŁAJ, DZIENIS 2004).

Increasing water quality protection requirements made it necessary to improve the efficiency of municipal (SMOCZYŃSKI, WARDZYŃSKA 1996) and industrial effluent treatment. Apart from biological treatment, another common method of pollutant load removal is chemical coagulation. A certain alternative to chemical coagulation may be electrochemical coagulation. Electrocoagulation has been already applied to treat water containing textile waste (CIARDELLI, RANIERI 2001, LIN, PENG 1996), dyes (JIA et al. 1999), tannery waste (NAUMCZYK 2001) or pulp and paper waste (SMOCZYŃSKI, ZAŁĘSKA-CHRÓST 2002). Theoretical models of electrocoagulation have also been developed (CHEN et al. 2002, MOLLAH et al. 2004). This suggests that this procedure may successfully compete with chemical coagulation (CHEN et al. 2002, HOLT et al. 2002).

Due to the specific production technology, the pulp and paper industry greatly contributes to environmental pollution, increasing the total annual amount of particulate matter, pollutant gases and industrial effluents. The qualitative and quantitative composition of pulp and paper waste depends on pulp production technology. Effluents from pulp and paper plants contain mostly dissolved organic compounds, such as alkali- and thiolignins, acids and rosin soaps as well as sulfur compounds – sulfates, sulfides and thioethers.

Pulp and paper wastewater can be treated by biological and chemical methods. However, the applied treatment procedures often do not meet the relevant criteria, particularly those pertaining to organic compound removal efficiency. Therefore, continuous research must be conducted in order to develop more effective methods, appropriate for a given type of waste material, as well as to search for new solutions that would enable to achieve the highest possible efficiency of pollutant removal under local conditions.

The paper presents an evaluation of electrocoagulation as a method for treating model wastewater whose composition resembles that of natural pulp and paper mill effluents.

Materials and Methods

Model wastewater was treated by electrocoagulation in a recirculation system. The study was carried out on a laboratory scale. The wastewater was prepared by mixing (at an increased temperature) Na_2SO_4 and Na_2CO_3 solutions with pine sawdust. The samples were then diluted with water, to obtain COD concentrations comparable to those recorded at pulp and paper plants. The initial COD values in electrocoagulated wastewater were 1840, 1400, 1023 and 750 mg dm^{-3}.

The above procedure of sample preparation permitted high reproducibility of the properties and parameters of model wastewater. The properties of natural wastewater change with time, which reduces the precision and replicability of experimental results. The use of model wastewater whose composition resembled that of natural pulp and paper mill effluents allowed to perform long-term electroagulation experiments.

Electrochemical coagulation was carried out in a continuous recirculation system, to facilitate contact between flocs of metal hydroxide produced during electrolysis and pollutants absorbed on its surface, as well as to ensure full control over the wastewater treatment process. The apparatus presented in Figure 1 was used.

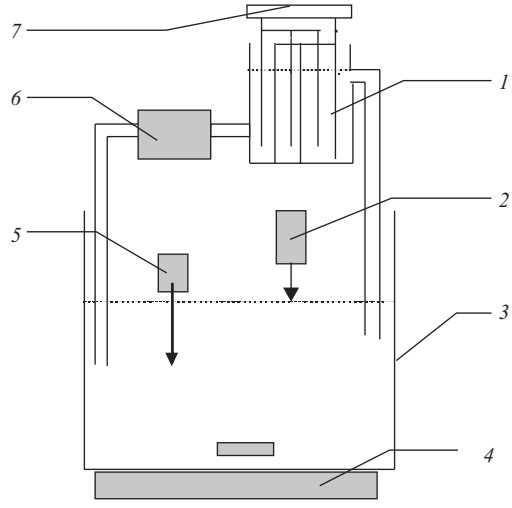


Fig. 1. A sketch of the laboratory recirculating system for wastewaters electrocoagulations: 1 – electrolyser, 2 – 1m HCl, 3 – container of wastewaters, 4 – magnetic stirrer, 5 – pH-meter, 6 – dosing pump, 7 – steering system

The main element of the apparatus was an electrolytic tank with six aluminum electrodes ($10 \times 1 \times 0.1$ cm). The spacing between the electrodes was 1 cm, to facilitate contact between them and the treated effluents. The wastewater was constantly pumped from the waste tank into the electrolytic tank and back into the waste tank, using a metering pump. In the tank the wastewater was stirred with a magnetic stirrer, to prevent sludge sedimentation and to make sure the sludge is pumped together with the effluents. The control-supply system of own design was used to stabilize current intensity and optimize the frequency of current direction changes. The experiment was performed at constant current intensity I = 0.1 A and variable voltage. The wastewater pH was adjusted to the required range (approx. 5.6-6.0) using 1 M HCl. Since $Al(OH)_3$ has a minimum solubility at pH 5.6-5.8 (STUMM, MORGAN 1996), conducting the process within the above pH range enabled to achieve maximum wastewater treatment efficiency, and to reduce Al residue in the effluent to a minimum. After 0.5 h sedimentation samples were taken above the sludge layer for analysis.

The suitability of electrocogulation for wastewater treatment was estimated based on the following parameters: COD, turbidity, suspended solids ands color, determined by a standard method (APHA, AWWA 1995), using a DR 2000 spectrophotometer (HACH), under laboratory conditions, at a temperature T = 293 ± 1K.

Results

The paper presents the results of studies on electrocoagulation carried out in a recirculation system, at constant current intensity I = 0.1 A. Four groups of model wastewater were used, whose initial COD values were 1840 mg dm^{-3}, 1400 mg dm^{-3}, 1023 mg dm^{-3} and 750 mg dm^{-3}.

Two major types of processes occur during wastewater electrolysis (LIN, PENG 1996, CHEN et al. 2000):

1. redox reactions at electrodes, with simultaneous oxidation and reduction in the solution,

2. anodic dissolution of electrode material, which due to solution alkalization causes classical coagulation of colloidal substances in water or wastewater.

The following main electrode processes (VIK et al. 1984) take place during electrolysis of an aqueous solution with a soluble anode, in the presence of Cl- (FØYN 1980):

at the anode (+):

$m/3\ Al^{o} - m\ e \rightarrow m/3\ Al^{3+}$

(n-m) Cl^- – (n-m) e → (n-m)/2 Cl_2

at the cathode (–):

$n\ H_2O + n\ e \rightarrow n/2\ H_2 \uparrow + nOH^-$

They are accompanied by a by-process, i.e. oxygen cathodic polarization:

1/2 O_2 (dissolved in water) + H_2O + 2e → $2OH^-$

Metal cations produced at the anode and hydroxyl ions produced at the cathode form aluminum hydroxide. Hydroxyl ions produced as a result of oxygen cathodic polarization are responsible for the ultimate pH of treated wastewater, since they cause an increase in pH during electrocoagulation.

Floc aggregation followed by sedimentation was observed in the solution during electrolysis.

All processes occurred within a maximum time of 21 600 s and were carried out with constant pH adjustment to 5.6-6.0 with 1M HCl. Such pH levels permitted, at least theoretically, the formation of aluminum polycations (BOTTERO, BERSILLION 1989) in electrocoagulated wastewater, since the pH of effluents electrocoagulated without adjustment via the addition of HCl, higher than 10 (GRØTERUD, SMOCZYŃSKI 1992), is not conducive to the formation of > +3 – valence aluminum polycations. Numerous authors (BOTTERO et al. 1988) reported the presence of these particularly effective forms e.g. in wastewater coagulated chemically with PAC.

Figures 2-5 present the course of wastewater treatment based on selected parameters of effluents determined during electrocoagulation, i.e. the supply of a specified electric charge to the wastewater solution. The Figures illustrate the efficiency of pollutant removal from effluents at different initial COD concentrations.

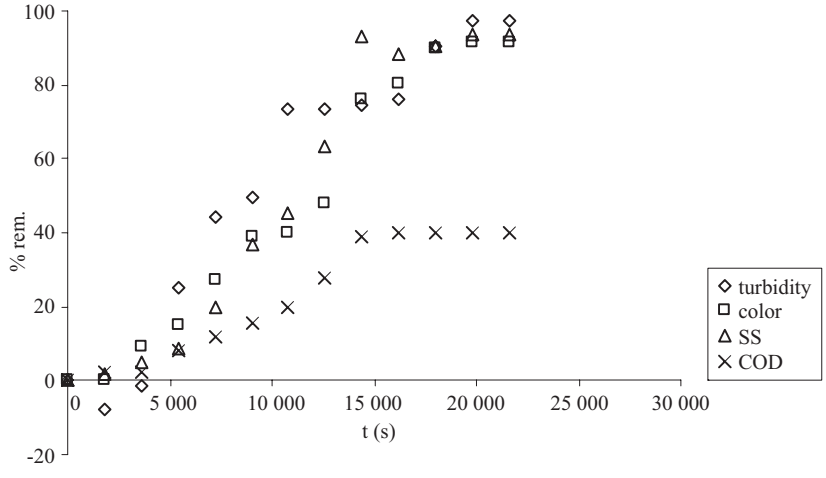


Fig. 2. Pollutant removal from wastewater at COD_o = 1840 mg dm^{-3}

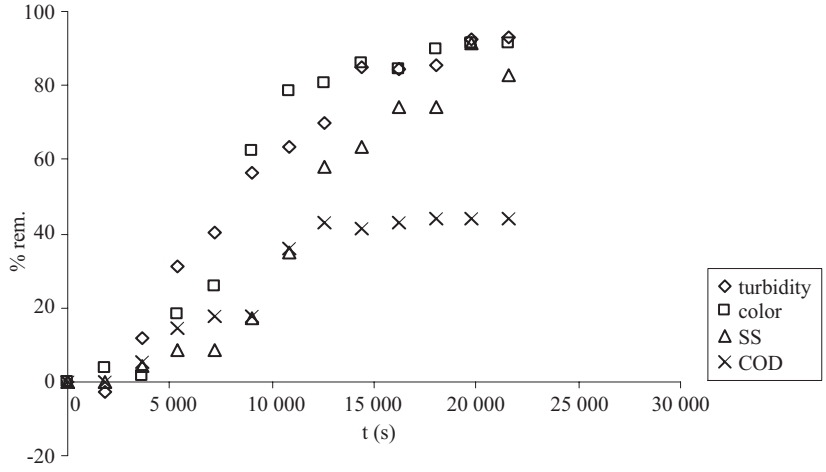


Fig. 3. Pollutant removal from wastewater at COD_o = 1400 mg dm^{-3}

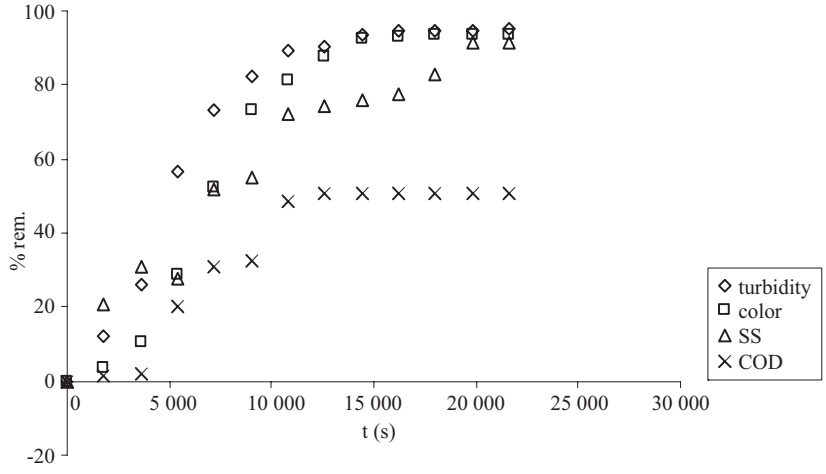


Fig. 4. Pollutant removal from wastewater at COD_o = 1023 mg dm^{-3}

Figures 2-5 show that during wastewater electrocoagulation in a recirculation system pollutant removal efficiency was proportional to the duration of the process and to an increase in the electric charge supplied to the solution, as reflected in the values of COD, turbidity, suspended solids and color. A comparison of relative COD removal from wastewater indicates that it decreased along with an increase in the initial COD concentrations. The maximum COD removal efficiency (60%) was observed for COD_o = 750 mg dm^{-3}. At COD_o = 1023 mg dm^{-3}, the ultimate COD concentration in treated effluents

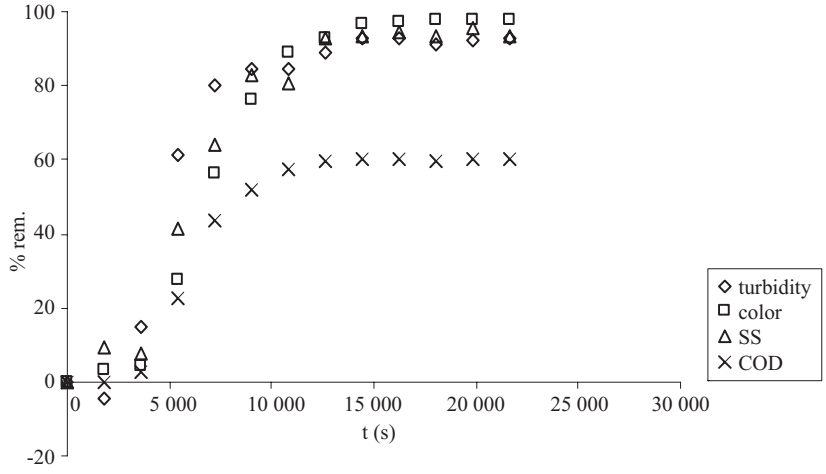


Fig. 5. Pollutant removal from wastewater at COD_o = 750 mg dm^{-3}

accounted for approximately 50% of COD_o. For both these types of effluents the time needed to achieve the above COD levels was 12 600 s. After that time wastewater parameters changed slightly only. At COD_o = 1400 mg dm^{-3} COD removal efficiency was lower (44%) and electrolysis lasted longer, namely for 16 200 s. At the highest initial COD concentration, 1840 mg dm^{-3}, under identical experimental conditions, COD removal efficiency was the lowest and oscillated around 40%. The same relationship was observed for the other wastewater parameters, i.e. turbidity, color and suspended solids: the lower the initial COD value of electrocoagulated effluents, the faster the rate of pollutant removal. The optimum treatment time, permitting the maximum efficiency of total pollutant removal, was as follows: at COD_o = 750 mg dm^{-3} – 14 400 s, at COD_o = 1023 mg dm^{-3} – 16 200 s, at COD_o = 1400 mg dm^{-3} – 18 000 s, at COD_o = 1800 mg dm^{-3} – 19 800 s. Higher COD_o levels required longer electrocoagulation times, i.e. higher electric charges. It should be noted that the supply of a higher electric charge to the solution means higher energy inputs and higher overall costs of the procedure.

Aluminum hydroxide produced during wastewater treatment by electrocoagulation has good sorptive properties. In the pH range of 3.0-9.0 aluminum hydroxide occurs in the state of colloidal dispersion. The charge of the precipitated colloid depends on the pH of the solution. In the pH range of 5.5-7.6 aluminum hydroxide has a positive charge (STUMM, MORGAN 1996). Thus, it destabilizes the negatively charged colloids responsible for turbidity and color, which are then adsorbed on its surface (GRØTERUD, SMOCZYŃSKI 1986, VIK 1982). At lower COD_o levels, i.e. lower total pollutant load, the ratio

between the number of pollutant molecules and the surface area of the adsorbent was lower, which increased the adsorption potential and improved treatment efficiency. Such a dependence was also observed during electrocoagulation of natural pulp and paper mill effluents (SMOCZYŃSKI, ZAŁĘSKA-CHRÓST 2002).

The common feature of the analyzed treatment processes was an increase in turbidity at the beginning of the procedure. This was most probably caused by the formation of small quantities of colloidal hydroxide. Due to a low electric charge ($Q = I \cdot t$) small amounts of Al^{3+} could pass into the solution. Low concentrations of hydroxide hindered its effective destabilization as well as agglomeration of positively charged aluminum micelles and negatively charged wastewater micelles. In consequence, total turbidity was the sum of turbidity of both types of colloids. The overcoming of the charge barrier enabled destabilization of the analyzed system, followed by aggregation of wastewater colloids and gel sedimentation, which in the studied case permitted approximately 99% turbidity removal.

Table 1 presents the efficiency of COD removal from wastewater during electrocoagulation.

Table 1

COD removal from wastewater coagulated in a recirculation system

COD_o (mg dm^{-3})	ΔCODmax. (mg dm^{-3})	a Q/1 mg COD (C mg^{-1})	ΔCOD under optimal conditions (mg dm^{-3})	b Q/1 mg COD (C mg^{-1})
750	452	2.88	446	1.68
1023	517	2.11	506	1.23
1400	620	1.54	600	0.90
1840	740	1.17	720	0.78

Table 1 shows changes in pollutant load responsible for COD values. The first part of this Table presents the maximum efficiency of COD removal from particular types of effluents over a maximum time of t = 21 600 s. The coefficient a = Q/1 mg COD denotes an electric charge per unit COD of electrocoagulated effluents, and provides information on the economic indices of the process. The value of electric charge Q is directly proportional to the amount of energy used during electrocoagulation, and affects the total costs of the procedure. Therefore, the lower this coefficient, the lower the energy input. In the case under consideration, the lowest cost was achieved at COD_o = 1840 mg dm^{-3}. The next part of Table 1 presents changes in COD under optimal conditions, i.e. calculated for the time of electrolysis after which

no further changes in COD levels are observed. It was found that the values of coefficients b and a differed slightly only. The minimum value of coefficient b, similarly as the minimum value of coefficient a, was recorded for wastewater samples with the highest COD_o.

Figure 6 shows the removal of pollutants affecting the other parameters of treated wastewater, under optimal conditions, i.e. at the lowest possible energy inputs.

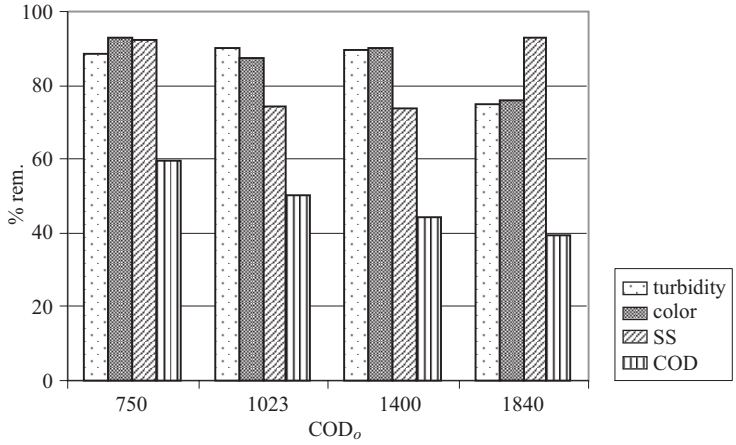


Fig. 6. Removal of turbidity, color, suspended solids and COD from wastewater inder optimal conditions

The best results, i.e. the highest pollutant removal efficiency, were achieved at COD_o = 750 mg dm^{-3}. At COD_o = 750, 1023 and 1400 mg dm^{-3}, the efficiency of turbidity and color removal was at a comparable level, i.e. about 90% and 87-92%, respectively. The worst results of turbidity and color removal under optimal conditions were noted at COD_o = 1840 mg dm^{-3}. However, the efficiency of suspended solids removal was the highest at COD_o = 750 and 1840 mg dm^{-3}. Generally, COD removal efficiency was highly dependent on the initial load of crude sewage.

The determination of the optimum electrocoagulation parameters for different types of effluents may provide a basis for planning and calculation of the overall costs of this process, taking into account the expected final results.

Conclusions

The method of continuous recirculation, applied in the study, permitted effective treatment of pulp and paper mill effluents by electrocoagulation. The maximum efficiency of pollutant removal was 40 to 60% for COD, and 90 to 99% for the other parameters. The efficiency of electrolytic wastewater treatment depended on the values of electric charge (in accordance with Faraday's law) and on the initial values of COD. Electrocoagulation may offer an alternative to other methods employed to treat pulp and paper wastewater, since it provides full control over the process. Repeated wastewater recirculation in a closed system enables to achieve the desired treatment results. Another advantage of this procedure is that it permits full automation of wastewater treatment and, in contrast to chemical coagulation, does not require very precise coagulant dosage. Since the composition and pollutant load of wastewater may vary widely under industrial conditions, it is very difficult to determine the inorganic coagulant dose with sufficient accuracy in the process of chemical coagulation. In addition, the overall costs of electrocoagulation do not include the costs of coagulant storage and transportation, because the typically used inorganic coagulant $Al_2(SO_4)_3 \cdot 18H_2O$ contains only 10% Al.

Translated by ALEKSANDRA POPRAWSKA

Accepted for print 11.09.2007

References

APHA, AWWA, WEF 1995. *Standard Methods for the Examination of Water and Wastewater*. 19th ed., Washington, DC: APHA, AWWA and WEF.

BOTTERO J., BERSILLION J.L. 1989. *Aluminum and Iron III Chemistry. Some Implications for Organic Substance Removal. Aquatic Humic Substances. Influence on Fate and Treatment of Pollutants.* Adv. Chem., Ser. 219: 425-442.

BOTTERO J.Y., TCHOUBAR D., CASES J.M., FRIPIAT J.J., FIESSINGER F. 1988. *New Developments in Knowledge of Aluminium Colloids, Interfacial Phenomena in Biotechnology and Materials Processing*. Elsevier Sci. Publishers B.V., Amsterdam, pp. 459-479.

CHEN X., CHEN G., YUE P.L. 2000. *Separation of pollutants from restaurant wastewater by electrocoagulation.* Separation Purification Technology, 19: 65-76.

CHEN X., CHEN G., YUE P.L. 2002. *Investigation on the electrolysis voltage of electrocoagulation.* Chem. Engin. Sci., 57: 2449-2455.

CIARDELLI G., RANIERI N. 2001. *The treatment and reuse of wastewater in the textile industry by means of ozonation and electrocoagulation.* Water Res., 35: 567-572.

FØYN E. 1980. *Etter 20 aar paa is: Tid for revurdeirng av den elektrolytiske renseprosess.* Technisk Ukeblad, 15a, 4, 48 (Norwegian).

GRØTERUD O., SMOCZYŃSKI L. 1986. *Removal of phosphorus and residual aluminium by recirculating electrolysis of wastewater.* Vatten, 42: 293-296.

GRØTERUD O., SMOCZYŃSKI L. 1992. *Purification of wastewater by electrolysis at continuous flow*. Vatten, 48: 36-40.

HOLT P., BARTON G., WARK M., MITCHELL A., 2002. *A quantitative comparison between chemical dosing and electrocoagulation.* Colloids and Surfaces, 211: 233-248.

JIA J., YANG J., LIAO J., WANG W., WANG Z. 1999. *Treatment of dyeing wastewater with ACF electrodes.* Water Res., 33: 881-884.

LIN S.H., PENG C.F., 1996. *Continuous treatment of textile wastewater by combined coagulation, electrochemical oxidation and activated sludge.* Water Res., 30(2): 587-592.

MOLLAH M., MORKOVSKY P., GOMES J.A.G., KESMEZ M., PARGA J., COCKE D. 2004. *Fundamentals, present and future perspectives of electrocoagulation*. Journal of Hazardous Materials, ser. B114: 199-210.

SMOCZYŃSKI L., ZAŁĘSKA-CHRÓST B. 2002. *Electrocoagulation of pulp and paper wastewater in a recirculation system.* Pol. J. Natur. Sc., 12(3): 293-301.

SMOCZYŃSKI L., WARDZYŃSKA R. 1996. *Study on macroscopic aggregation of silica suspensions and sewage.* J. Colloid Interf. Sci., 183: 309-314.

STUMM W., MORGAN J.J. 1996. *Aquatic Chemistry.* John Wiley & Sons Inc., New York.

SZPYRKOWICZ L., NAUMCZYK J., ZILIO-GRANDI F. 1995. *Electrochemical treatment of tannery wastewater using Ti/Pt and Ti/Pt/Ir electrodes.* Water Res., 29(2): 517-524.

TAŁAŁAJ A., DZIENIS L. 2004. *Evaluation of landfill pollution expansion in groundwaters*. Pol. J. Natur. Sci., 17(2): 477-489.

VIK E.A., CARLSON D.A., EIKUM A.S., GJESSING E.T. 1984. *Electrocoagulation of potable water.* Water Res., 18(11): 1355-1360.

POLISH JOURNAL OF NATURAL SCIENCES
Abbrev.: Pol. J. Natur. Sc., Vol 23(2): 461-481, Y. 2008

DOI 10.2478/v10020-008-0036-9

INFLUENCE OF WATER TEMPERATURE ON EGGS INCUBATION TIME AND EMBRYONIC DEVELOPMENT OF FISH FROM GENUS *LEUCISCUS*

Krzysztof Kupren, Andrzej Mamcarz, Dariusz Kucharczyk, Maja Prusińska, Sławomir Krejszeff

Department of Lake and River Fisheries
University of Warmia and Mazury in Olsztyn

K e y w o r d s: Cyprinidae, embryonic development, temperature, *Leuciscus cephalus* (L.), *Leuciscus idus* (L.), *Leuciscus leuciscus* (L.).

A b s t r a c t

Wild living spawners of dace *Leuciscus leuciscus* (L.), ide *L. idus* (L.) and chub *L. cephalus* (L.), were obtained from rivers of central (the Pisa River basin) and northern (the Pasłęka River basin) Poland and next transported to a hatchery for artificial reproduction. The obtained eggs were dry fertilized and next incubated in water as different constant temperatures ranging from 4.5 to 29.0°C. The hatched embryos were kept at the same temperatures until the moment of complete yolk sac resorption and later fed *ad libitum* on live nauplial *Artemia* sp. stages. The power function was applied for determination of the relation between the temperature and time until and achievement of each of the thirteen characteristic stages of embryonic development. It was shown that the pace of embryonic development increased with the temperature increase. In the majority of cases, at identical thermal conditions, both the incubation time and the pace of embryonic development were much less diversified among individual of the same species originating from different population than between the individual species. The obtained data finds application in optimization of early raring process and as a consequence will be an important tool in protection of endangered species.

WPŁYW TEMPERATURY WODY NA CZAS TRWANIA INKUBACJI IKRY ORAZ ROZWOJU EMBRIONALNEGO RYB Z RODZAJU *LEUCISCUS*

Krzysztof Kupren, Andrzej Mamcarz, Dariusz Kucharczyk, Maja Prusińska, Sławomir Krejszeff

Katedra Rybactwa Jeziorowego i Rzecznego
Uniwersytet Warmińsko-Mazurski w Olsztynie

S ł o w a k l u c z o w e: karpiowate, rozwój embrionalny, temperatura, *Leuciscus cephalus* (L.), *Leuciscus idus* (L.), *Leuciscus leuciscus* (L.).

Address: Krzysztof Kupren, University of Warmia and Mazury, 1, ul. Michała Oczapowskiego 5, 10-957 Olsztyn, Poland, phone: (089) 523 44 36, e-mail: krzysztof.kupren@uwm.edu.pl

Abstrakt

Dziko żyjące tarlaki jelca *Leuciscus leuciscus (*L.), jazia *L. idus* (L.) i klenia *L. cephalus* (L.) pozyskano z rzek Polski centralnej (zlewnia Pisy) i północnej (zlewnia Pasłęki), a później przetransportowano do wylegarni w celu przeprowadzenia sztucznego rozrodu. Otrzymaną ikrę zapłodniono na sucho, a następnie inkubowano w wodzie o różnych stałych temperaturach z zakresu 4,5 – 29°C. Wyklute zarodki przetrzymywano w tych samych temperaturach do momentu całkowitej resorpcji woreczków żółtkowych oraz karmiono *ad libitum* żywymi stadiami nauplialnymi *Artemia* sp. Zależność między temperaturą a czasem do wyklucia i osiągnięcia każdego z 13 charakterystycznych stadiów rozwoju embrionalnego określono za pomocą funkcji potęgowej. Wykazano, że tempo rozwoju embrionalnego wzrastało wraz ze wzrostem temperatury. W większości przypadków w jednakowych warunkach termicznych zarówno czas trwania inkubacji, jak i tempo rozwoju embrionalnego były wyraźnie mniej zróżnicowane między dwiema badanymi populacjami jednego gatunku niż między poszczególnymi gatunkami. Otrzymane dane znajdą zastosowanie w optymalizacji procesu podchowowego, a przez to będą stanowić ważne narzędzie ochrony zagrożonych populacji.

Introduction

During the recent years a clear decrease in numbers, or even disappearance of numerous local populations in case of the majority of reophylic fish species including dace, ide and chub has been recorded. The major causes of that situation are the disappearance of spawning grounds caused by pollution of aquatic environment, regulation of rivers and excessive fishing (BŁACHUTA 1998). Production of stocking material based on reproduction, incubation and initial raring under controlled conditions is, next to improvement of the environmental conditions, one of the possibilities in protection of the fish living in rivers (KUJAWA et al. 1998, 2000, KUCHARCZYK et al. 1999, KUCHARCZYK 2002, HARZEVILI et al. 2003). Temperature is the environmental factor that has the largest influence on the development of fish (KOKUREWICZ 1970, HERZIG, WINKLER 1986). Its influence on incubation of the oocytes of dace and ide was studied by KENNEDY (1969), FLOREZ (1972), MILLS (1980) and RECHULICZ et al. (2002). Data concerning chub was presented by PENAZ (1968), PENAZ and STERBA (1969), ECONOMOU et al. (1991), as well as CALTA (2000). In the majority of cases, particularly in case of dace and chub, those studies concerned only to a minor extent the process of embryonic development under different thermal conditions. Additionally, until now, no comparative studies on three so closely related species were carried out during the earliest stages of ontogenesis occurring under different thermal conditions.

Determining the functional dependence between temperature and the time of embryonic development as well as showing the full range of temperatures tolerated by the embryos is extremely important for practical purposes as well as from the ecological and physiological point of view (HERZIG, WINKLER 1986). The studies carried out during this work aimed at investigating the influence of similar thermal conditions on incubation duration, embryonic and early

larval development of closely related species of genus *Leuciscus*, which, since recently have become important objects in European aquaculture.

Materials and Methods

Spawners of dace, ide and chub were obtained just before the time of natural spawning (February – June 2003 and 2004) from rivers situated in central (the Pisa River basin) (dace: 10 females and 12 males with body weight of 70-345 g, ide: 25 females and 14 males 320-1200 g, chub: 20 females and 16 males 136-365 g) and northern Poland (the Pasłęka River basin) (dace: 13 females and 10 males with body weight of 80-295 g, ide: 12 females and 14 males, 300-1150 g, chub: 14 females and 12 males with body weight of 160-390 g). Next, they were transported to the hatchery of the Chair of Lake and River Fishery of the University of Warmia and Mazury and placed in 1000 dm^3 basins equipped with lighting and thermal control in which the water temperature was gradually increased (KUJAWA et al. 1999). At the moment when the oocytes in the ovaries of the females reached the appropriate stage of maturity (stage II – III) (BRZUSKA, BIENIARZ 1977), the individuals of both sexes were subjected to hormonal stimulation using ovopel for that purpose according to the methodology described by KUCHARCZYK (2002).

Following the second hormonal injection the water temperature in the basins with spawners was increased to 12.0°C for dace, 14.5°C for ide and 17.5°C for chub. The eggs obtained were fertilized by means of thè dry' method with semen originating from the batch sample obtained from at least a few males. The water temperature during fertilization was the same as in the basins containing spawners after the second injection.

Eggs incubation followed by initial raring of embryos hatched until the moment of total resorption of yolk sac were carried by means of aquarium method at different constant water temperatures (4.5, 7.5; 9.5; 12.3; 15.7; 19.0; 23.0; 25.0; 27.5 and 29.0°C). The time of thermal adaptation to the specific temperature was 1.5°C h^{-1}. Each experimental setup within the range of 4.5-19.0°C, in which the incubation was carried out consisted of two lighted, oxygenated 40 l aquaria placed in a basin with water equipped with thermal control. In case of the four highest temperatures (23.0; 25.0; 27.5 and 29.0°C) the incubation was carried out in the aquaria outside the basin. In each aquarium the spawn was incubated on dishes containing around 150-180 pieces per Petri dish, in two repetitions, and in a basket with spawn stuck in talc at ca. 500-800 units allocated for controls. At the moment of appearance of eyes in the embryos the dishes with incubated spawn were placed in baskets of fine mesh where they stayed until completion of the experiment. As of the

moment of straightening the head the fishes were fed *ad libitum* on live nauplial stages of *Artemii* sp.

During incubation, water temperature was measured with the accuracy of up to 0.1°C four times daily. The temperature fluctuations did not exceed 0.3°C. Water in the aquaria with lower temperatures (7.5-12.3°C), was replaced every two-four days, that at higher temperatures – once or twice per day. During that operation dead spawn was also removed. Oxygen content in water ranged from 8.5 to 11.2 mg O_2 dm^3. Ammonia was not detected during the entire experimental period.

For the purpose of determination and comparison of dace, ide and chub development during the earliest stages of ontogenesis, based on own observations as well as those by other authors (DZIEKOŃSKA 1956, PENAZ et al. 1981, 1982, ŁUCZYŃSKI, KIRKLEWSKA 1984, TROTTER et al. 2003) 13 relatively easily identifiable stages of development were selected. Those stages were numbered from 0 to XIII (Table 1). The egg incubation time measured during the experiment was the time between fertilization and hatching of 50% of the individuals (KAMLER 2002). Embryonic development continued from fertilization until commencement of exogenous feeding (BALON 1975b, PENAZ et al. 1983). Additionally, the time necessary for full resorption of yolk sac by the embryos was also recorded.

Table 1

Description if development stages of dace *L. leuciscus* (L.), ide *L. idus* (L.) and chub *L. cephalus* (L.) used for determination off embryonic development advancement

Developmental stage (No)	Stage description
0	fertilization
I	blastula; 128 cells
II	50% epiboly
III	blastopore closure
IV	onset of yolk sac narrowing
V	finfold appearance
VI	onset of head separation from yolk sac
VII	onset of eye pigmentation
VIII	onset of body pigmentation
IX	swimm bladder primordium apperance
X	swimm bladder dilation (filled by the water)
XI	swimm bladder inflation (filled by the air)
XII	exogenous feeding
XIII	yolk sac resorption

Samples of the minimum size of 7 individuals were collected depending on water temperature and embryogenesis advancement a number of times per day. During the first day the samples were collected every 2 hours. Next, until appearance of color in the eyes of the embryos that frequency was 3 to 6 times

per day. As of that moment until the end of the experiment samples were collected 2 times (temperatures under 12.3°C), or 4 times per day (water temperature between 15.7 and 19.0°C).

The embryos collected were preserved in 4% formaldehyde solution (TAKIZAWA et al. 1994) and placed in separate tubes. The advancement of development was determined on current bases during spawn incubation and during microscopic analysis of preserved embryos. The observations were carried out under stereoscopic microscope connected to a digital camera using magnifications from 10 to 40 times. The results were documented and analyzed using computer software Olympus DP-Soft, based on Analysis® software.

A given group of eggs and later free-flowing embryos in the sample were allocated to a specific stage of development when ≥ 50% of individuals reached the criteria for a given stage (ŁUCZYŃSKI, KIRKLEWSKA 1984, GADOMSKI, CADDELL 1996).

The time necessary for achievement of individual development stages (0-XIII) by developing embryos was compared at optimum temperatures that differed for each of the studied species (12.3°C for dace, 15.7°C for ide and 19.0°C for chub) (MAMCARZ et al. 2005).

The dependence between water temperature and eggs incubation time and achievement of a given development stage can be expressed by means of a number of different models. Based on earlier studies (ELIOT et al. 1987, HAMEL et al.1997) that simple dependence was expressed by power function:

$$y = ax^{-b}$$

where:
y – embryonic development time (day),
x – incubation temperature (°C)
while a and b are constant values.

To increase transparency of results presented it was decided to designate spawners from central Poland as group C and those from the north of the country as group N (e.g. dace C, chub N). That designation will be applied further in this paper.

Results

In the highest of the tested water temperatures (19.0°C) a half of dace C embryos hatched after 7.2 days and in the lowest temperature (7.5°C) after 43 days of incubation (Figure 1a). In case of dace N release of 50% of embryos

from eggs shells at the highest (23.0°C) and lowest (7.5°C) water temperature occurred after 3.2 and 40 days respectively (Figure 1b). Depending on water temperature dace embryos hatching time for dace C was from 2.7 (19.0°C) to 7.9 days (9.5°C), and the embryos of dace N from 0.7 (23.0°C) to 7.1 (9.5°C) days (Figure 2 a, 2 b).

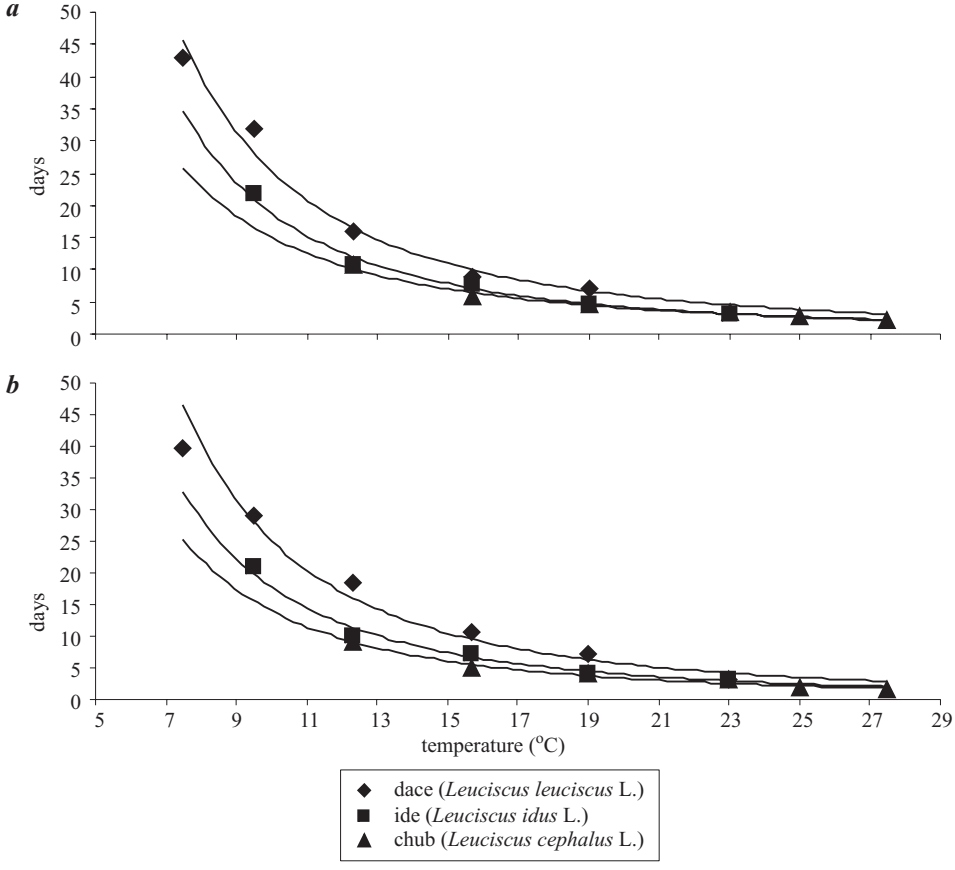


Fig. 1. Dependence between water temperature (°C), and time (days) from fertilization moment until hatching of ≥ 50% embryos of dace *L. leuciscus* (L.), ide *L. idus* (L.) and chub *L. cephalus* (L.) originating from central (a) and northern (b) Poland. Parameters of the curves are presented in Table 2

Hatching of ide C embryos occurred the earliest after 3.2 (23°C), and the latest after 22.8 days (9.5°C) (Figure 1a). In the same water temperatures (23.0 and 9.5°C) a half of hatched ide N embryos could be observed after 3 and 21.3 days (Figure 1b). Release of embryos from egg membranes in case of the central group took from 1.2 (23°C) to 3.5 (12.3°C) days and the north population from 1.2 (23°C) to 4.2 (9.5°C) days (Figure 2a, 2b).

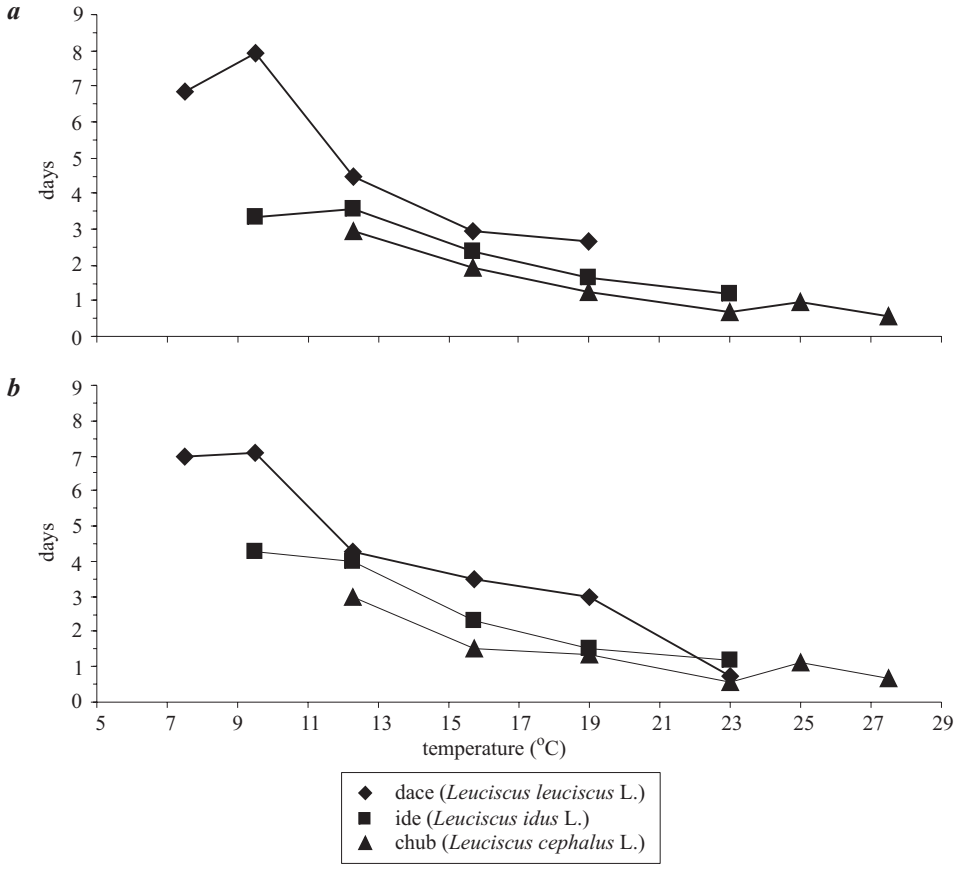


Fig. 2. Duration (days) of hatching of dace *L. leuciscus* (L.), ide *L. idus* (L.) and chub *L. cephalus* (L.) embryos originating from central (a) and northern (b) Poland, incubated at different constant temperatures

In the water of the highest temperature (27.5°C) hatching of chub C embryos occurred after 2.1 days and chub N after 1.5 days (Figure 1a, 1b). In the lowest water temperature (12.3°C), in which live, normally developed embryos were obtained, leaving the egg membranes occurred after 10 and 9 days from fertilization respectively (Figure 1a, 1b). The hatching time of chub C and N embryos was between 0.6 (23°C) and 2.9 (12.3°C) as well as 0.6 (23°C) and 3.0 (12.3°C) days respectively (Figure 2a, 2b).

In case of all the studied species the time from fertilization until hatching of 50% of embryos shortened with the increase of temperature. The relation between the incubation time until hatching of 50% of the individuals and water temperature was curvilinear (Figure 1a, 1b, Table 2). In the majority of the

observed cases with the increase of incubation temperature the time needed for all embryos leaving the egg membranes also shortened (Figure 2a, 2b). Among the analyzed species, in case of identical thermal conditions the chub hatched the earliest and the shortest. Ide was the second and dace was the last (Figure 1, 2). Those observations apply to fishes originating from two parts of the country. For example at water temperature of 15.7°C (favorable for spawn incubation simultaneously for all three species) hatching of a half of the individuals of dace, ide and chub C occurred in the following order: chub hatched first after 5.8 days, next ide hutched after 7.7 days and finally dace hutched after 8.9 days (Figure 1a). In case of fish originating from N group spawners the hatching occurred after 5.7 and 10 days respectively (Figure 1b).

Table 2

Values of constants for the function $y = ax^b$, describing the dependence between incubation temperature (°C) and time (days) until hatching of 50% of embryos of dace *L. leuciscus* (L.), ide *L. idus* (L.) and chub *L. cephalus* (L.) originating from central (a) and northern (b) Poland. The table also includes the coefficient of determination (R^2)

Species	Temperature range (°C)	Equations parameters		R^2
		a	b	
Central Poland				
Dace	7.5-19.0	2963.9	-2.07	0.98
Ide	9.5-23.0	2766.9	-2.16	0.99
Chub	12.3-27.5	961.65	-1.83	0.99
Northern Poland				
Dace	7.5-23.0	4149.2	-2.21	0.97
Ide	9.5-23.0	2858.2	-2.21	0.99
Chub	12.3-27.5	1609.1	-2.06	0.96

All the listed development stages in case of dace C and N individuals were observed at water temperatures from 12.3 to 19.0°C. In the water with the lowest temperature (12.3°C) the total embryonic development time for group C individuals took 23.7 days. The larvae reached the stage of yolk sac resorption after 21.7 days. At the highest temperature (19.0°C) those values were 9.9 and 12.1 days respectively (Figure 3a, 3b). At the lowest water temperature commencement of exogenus feeding by dace N was observed after 22.0 days and yolk sac resorption after 23.0 days. At the highest temperature the group N fishes reached those stages after 9.5 and 11.7 days respectively (Figure 3a, 3b).

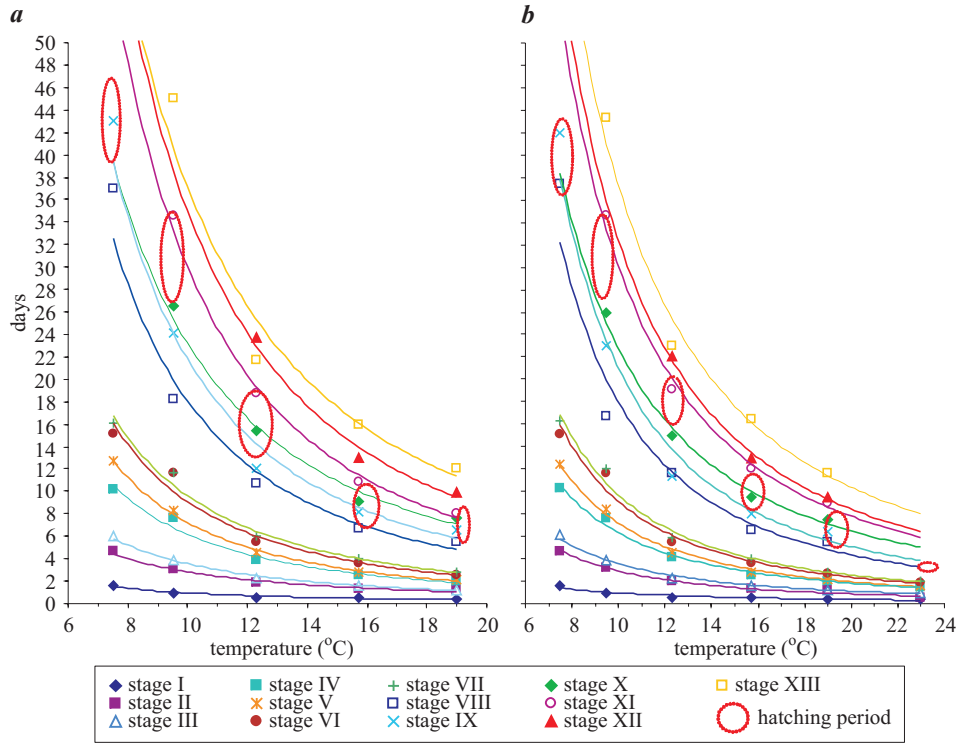


Fig. 3. Dependence between water temperature (°C), and time (days) from fertilization moment until achievement by ≥ 50% of individuals of dace *L. leuciscus* (L.) originating from central (a) and northern (b) Poland of individual development stages (0-XIII). Parameters of the curves are presented in Table 3 and Table 4

Early individual development of ide obtained from two parts of Poland going all the way through (stages 0-XIII) was observed in waters at temperatures from 12.3 to 23.0°C (Figure 4a, 4b). At 12.3°C the intake of external food by the embryos occurred after 23.7 days and total resorption of yolk sac occurred after 24 days from fertilization. At the highest water temperature (23.0°C) those values were 6.8 and 7.5 days respectively (Figure 4 a). In case of ide N the above-named development stages could be observed after 21.3 and 23 days respectively (at 12.3°C) and after 6.1 and 7.5 days of initial raring (at 23.0°C) – Figure 4b.

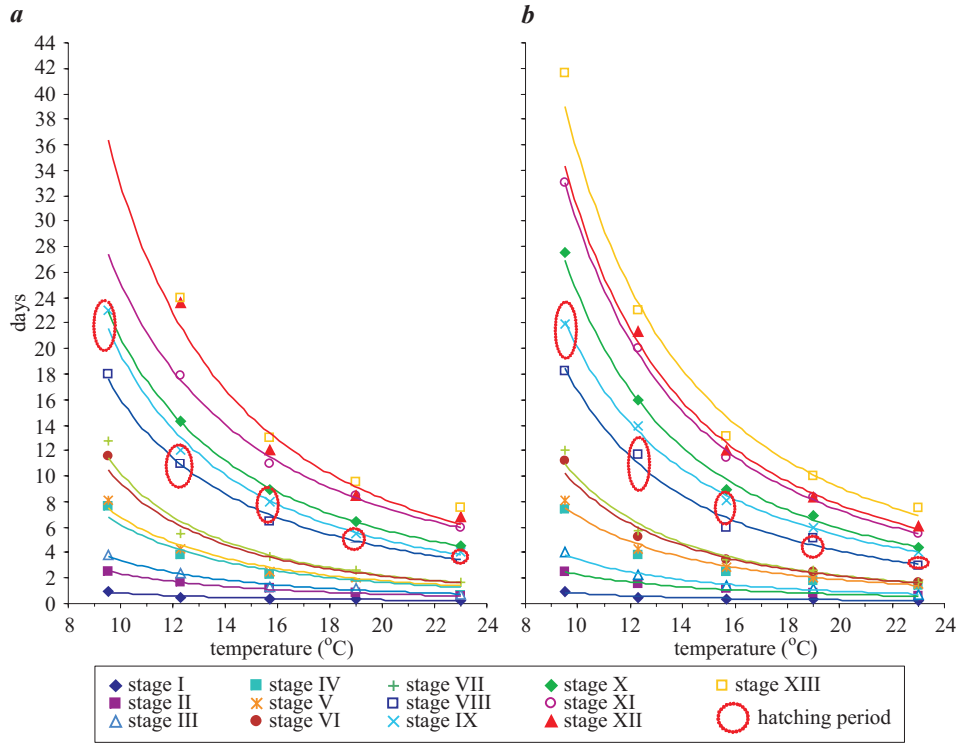


Fig. 4. Dependence between water temperature (°C), and time (days) from fertilization moment until achievement by ≥ 50% of individuals of ide *L. idus* (L.) originating from central (a) and northern (b) Poland of individual development stages (0-XIII). Parameters of the curves are presented in Table 3 and Table 4

Embryonic development of both geographic groups of chub covered was observed within the entire range of tolerated temperatures (12.3-27.5°C) (Figure 5a, 5b). At the lowest temperature the embryonic development of fishes obtained from group C spawners lasted 36.7 days and the total resorption of yolk sac occurred after 35 days from fertilization. At 27.5°C the larvae started taking the external fodder after 5.8 days and they exhausted the reserves of yolk after 5.5 days (Figure 5a). In case of chub N at the lowest and the highest temperatures those stages were reached after 35.5 and 34.2 and 5.0 and 5.5 days respectively (Figure 5b).

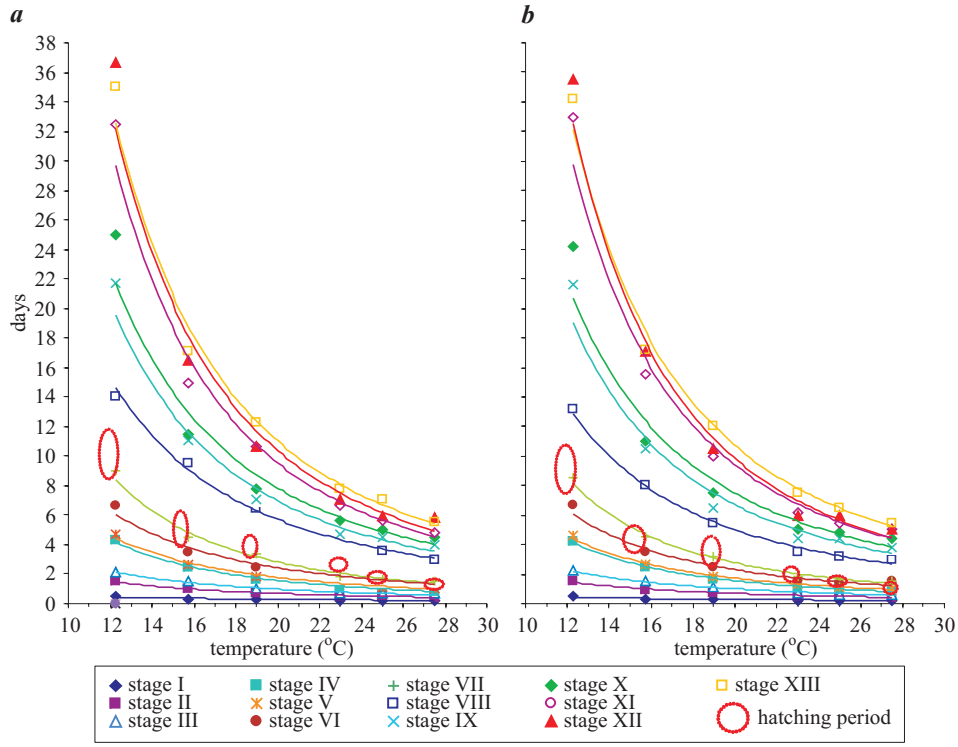


Fig. 5. Dependence between water temperature (°C), and time (days) from fertilization moment until achievement by ≥ 50% of individuals of chub *L. cephalus* (L.) originating from central (a) and northern (b) Poland of individual development stages (0-XIII). Parameters of the curves are presented in Table 3 and Table 4

With temperature increase the time needed for reaching consecutive development stages by the developing embryos (embryonic development rate) shortened. (Figures 3-5). The parameters of the function describing the dependence between the initial raring temperature and the embryogenesis pace between fertilization and achievement of a given development stage are presented in Table 3 and Table 4. In the majority of analyzed temperatures the total time of embryonic development was the shortest for ide and the longest for chub.

Table 3

Values of constants for the function $y = ax^b$, describing the dependence between incubation temperature (°C) and time (days) until the moment of achievement of individual development stages by ≥ 50 of individuals of dace *L. leuciscus* (L.), ide *L. idus* (L.) and chub *L. cephalus* (L.) from central Poland. The table also includes the coefficient of determination (R^2)

Species	Temperature range (°C)	Developmental stage (No)	Equations parameters		R^2
			a	*b*	
Dace	7.5-19.0	I	35.11	-1.56	0.97
	7.5-19.0	II	97.52	-1.54	0.98
	7.5-19.0	III	186.44	-1.72	0.99
	7.5-19.0	IV	445.55	-1.86	0.99
	7.5-19.0	V	692.91	-1.98	1.00
	7.5-19.0	VI	885.68	-1.99	0.98
	7.5-19.0	VII	816.78	-1.93	0.99
	7.5-19.0	VIII	2047.21	-2.06	0.98
	7.5-19.0	IX	2509	-2.06	0.98
	9.5-19.0	X	1633	-1.85	0.98
	9.5-19.0	XI	3992.61	-2.12	0.99
	12.3-19.0	XII	3743.93	-2.03	0.98
	9.5-19.0	XIII	2594.33	-1.85	0.96
Ide	9.5-23.0	I	23.29	-1.46	0.95
	9.5-23.0	II	105.48	-1.65	1.00
	9.5-23.0	III	215.99	-1.80	0.99
	9.5-23.0	IV	486.32	-1.89	0.97
	9.5-23.0	V	548.29	-1.91	0.98
	9.5-23.0	VI	1233.60	-2.12	0.99
	9.5-23.0	VII	1584.41	-2.19	0.98
	9.5-23.0	VIII	1118.43	-1.84	0.99
	9.5-23.0	IX	1769.54	-1.96	0.99
	12.3-23.0	X	1447.53	-1.84	1.00
	12.3-23.0	XI	1338.40	-1.73	1.00
	12.3-23.0	XII	3245.40	-1.99	0.97
Chub	12.3-23.0	XIII	2367.21	-1.86	0.97
	12.3-27.5	I	3.99	-0.86	0.91
	12.3-27.5	II	64.21	-1.51	0.99
	12.3-27.5	III	108.83	-1.57	0.98
	12.3-27.5	IV	624.24	-2.00	0.99
	12.3-27.5	V	465.53	-1.86	0.99
	12.3-27.5	VI	659.49	-1.87	0.98
	12.3-27.5	VII	2294.90	-2.23	0.98
	12.3-27.5	VIII	1920.81	-1.94	1.00
	12.3-27.5	IX	4020.42	-2.12	0.98
	12.3-27.5	X	4190.61	-2.10	0.97
	12.3-27.5	XI	10431.00	-2.34	0.99
	12.3-27.5	XII	10974.00	-2.32	0.97
	12.3-27.5	XIII	8689.70	-2.23	0.99

Table 4

Values of constants for the function $y = ax^b$, describing the dependence between incubation temperature (°C) and time (days) until the moment of achievement of individual development stages by ≥ 50 of individuals of dace *L. leuciscus* (L.), ide *L. idus* (L.) and chub *L. cephaluss* (L.) from northern Poland. The table also includes the coefficient of determination (R^2)

Species	Temperature range (°C)	Developmental stage (No)	Equations parameters		R^2
			a	b	
Dace	7.5-23.0	I	21.38	-1.35	0.93
	7.5-23.0	II	157.43	-1.73	0.98
	7.5-23.0	III	171.43	-1.68	0.99
	7.5-23.0	IV	387.25	-1.79	0.99
	7.5-23.0	V	547.94	-1.88	0.99
	7.5-23.0	VI	799.73	-1.94	0.99
	7.5-23.0	VII	810.59	-1.92	0.99
	7.5-19.0	VIII	1960.70	-2.04	0.97
	7.5-19.0	IX	2338.80	-2.05	0.97
	9.5-19.0	X	1482.50	-1.81	0.99
	9.5-19.0	XI	2680.81	-1.95	0.99
	12.3-19.0	XII	2827.83	-1.94	0.99
	9.5-19.0	XIII	2602.10	-1.84	0.98
Ide	9.5-23.0	I	23.29	-1.46	0.95
	9.5-23.0	II	98.34	-1.63	1.00
	9.5-23.0	III	231.67	-1.83	0.99
	9.5-23.0	IV	405.41	-1.82	0.98
	9.5-23.0	V	510.66	-1.87	0.99
	9.5-23.0	VI	1108.00	-2.08	0.99
	9.5-23.0	VII	1376.72	-2.15	0.99
	9.5-23.0	VIII	1746.10	-2.02	0.99
	9.5-23.0	IX	1637.00	-1.91	1.00
	9.5-23.0	X	2653.72	-2.03	1.00
	9.5-23.0	XI	3163.13	-2.03	1.00
	12.3-23.0	XII	3077.60	-2.00	0.99
	9.5-23.0	XIII	3148.90	-1.95	0.99
Chub	12.3-27.5	I	3.78	-0.85	0.90
	12.3-27.5	II	66.25	-1.52	0.98
	12.3-27.5	III	126.53	-1.61	0.99
	12.3-27.5	IV	619.24	-2.00	1.00
	12.3-27.5	V	509.50	-1.90	1.00
	12.3-27.5	VI	868.51	-1.98	0.96
	12.3-27.5	VII	2088.20	-2.21	0.99
	12.3-27.5	VIII	1637.31	-1.93	0.99
	12.3-27.5	IX	4111.53	-2.14	0.97
	12.3-27.5	X	3976.12	-2.09	0.96
	12.3-27.5	XI	11432.00	-2.37	0.98
	12.3-27.5	XII	15724.00	-2.46	0.98
	12.3-27.5	XIII	9307.61	-2.26	0.99

Comparing the times after which the embryos and larvae of the three given species reached the individual development stages at optimum temperatures characteristic for them (12.3°C for dace, 15.7 for ide and 19.0°C for chub) the following regularities were observed (Figure 6a, 6b, 6c). At 12,3°C,

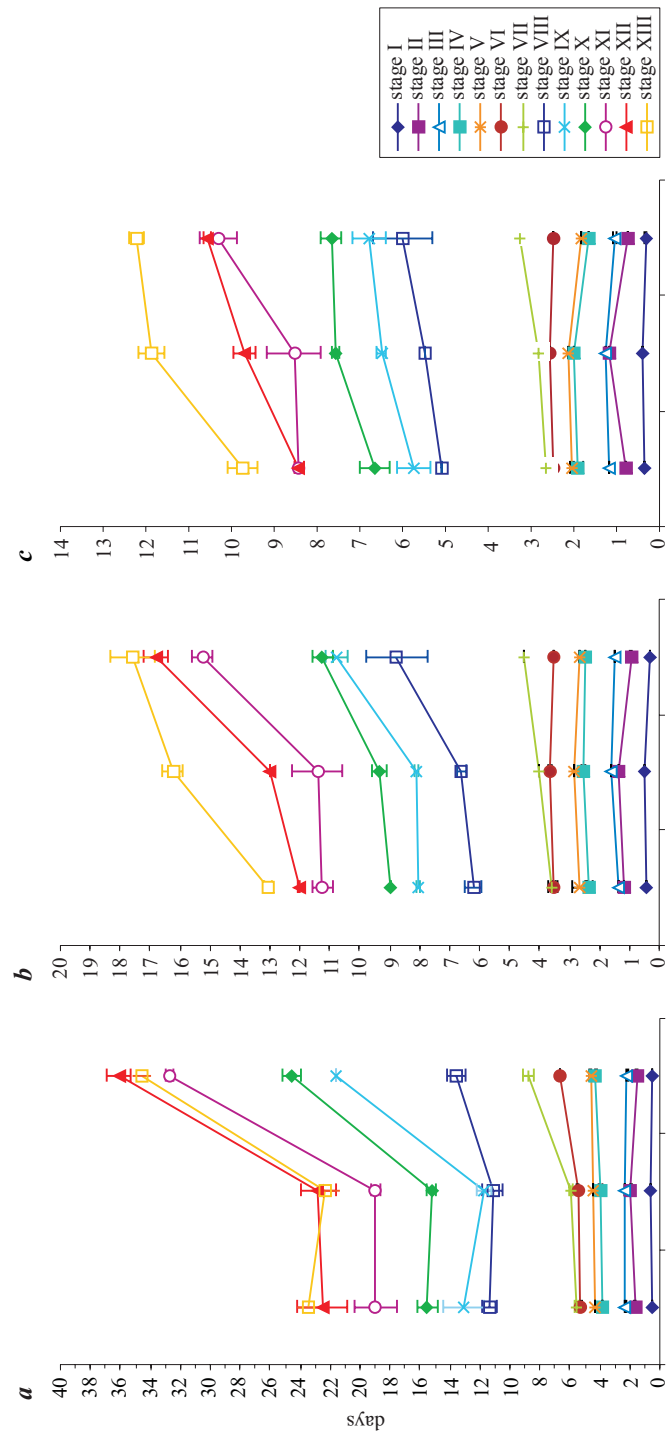


Fig. 6. Average times (days), after which in the water at 12.3°C (a), 15.7°C (b) and 19.0 °C (c) ≥ 50% of embryos and larvae of dace *L. leuciscus* (L.), ide *L. idus* (L.) and chub *L. cephalus* (L.) from central and northern Poland achieved individual development stages (0-XIII) (average values ±0)

the one the most appropriate for the eggs of dace, embryonic development of that species progressed all the time at almost identical pace as in case of the ide (Figure 6a). In two higher temperatures of 15.7 and 19.0°C the situation looked very similar until commencement of active swimming (stage of swim blader inflation) (Figure 6b, 6c). As of that moment the consecutive stages of dace development appeared with an obvious delay. The chub, until the moment of the head separation from the yolk sac developed at the same pace as the other two species. The further development stages were usually observed with obvious delay, which, depending on the temperature, could reach even some days. The observed lack of synchronization in development of individual of the same species originating from different parts of Poland appeared most frequently at the moment of commencement of active swimming and intake of external food by the larvae (Figure 6a, 6b, 6c). Additionally, in the majority of temperatures applied the pigment cells on the body surface of chubs C appeared slightly later than in the N individuals, e.g. at 15.7°C the difference was 1.5 days and at 19.0°C 1 day (Figure 6b, 6c).

Discussion

Water temperature can influence the developing fish embryos in many different ways. Its influence on the moment of embryos; hatching can be easily observed and, as a consequence, the differences in the level of advancement of their development can be easily noticed. Increase in incubation temperature influences shortening of the time until hatching while its decrease delays that moment. The best developed fishes hatch at optimum temperatures for a given species. In other temperatures the individuals leaving the egg shells usually show a lower level of development advancement (KOKUREWICZ 1969, 1970, KUCHARCZYK 1997). The reasons of early hatching of embryos at higher temperatures are their mobility in warmer water and earlier excretion of hatching enzyme. Such characteristic behaviors of embryos incubated at higher water temperatures were commonly observed for many fish species (BLAXTER 1969, 1992, PENAZ 1974, KAMLER 1992).

In the vast majority of cases, at the same water temperature, the longest incubation time was characteristic for dace while the shortest for the chub. As a consequence the embryos of dace were the most advanced in their development at the time of hatching (usually stage IX-X of embryonic development) and those of chub were the least developed (usually stage VII of embryonic development) (Figure 3-5). As a result of long stay in the egg, the hatched embryos of dace already a few hours after leaving the egg shells started filling

the swim bladders and active swimming. Freshly hatched ides are unable to swim actively for an extended time. Thanks to the presence of cement glands that are absent in dace and chub embryos they stay glued to the substrate for a couple of days. The hatching chubs were missing the pigment on their bodies entirely while the blood vessels of their yolk sacs were not fully developed yet. They did not have the ability of free swimming for a long time. The results of observations by KRYŻANOWSKI (1949), KENNEDY (1969), FLOREZ (1972), PENAZ and STERBY (1969), ECONOMOU et al. (1991) and CALTA (2000) are consistent with those findings. The dependence between temperature and embryonic development time of dace, ide and chub from hatching is very similar to that observed in case of other cyprinid species, e.g. bream *Abramis brama* (L.) (KUCHARCZYK et al., 1997), roach *Rutilus rutilus* (L.) or perch *Tinca tinca* (L.) (HERZIG, WINKLER 1986).

The time passing from fertilization until hatching of all embryos was similar in two populations of each of the studied species and similar to the length of hatching process it was shortened with the increase of temperature. Certain deviations from that principle were, however, observed in case of dace and ide embryos. Individuals of those species incubated at the lowest temperatures hatched faster than those from the higher temperatures. A similar phenomenon was observed in case of vimba *Vimba vimba* (L) and Bavarian *Chalcalburnus chalcoides* (Herzig, Winkler 1986). Those anomalies can be explained by disorders appearing in unfavorable conditions in metabolism and accelerated excretion of hatching enzyme.

As depending on species and water temperature leaving the egg shells by developing embryos can occur at different moments of ontogenesis, the moment of hatching was not classified as one of the covered stages of embryonic development. This approach to the issue allowed analysis of embryonic development according to the two definitions of that period most frequently encountered in literature. According to one of them hatching represents the moment of ontogenesis completion while according to the other one ontogenesis ends when intake of exogenous food commences (URHO 2002).

Analysis of early ontogenesis of three studied fish species confirms that the embryos of fish reproducing in water of lower temperatures, in low temperatures of initial raring develop faster than the embryos of species spawning in warm water (HERZIG, WINKLER 1986). We can observe that on the example of dace and chub for which the optimum temperatures for development differ significantly. For example at 12.3°C the development pace of chub in comparison to its duration in case of dace is very clearly decelerated. The moment of commencement of exogenous feeding by chubs at that temperature comes ca. 13 days later than in case of dace. In case of young

chubs it comes, in average, after 36 days and in dace larvae after just around 23 days. The situation at water temperature of 19.0°C, that is optimal for chub, is different. The time from fertilization until achievement of the majority of development stages at that temperature is equalized and the difference in the moment of food intake is less than one day only. In dace it comes, in average, after 9.7 days and in chub after around 10.5 days. The reason for that phenomenon could be the disturbances of metabolism caused by, e.g. irregularities in the process of synthesis or functioning of various enzymes the functioning of which is closely dependent on the temperature occurring in extreme temperatures (TAGAWA, HIRANO 1987). The abovementioned disturbances also had a clear influence on increased embryos; mortality. Low temperatures of initial raring also did not allow the developing fishes to intake exogenous food (temperatures under 9.5°C).

The differences observed in embryogenesis of those three closely related species including both the different incubation time and different pace of the development itself can be explained by adaptation to natural environment conditions. That adaptation is also expressed by different strategies of embryos; behavior after hatching. Ide embryos, after leaving egg shells, following a short rest stage, started the period of increase mobility. In a short time they swam towards the surface and using well-developed cement glands characteristic for phytolitoral fish, they stuck to the substrate frequently changing the position in case of every disturbance. That increased activity was expressed by a relatively short duration of yolk sac resorption and initiation of external food intake. Young ides that were the most exposed ones to a diversity of threats among the three studied species seem to be genetically adapted to fast embryogenesis. Within a short time they obtain pigment on their body and start swimming, which helps them in hiding and possible escape from predators. That relatively fast embryonic development is characteristic for many phytolitoral fish species (KRYŻANOWSKIJ 1949, KOBLICKAJA 1981, PENAZ et al. 1983).

A slightly later moment of food intake and resorption of yolk sac by dace is most probably caused by embryos of that species staying inside the egg until late development stages. That involved the lower demand of those embryos for energy resulting in later commencement of exogenous feeding. A similarly long stay inside the oocytes is rarely found in cyprinids although almost identical incubation time was observed at the same temperatures by KAMLER et al. (1998) in case of *Chondrostoma nasus* (L.)

The delayed, as compared to dace and ide, achievement of individual development stages by chub can be explained by the adjustment to development near the stony bottom with minor threat from predators. Chub embryos released from egg shells, similar to freshly hatched dace, did not have the

cement glands and after hatching fell to the bottom. A large yolk sac keeps young fishes close to the bottom, in gaps between stones and protects them against being carried away by the tide. The lack of pigmentation on the body observed throughout of the majority of yolk sac resorption period may result from staying in the dark and strong photophobia delays the moment of filling the swim bladder. Similar behavior is observed by hatched *Chondrostoma nasus* (L.) and barbel *Barbus barbus* (L.) (KRYŻANOWSKIJ 1949, BALON 1975b, KAMLER et al.1998). That to a high extent settled lifestyle results in a slower rate of consumption of reserve materials accumulated in the yolk sac and delays the moment of exogenous, which, contrary to the other two species occurs almost simultaneously with total resorption of yolk sac. A similar pace of embryogenesis id characteristic for *Chondrostoma nasus* (KAMLER et al.1998).

The data collected during the study concerning the incubation time and the embryonic development ending with commencement of exogenous food intake, supplementing the missing information concerning the biology of early life stages of three closely related species of genus *Leuciscus* will be extremely useful in planning and performance of stocking material production.

Conclusion

1. The possibility of artificial reproduction of dace, ide and chub after two applications of hormonal injection (ovopel) was confirmed.

2. Incubation of spawn at the same water temperatures took the longest for dace, next for ide and the shortest for chub (e.g. at water temperature of 15.7°C hatching occurred for dace after 9.5, ide after 7.2 and chub after 5.5 days). The duration of stay inside the egg was reflected by advancement of hatched embryos in development.

3. On the basis of the conducted studies on early development (until resorption of yolk sac) 13 development stages of dace, ide and chub characterizing the advancement of their development were identified.

4. Under the same thermal conditions the embryonic development progressed and is completed the fastest for ide, it takes slightly longer for dace and the longest for chub (e.g. at water temperature of the 15.7°C moment of food intake by ide embryos occurs after around 12 days, for dace after 13 and for chub after 17 days. At extreme temperatures for a given species the rate of developmental transformations is disturbed.

5. Minimal differences in all the analyzed phenomena (moment and time of hatching of embryos, rate of embryonic development) were found between two geographic populations of each of the three species studied.

Acknowledgements

This study was supported by Project No. 2PO4F07727 financed by State Committee for Scientific Research.

Translated by JERZY GOZDEK

Accepted for print 3.09.2007

References

BALON E.K. 1975a. *Reproductive guilds of fishes*. J. Fish. Res. Bd. Can., 32: 821-864.

BALON E.K. 1975b. *Terminology of intervals in fish development*. J. Fish. Res. Bd. Can., 32 (9): 1663-1670.

BALON E.K. 1990. *Epigenesist of an epigeneticist: the development of some alternative concepts on the early ontogeny and evolution of fishes*. Guelph. Ichtiol. Rev., 1: 1-42.

BLAXTER J.H.S. 1969. *Development: eggs and larvae. Reproduction and Growth*, Fish Physiology, 3: 177-252.

BLAXTER J.H.S. 1992. *The effect of temperature on larval fishes*. Neth. J. Zool., 42: 336-357.

BŁACHUTA J. 1998. *Rola i znaczenie rodzimych gatunków ryb reofilnych w ekosystemach rzek. Karpiowate ryby reofilne*. Mat. Konf., Wyd. PZW, Warszawa, ss. 17-24.

BRYLIŃSKA M. 2000. *Ryby słodkowodne Polski*. PWN, Warszawa.

BRZUSKA E., BIENIARZ K. 1977. *Metoda przyżyciowego określania dojrzałości płciowej samic karpia w związku z iniekcjami homogenatu przysadki mózgowej karpia*. Oprac. broszurowe IRŚ, 105: 3-27.

CALTA M. 2000. *Morphological development and growth of chub* (*Leuciscus cephalus* L.) *larvae*. J. Appl. Ichthyol., 16: 83-85.

DZIEKOŃSKA J. 1956. *Studies on embryonic development of fish*. I. *Observation on the spawning and the embryonic development of bream* (*Abramis brama* L) *in the Vistula delta*. Pol. Arch. Hydrobiol., 3: 291-305.

ECONOMOU A.N., DAOULAS C., PSARRAS T. 1991. *Growth and morphological development of chub, Leuciscus cephalus L. during the first year of life*. J. Fish Biol., 39: 398-408.

ELLIOTT J.M., HUMPESCH U.H., HURLEY M.A. 1987. *A comparative study of eight mathematical models for the relationship between water temperature and hatching time of eggs of freshwater fish*. Archive für Hydrobiolgie., 109: 257-277.

FLOREZ F. 1972. *The effect of temperature on incubation time, growth and lethality of embryos, larvae and juveniles of the ide, Idus idus* (L.). Rep. Inst. Freshw. Res. Drottningholm, 52: 50-64.

GADOMSKI D.M., CADDELL S.M. 1996. *Effects of temperature on the development and survival of eggs of four coastal California fishes*. Fish. Bull. 94: 41-48.

HAMEL P., MAGNAN P., EAST P., LAPOINTE M., LAURENDEAU P. 1997. *Comparison of different models to predict the in situ embryonic developmental rate of fish, with special reference to white sucker* (*Catostomus commersoni*). Can. J. Fish. Aquatic Sci., 54: 190-197.

HARZEVILI A.S., CHARLEROY D. DE, AUVERX J., VUGHT I., SLYCKEN J. VAN 2003. *Larval rearing of chub, Leuciscus cephalus* (L.) *using decapsulated Artemia as direct food*. J. Appl. Ichthyol., 19: 123-125.

HERZIG A., WINKLER H. 1986. *The influence of temperature on the embryonic development of three cyprinid fishes, Abramis brama, Chalcaburnus chalcoides and Vimba vimba*. J. Fish Biol., pp. 28: 171-181.

KAMLER E. 1992. *Early life history of fish. An energetics approach*. Chapman & Hall, London, pp. 267.

KAMLER E., KECKEIS H., BAUER-NEMESCHKAL E. 1998. *Temperature-induced changes of survival, development and yolk partitioning in Chondrostoma nasus*. J. Fish Biol., 53: 658-682.

KAMLER E. 2002. *Ontogeny of yolk-feeding fish: an ecological perspective*. Reviews in Fish Biology and Fisheries, 12: 79-103.

KENNEDY M. 1969. *Spawning and early development of the dace Leuciscus leuciscus* (L.). J. Fish Biol., 1: 249-259.

KOBLICKAJA A.F. 1981. *Opredelitel molodi presnovodnykh ryb*. Legkaja i Pishhevaya Promyshlennost, Moskva.

KOKUREWICZ B. 1969. *The influence of temperature on the embryonic development of the perches* (*Perca fluviatilis* L.) *and Lucioperca lucioperca*. Zool. Pol., 19 (1): 47-67.

KOKUREWICZ B. 1970. *The effect of temperature on embryonic development of Tinca tinca* (L.) *and Rutilus rutilus* (L.). Zool. Pol., 20: 317-337.

KOKUREWICZ B. 1971. *Warunki termiczne, a rozród i rozwój niektórych gatunków ryb*. Oprac. broszurowe IRŚ, 47: 1-18.

KRYŻANOWSKIJ S.G. 1949. *Ekologo-morfologiceskie zakonomernosti razvitija karpovych, vjunovych i somovych ryb* (*Cyprinoidei i Siluroidei*). Tr. Inst. Morf. Zhivotn., 1: 5-332.

KUCHARCZYK D., ŁUCZYŃSKI M, KUJAWA R., CZERKIES P. 1997. *Effect of temperature on embryonic and larval development of bream* (*Abramis brama* L.). Aquat. Sci., 59: 214-221.

KUCHARCZYK D., KUJAWA R., MAMCARZ A., WYSZOMIRSKA E., ULIKOWSKI D. 1999. *Artificial spawning of ide* (*Leuciscus idus* L.) *under controlled conditions*. EJPAU 2 (2),

KUCHARCZYK D. 2002. *Rozród kontrolowany i androgeneza wybranych gatunków ryb karpiowatych*. Rozprawy i Monografie, 63: 1-81.

KUJAWA R., KUCHARCZYK D., MAMCARZ A. 1999. *A model system for keeping spawners of wild and domestic fish before artificial spawning*. Aquacult. Engineering, 20: 85-89.

KUJAWA R., KUCHARCZYK D., MAMCARZ A., SKRZYPCZAK A. 1998. *The rearing methods of ide* (*Leuciscus idus* L.) *and dace* (*Leuciscus leuciscus* L.) *on artificial diets*. Europ. Aquacult. Soc., Spec. Pub., 26: 153-154.

KUJAWA R., KUCHARCZYK D., MAMCARZ A. 2000. *The rearing methods of chub* (*Leuciscus leuciscus* L.) *on artificial diets*. Europ. Aquacult. Soc. Spec. Publ., 28: 356-356.

ŁUCZYŃSKI M., KIRKLEWSKA A. 1984. *Dependence of Coregonus albula embryogenesis rate on the incubation temperature*. Aquacult., 42: 43-55.

MAMCARZ A., KUPREN K., KUCHARCZYK D., KUJAWA R. 2005. *Influence of temperature on, body size and yolk sac volumes of three larval rheophilic cyprinids* (genus *Leuciscus*). Europ. Aquacult. Soc., Spec. Pub., 36: 310-313.

MANN R.H.K. 1996. *Environmental requirements of European non-salmonid fish in rivers*. Hydrobiol., 323: 223-235.

MILLS C.A. 1980. *Spawning and rearing eggs of the dace Leuciscus leuciscus* (L.). Fish. Mgmt., 1: 67-72.

PENAZ M. 1968. *Development of the chub*, *Leuciscus cephalus* (*Linnaeus, 1758*) *in the post-hatching period*. Zool. Listy, 17: 269-278.

PENAZ M., STERBA O. 1969. *Notes to the incubation period, growth and mortality of the chub*, *Leuciscus cephalus* (*Linne, 1758*), *in the early life-history stages*. Vestnik Ceskoslovenske Spolecnosti Zoologicke, 33: 56-70.

PENAZ M. 1974a. *Influence of water temperature on incubation and hatching in Chondrostoma nasus* (*Linnaeus, 1758*). Zool. Listy, 23: 53-59.

PENAZ M., WOHLGEMUTH E., HAMACKOVA J., KOURIL J. 1981. *Early ontogeny of the tench*, *Tinca tinca*. I. *Embryonic period*. Folia Zool., 30(2): 165-176.

PENAZ M., WOHLGEMUTH E., HAMACKOVA J., KOURIL J. 1982. *Early ontogeny of the tench*, *Tinca tinca*. II. *Larval period*. Folia Zool., 31(2): 175-180.

PENAZ M., PROKES M., KOURIL J., HAMACKOVA J. 1983. *Early development of the carp*, *Cyprinus carpio*. Acta Sci. Nat. Acad. Sci. Bohem. Brno, 17: 1-39.

RECHULICZ J., OSTASZEWSKA T., WOJDA R. 2002. *Water temperature effects on incubation of ide* (*Leuciscus idus* L.) *eggs and selected parameters of the larvae*. Acta Sci. Pol. Piscaria, 1: 35-46.

TAGAWA M., HIRANO T. 1987. *Presence of thyroxine in eggs and changes in its content during early development of chum salmon*, *Onchorynchus keta*. Gen. Compar. Endocr., 68: 129-135.

TAKIZAWA K., FUJITA Y., OGUSHI Y., MATSUNO S. 1994. *Relative change in body length and weight in several fish larvae due to formalin fixation and preservation*. Fisheries Sciences, 60: 355-359.

TARGONSKA-DIETRICH K., ZIELAZNY T., KUCHARCZYK D., MAMCARZ A., KUJAWA R. 2004. *Out-of-season spawning of cultured ide* (*Leuciscus idus* L.) *under controlled conditions*. EJPAU 7 <www.ej-pau.media.pl>.

TROTTER A.J., PANKHURST D.A., MOREHEAD D.T., BATTAGLENE S.C. 2003. *Effect of temperature on initial swim bladder inflation and related development in cultured striped trumpeter* (*Latris lineata*) *larvae*. Aquacult., 221: 141-156.

URHO L. 2002. *Characters of larvae – what are they*? Folia. Zool., 51(3): 161-186.

POLISH JOURNAL OF NATURAL SCIENCES
Abbrev.: Pol. J. Natur. Sc., Vol 23(2): 482-495, Y. 2008

DOI 10.2478/v10020-008-0037-8

THE EFFECT OF PRESSURIZATION ON SELECTED PROPERTIES OF YOGHURTS*

Agnieszka Jankowska, Arnold Reps, Anna Proszek, Krystyna Wiśniewska

Chair of Food Biotechnology
University of Warmia and Mazury in Olsztyn

K e y w o r d s: pressurization, yoghurt, Lactobacillus delbrueckii ssp. bulgaricus, Streptococcus thermophilus.

A b s t r a c t

Analyses carried out within the study indicated that the microflora composition of a yoghurt starter culture affects the organoleptic traits and antibacterial properties of yoghurt.

Yoghurts obtained from five different commercial starter cultures were pressurized at 100-1000 MPa/ 15 min at room temperature.

The pressure applied and the type of starter culture used to produce yoghurts were found to have an impact on the survival of bacteria as well as acidity, antibacterial activity and the organoleptic properties of the yoghurt produced.

The results obtained in this study indicate that in order to prolong shelf-life of yoghurts, the pressure applied should not exceed 400 MPa. Higher pressures result in a considerable inactivation of microflora, especially of *Lactobacillus delbrueckii* ssp. *bulgaricus*, reduction of the antibacterial properties and deterioration of the organoleptic properties of yoghurts.

WPŁYW PRESURYZACJI I SZCZEPIONKI NA WYBRANE WŁAŚCIWOŚCI JOGURTU

Agnieszka Jankowska, Arnold Reps, Anna Proszek, Krystyna Wiśniewska

Katedra Biotechnologii Żywności
Uniwersytet Warmińsko-Mazurski

S ł o w a k l u c z o w e: presuryzacja, jogurt, *Lactobacillus delbrueckii* ssp. *bulgaricus Streptococcus thermophilus*.

Address: Agnieszka Jankowska, University of Warmia and Mazury, ul. Jana Heweliusza 1, 10-759 Olsztyn, Poland, phone: 48(089) 523 47 35, e-mail: agajan@uwm.edu.pl
* This research has been supported by Grant No. 2 P06T 104 26

A b s t r a k t

Podczas przeprowadzonych badań stwierdzono, że skład mikroflory szczepionki jogurtowej ma wpływ na cechy organoleptyczne oraz właściwości antybakteryjne jogurtu.

Jogurty otrzymane z pięciu różnych handlowych szczepionek poddano działaniu ciśnień 100-1000 MPa/ 15 min w temperaturze pokojowej.

Stwierdzono, że wysokość zastosowanego ciśnienia oraz rodzaj szczepionki użytej do produkcji jogurtu ma wpływ na przeżywalność bakterii, kwasowość, aktywność antybakteryjną oraz cechy organoleptyczne jogurtu.

Na podstawie wyników uzyskanych w trakcie realizacji doświadczenia można stwierdzić, że w celu wydłużenia czasu przechowywania jogurtu należałoby stosować ciśnienie nie wyższe niż 400 MPa. Wyższe ciśnienie powoduje znaczną inaktywację mikroflory, a szczególnie pałeczek *Lactobacillus delbrueckii ssp. bulgaricus*, redukcję właściwości antybakteryjnych, oraz pogorszenie cech organoleptycznych jogurtu.

Introduction

Milk and milk products serve a key function in proper human nutrition. Recently, the consumption of fermented milks, including yoghurt, has been increasing and is linked with a growing awareness by consumers of the beneficial effect of these products on human health.

As demonstrated in ample scientific investigations, the fermented milks are characterized by high dietetic and therapeutic properties, including: higher availability of nutrients compared to milk (BUTTRISS et al. 1997), a positive effect on the gastrointestinal tract of humans (HOLT 2003), hypocholesterolemic activity (*IDF* 1998), potential for the reduction or elimination of lactose intolerance (MARTINI et al. 1987) and for the enhancement of body resistance (*IDF* 1998). The beneficial effects of the fermented milks result from the presence of specific groups of microorganisms. In the production of yoghurts, *Lactobacillus delbrueckii ssp. bulgaricus* and *Streptococcus thermophilus* strains are often supplemented with probiotic bacteria, including *Bifidobacterium sp.* and *Lactobacillus acidophilus* (LOUTENS-HATTING, VILJOEN 2001).

After fermentation, the metabolic activity of bacteria, which determines the organoleptic properties and nutritive value of yoghurt in the fermentation process, leads to the accumulation many of substances, including lactic acid, which could shorten a shelf-life of yoghurts (BIROLLO et al. 2000).

Thermal treatments applied for yoghurt preservation are unacceptable. According to *FAO/WHO* (2000) recommendations, the number of characteristic microflora should account for at least 107 cfu g^{-1}, including 106 cfu g^{-1} of *Lactobacilus delbrueckii ssp. bulgaricus* throughout the entire declared shelf-life.

Food technology has long been searching for new non-thermal methods of preservation. One of them is pressurization, namely the technology of high pressures.

The first reports on the application of high pressure for food preservation appeared at the end of the XIX century when Hite (1899) undertook trials of pressure sterilization of milk, meat, fruit and vegetables. More extended studies with the use of that method were carried out already in the 1990s. They confirmed the destructive effect of high pressure on microorganisms and prompted studies into the application of this technique for food preservation (KNORR 1993).

The effect of high pressure on food products is linked with the control of bacterial activity, enhancement or inhibition of enzymatic and chemical reactions and changes in the properties of a number of food components as a result of conformation transformations (HOOVER et al. 1989).

High pressure treatment causes the inactivation of pathogenic and technologically-harmful bacteria in milk. The efficacy of short time pasteurization i comparable to the pressurization at 500-600 MPa for a few minutes (JOHNSTON 1995).

Spores are more resistant to pressure than vegetative forms of bacteria and survive even the pressurization at 1000 MPa. In order to inactivate bacterial spores, a two-step pressurization is required. At first lower pressure causes the germination of spores. Then higher pressure inactivates the germinated vegetative forms. KNORR (1993), GOULD and TYTUL (1995), indicated that it is necessary to combine high pressure and temperature treatments for inactivation of all bacteria spores.

In yoghurt technology, high pressure may be used for the preservation of processing milk (LANCIOTTI et al. 2004), which improves the properties of yoghurt curd, and for the preservation of the end product (TANAKA, HATANAKA 1992, ANCOS DE et al. 2000).

In this study, a trial was made to determine the effect of high pressures on yoghurt produced from several yoghurt starter cultures available on the Polish market.

Methods

The experimental material were yoghurts produced from commercial starter culture in the form of lyophilized YC-X16 and YC-X11 (CHr. Hansen), MYE 92 and MYE 95 (Danisco-Biolacta Ltd.), and in the form of a frozen concentrate StAr 501 (CSK Food Enrichment).

Yoghurts were prepared following the procedures recommended by the producers. Sterile milk with 2% fat content and 2% addition of powdered milk free of inhibiting substances were inoculated with the above-mentioned yoghurt culture (5%). Fermentation was carried out at a temperature of 42°C until typical, homogenous curd was obtained, which was then stored at the same temperature for 2 hours.

Yoghurts cooled to a temperature of 4-7°C were poured into plastic containers, then pressurized in a high-pressure generator at a room temperature using high pressure range from 100 to 1000 MPa, in 100 MPa intervals, for 15 min.

The non-pressurized yoghurts, as well as those immediately after pressurization were determined for:

– the number of Streptococcus thermophilus on M17-Agar medium (by Merck), at an incubation temperature of 30°C, for 72 hours;

– the number of *Lactobacillus delbrueckii ssp. bulgaricus* on MRS-Agar medium (by Merck), at an incubation temperature of 37°C, for 72 hours;

– antibacterial properties with the modified well method against 12 test strains;

– active acidity pH and titratable acidity (°SH).

The yoghurts were also subjected to the organoleptic evaluation according to the Polish Norm (*Mleko...* PN-83/A-86061).

All experiments and analyses were duplicated. The results presented are averages of all available replicates.

Results and Discussion

Survivability of yoghurt bacteria

The number of *Streptococcus thermophilus* was in conformity with the standard and ranged from 7.00 log cfu g^{-1} in the yoghurt produced from the MYE 95 culture to 9.38 log cfu g^{-1} in that prepared from the CSK 501 culture. The number of *Lactobacillus delbrueckii* ssp. *bulgaricus* met the recommended standard values in the yoghurt produced from the YC-X16 inoculum – 8.34 log cfu g^{-1}, in that prepared from the MYE 92 culture – 7.64 log cfu g^{-1} as well as in that produced from the YC-X11 culture – 6.25 log cfu g^{-1}. In the other yoghurts, the numbers of lactobacilli were lower than 106 cfu g^{-1} (Figure 1 a, b, c, d, e).

Yoghurt pressurization at 100-400 MPa resulted in a negligible reduction in the number of *Streptoccocus thermophilus* – by ca. ≥ one order of magnitude. The pressurization at 500-1000 MPa had a more diversified effect on those bacteria, depending on the starter culture used for yoghurt production.

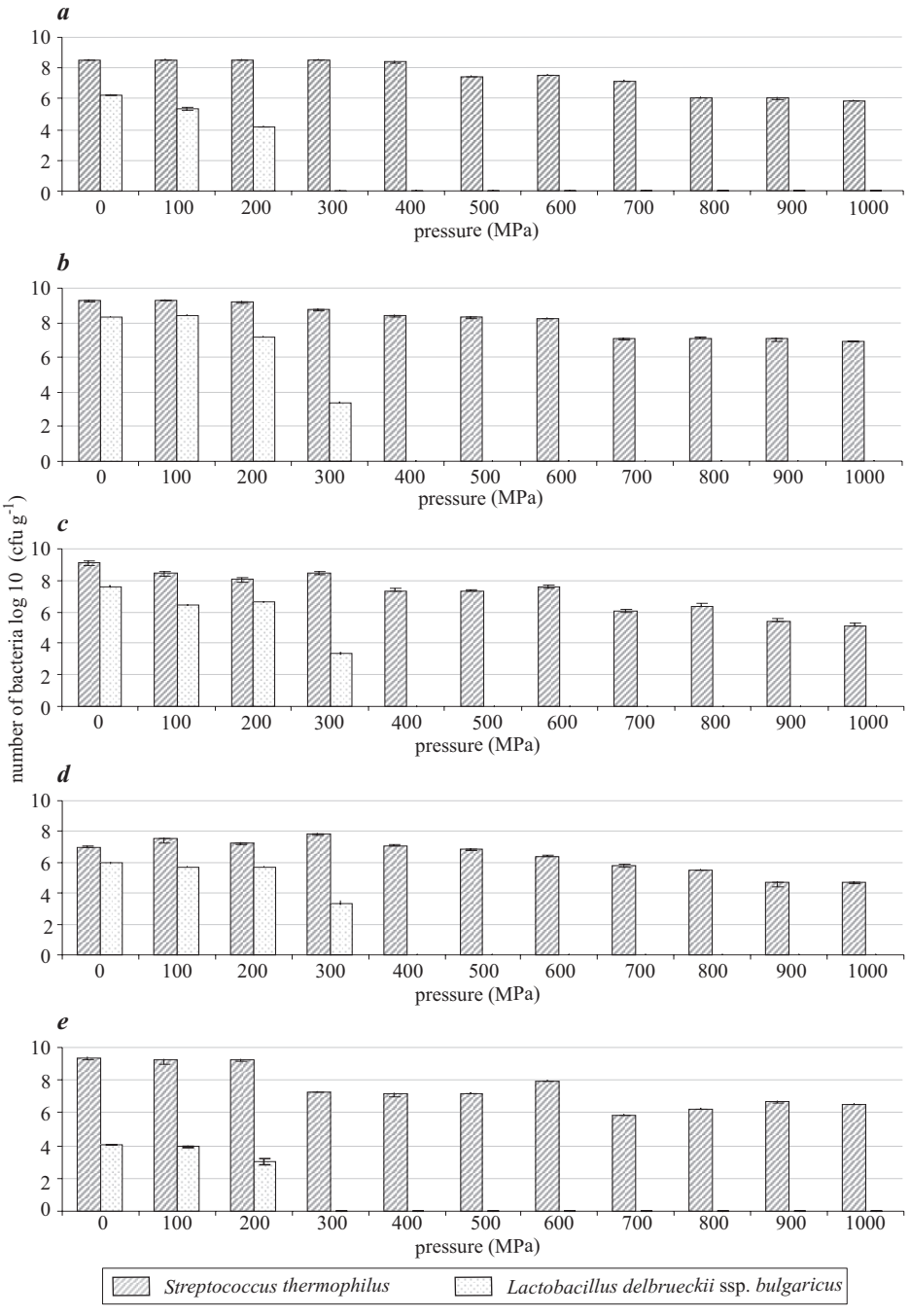


Fig. 1. The number of bacteria in yoghurts after pressurization at 100-1000 MPa/15 min.: *a* – YC-X11, *b* – YC-X16, *c* – MYE92, *d* – MYE95, *e* – CSK501

Upon pressure treatment at 1000 MPa, the highest survivability of streptococci was observed in the YC-X16 yoghurt – 74.66 %log, whereas the lowest one in that produced from the MYE 92 inoculum – 56.60 %log. The *Lactobacillus delbrueckii* ssp. *bulgaricus* strain appeared to be more susceptible to the pressure treatment. In yoghurts made with the use of YC-X11 and CSK 501 cultures, complete inactivation of lactobacilli occurred at the pressure of 300 MPa. In the three other yoghurts, pressure treatment at 400 MPa caused a total reduction in the lactobacilli number.

A number of studies carried out to date have pointed to the higher resistance of streptococci than lactobacilli to the high pressure treatment, which has been ascribed to a higher resistance of the spherical cell wall to stress induced by increasing pressure (LUDWIG 2002). After yoghurt pressurization at 200 MPa/15 min, REPS et al. (1999) observed the total inactivation of *Lactobacillus delbrueckii* ssp. bulgaricus rods and a decrease in the number of streptococci by as little as one order of magnitude. The inactivation of bacteria upon high pressure depends on the type of starter culture used for the production of yoghurt, which in turn is linked with its strain composition. In examining the effect of pressurization at 400 MPa/ 15 min, REPS et al. (2001) observed highly diversified survivability – ranging from 35.3 to 99.9 %log – of seven different strains of yoghurt streptococci *Streptococcus thermophilus*.

The effect of pressurization on yoghurt acidity

The acidity of all investigated yoghurts met the recommended norms (Table 1). The active acidity pH of the analyzed yoghurts ranged from 4.305 in the YC-X16 yoghurt to 4.550 in yoghurts produced from MYE 95 culture, whereas the titratable acidity accounted for 44.0 °SH in the yoghurt prepared from the YC-X11 culture, to 32.0 °SH in that produced from the CSK 501 culture.

The pressurization appeared to affect yoghurt acidity to a slight extent, evoking a negligible increase in pH of the analyzed yoghurts that ranged from 0.025 (MYE 92) to 0.080 (YC-X16) unit. – Table 1.

KRUK et al. (1999) demonstrated that the pressure treatment at 200-1000 MPa did not affect milk pH and caused only small changes in the conductance and a slight increase in the freezing point. In contrast, JOHNSON et al. (1992) observed a slight decrease in the acidity of defatted milk (by 0.01-0.03 pH unit) subjected to pressure treatment at 20-60 MPa for one hour. According to SCHRADER et al. (1997), negligible fluctuations in the active acidity are due to changes in the structure and dynamic equilibrium of proteins as well as the composition of mineral salts of milk. In addition, the decreased milk acidity

Table 1

The effect of pressurization (100-1000 MPa) on the acidity of yoghurts

Pressure (Mpa)	YC-X11		YC-X16		MYE 92		MYE 95		CSK 501	
	pH	°SH	pH	°SH	pH	°SH	pH	°SH	pH	°SH
0	4.315±0.007	44.0±1.4	4.305±0.007	43.0±0.0	4.545±0.007	34.0±0.0	4.550±0.014	35.0±0.0	4.500±0.000	32.0±0.0
100	4.330±0.000	43.0±0.0	4.315±0.007	42.5±0.7	4.525±0.035	35.5±0.7	4.570±0.000	35.5±0.7	4.540±0.000	34.5±0.7
200	4.355±0.021	42.5±0.7	4.380±0.014	42.0±0.0	4.540±0.000	36.0±0.0	4.550±0.014	35.5±0.7	4.550±0.000	35.0±0.0
300	4.355±0.021	43.0±0.0	4.385±0.007	42.0±1.4	4.550±0.000	36.0±0.0	4.550±0.000	35.5±0.7	4.550±0.000	35.5±0.7
400	4.345±0.021	42.5±0.7	4.380±0.028	42.0±0.0	4.560±0.000	36.0±0.0	4.560±0.000	35.0±0.0	4.450±0.007	35.0±0.0
500	4.335±0.021	43.0±0.0	4.370±0.014	41.5±0.7	4.570±0.000	35.5±0.7	4.545±0.021	35.5±0.7	4.550±0.007	35.5±0.7
600	4.340±0.028	43.0±1.4	4.360±0.000	42.5±0.7	4.560±0.000	36.0±0.0	4.560±0.000	35.5±0.7	4.560±0.000	36.0±0.0
700	4.330±0.014	43.5±0.7	4.340±0.000	42.0±1.4	4.560±0.000	36.5±0.7	4.560±0.000	36.0±0.0	4.560±0.000	35.5±0.7
800	4.330±0.014	43.5±0.7	4.340±0.000	42.0±0.0	4.570±0.000	36.0±0.0	4.570±0.000	35.5±0.7	4.550±0.000	35.5±0.7
900	4.330±0.000	43.0±0.0	4.330±0.000	42.0±1.4	4.560±0.000	36.5±0.7	4.560±0.000	35.5±0.7	4.535±0.007	35.0±0.0
1000	4.335±0.007	43.0±0.0	4.330±0.000	42.0±1.4	4.560±0.000	36.5±0.7	4.590±0.000	36.0±0.0	4.530±0.000	35.5±0.7

format of values: mean ± standard deviation

observed after pressurization is likely to result from partial dissociation of colloidal phosphate (Huppertz et al. 2002).

The effect of pressurization on antibacterial activity

The application of an inoculum with appropriately composed microflora enables either elimination or inhibition of the growth of pathogenic and toxicogenic as well as proteolytic bacteria in fermented food products. The prevention of excessive multiplication of undesirable bacteria results from the production of metabolites by lactic acid bacteria, including: short--chain fatty acids (actic acid, acetic acid) and other acids, hydrogen peroxide, ethanol, carbon dioxide, diacetyl, bacteriocins and other antibiotic substances (BIELECKA et al. 1982).

The antibacterial activity of yoghurt bacteria is not only of technological significance, but also exerts a stabilizing effect on microflora composition in the gastrointestinal tract of humans through the elimination of harmful microflora.

The strongest antibacterial activity was observed in yoghurts produced from YC-X11, YC-X16 and MYE 95 culture that inhibited the growth of all 11 test strains. Slightly lower antibacterial activity was reported for the yoghurt prepared from the MYE 92 culture which inhibited the growth of ten strains. The yoghurt obtained from the CSK 501 culture inhibited the growth of 5 test strains only (Table 2).

The pressure treatment at 200-1000 MPa considerably deteriorated the antibacterial activity of the yoghurts analyzed. At this pressure range, the YC-X11 yoghurt inhibited the growth of six strains, whereas yoghurt made of the YC-X16 culture reduced the growth of five strains, and those prepared from MYE 92, MYE 95 and CSK 501 inocula inhibited the growth of three test strains.

The causes of deteriorated antibacterial activity in pressurized yoghurts should be searched for in the decreased or totally reduced number of *Lactobacillus delbrueckii* ssp. *bulgaricus* rods responsible for the production of a number of substances with bactericidal activity, including: hydrogen peroxide in the amounts exceeding toxicity thresholds for multiple pathogens (GUDKOV 1986).

In addition, as demonstrated in ample studies, high pressures affect an enzymatic apparatus, this evoking changes in the metabolic activity of microorganisms (KOŁAKOWSKI et al. 1998, JANKOWSKA et al. 2001).

Table 2

The effect of high pressures (100-1000 MPa/ 15 min) on the antibacterial activity of yoghurts

Yoghurt	Prssure (Mpa)	Test strains										
		Ent. cloacae 17	*Ent. cloacae* 10	*Ent.* A1	*Ent.* A1/17	*Proteus* 16	*Proteus* J	*Klebsiella* 02/1133	*Klebsiella* 449	*E. coli* 366	*E. coli* 3	*E. coli* 323
		Inhibition zones [mm]										
1	2	3	4	5	6	7	8	9	10	11	12	13
YC-X11	0	13.5±0.7	12.5±0.7	27.5±0.7	26.5±0.7	14.0±0.0	21.0±0.0	13.0±0.0	18.0±0.0	28.5±0.7	32.0±0.0	17.5±0.7
	100	12.0±0.0	12.0±0.0	25.5±0.7	26.0±0.0	13.5±0.7	20.5±0.7	12.0±0.0	17.5±0.7	29.0±1.4	30.5±0.7	17.0±0.0
	200	–	11.0±0.0	23.0±0.0	24.5±0.7	12.0±0.0	18.0±1.4	–	18.0±1.4	25.0±0.0	28.0±0.0	14.0±0.0
	300	–	–	22.5±0.7	22.5±0.7	11.5±0.7	17.5±0.7	–	17.5±0.7	24.5±0.7	25.0±0.0	–
	400	–	–	22.0±0.0	22.0±0.0	11.0±0.0	17.0±0.0	–	17.0±0.0	24.0±0.0	24.0±0.0	–
	500	–	–	22.5±0.7	23.5±0.7	–	17.0±0.0	–	17.0±0.0	21.0±1.4	24.5±0.7	–
	600	–	–	22.5±0.7	24.0±1.4	–	17.5±0.7	–	17.5±0.7	20.0±0.0	24.5±0.7	–
	700	–	–	21.0±0.7	24.0±0.0	–	16.5±0.7	–	16.5±0.7	20.5±0.7	24.5±0.7	–
	800	–	–	21.0±0.0	23.5±0.7	–	16.5±0.7	–	16.5±0.7	20.5±0.7	22.0±0.0	–
	900	–	–	21.0±1.4	23.0±0.0	–	16.0±0.0	–	16.0±0.0	19.0±0.0	21.0±1.4	–
	1000	–	–	20.0±0.0	22.0±0.0	–	16.0±0.0	–	16.0±0.0	29.5±0.7	19.0±0.0	–
YC-X16	0	16.5±0.7	11.0±0.0	26.0±0.0	25.5±0.7	11.5±0.7	21.5±0.7	11.5±0.7	15.5±0.7	26.0±0.0	14.0±0.0	11.0±0.0
	100	15.5±0.7	11.0±0.0	25.5±0.7	25.5±0.7	11.5±0.7	20.5±0.7	11.0±0.0	14.5±2.1	27.0±0.0	15.5±0.7	11.0±0.0
	200	13.5±0.7	–	21.5±2.1	21.5±0.7	–	16.5±0.7	–	12.5±0.7	24.0±0.0	11.5±0.7	–
	300	–	–	19.5±3.5	19.5±0.7	–	16.5±0.7	–	11.5±0.7	20.0±0.0	–	–
	400	14.0±1.4	–	22.0±0.7	22.0±0.0	–	18.5±0.7	–	12.0±0.0	23.0±0.0	–	–
	500	13.0±1.4	–	21.5±0.7	21.5±2.1	–	17.5±2.1	–	12.0±0.0	21.0±1.4	–	–
	600	13.5±0.7	–	21.5±0.7	21.5±0.7	–	18.0±1.4	–	11.5±0.7	22.0±2.8	–	–
	700	14.0±0.0	–	21.5±0.7	20.5±0.7	–	16.5±2.1	–	11.0±0.0	20.0±0.0	–	–
	800	–	–	19.0±0.0	19.0±1.4	–	11.0±0.0	–	11.0±0.0	20.0±0.0	–	–
	900	–	–	19.0±0.0	19.0±0.7	–	11.0±0.0	–	11.0±0.0	19.5±0.7	–	–
	1000	–	–	19.0±1.4	18.0±1.4	–	11.0±0.0	–	–	19.0±0.0	–	–

cont. table 2

1	2	3	4	5	6	7	8	9	10	11	12	13
MYE 92	0	13.5±0.7	12.0±0.0	30.0±0.0	29.0±1.4	18.5±0.7	11.0±0.0	–	16.5±0.7	29.0±1.4	14.5±2.1	12.5±0.7
	100	12.5±0.7	12.0±0.0	28.5±0.7	28.0±0.0	17.0±0.0	–	–	15.5±0.7	28.0±0.0	12.5±0.7	12.0±0.0
	200	–	–	26.0±0.0	24.5±0.7	15.5±0.0	–	–	13.5±0.7	24.5±0.7	12.0±0.0	–
	300	–	–	17.0±1.4	18.0±0.0	–	–	–	–	17.0±1.4	–	–
	400	–	–	18.5±0.7	20.0±0.0	–	–	–	–	18.5±0.7	–	–
	500	–	–	17.0±0.0	18.0±1.4	–	–	–	–	18.0±0.0	–	–
	600	–	–	17.0±1.4	18.0±0.0	–	–	–	–	17.5±0.7	–	–
	700	–	–	18.0±0.0	17.5±0.7	–	–	–	–	17.0±1.4	–	–
	800	–	–	17.0±1.4	17.5±0.7	–	–	–	–	16.0±0.0	–	–
	900	–	–	15.5±0.7	17.0±0.0	–	–	–	–	15.5±0.7	–	–
	1000	–	–	15.5±0.7	16.0±0.0	–	–	–	–	15.0±1.4	–	–
MYE 95	0	12.0±0.0	11.5±0.7	25.5±0.7	22.5±0.7	11.0±0.0	13.5±0.7	11.5±0.7	14.0±0.0	23.5±0.7	12.5±0.7	11.5±0.7
	100	12.0±0.0	11.0±0.0	24.5±0.7	23.0±0.0	11.5±0.7	14.0±0.0	11.5±0.7	13.5±0.7	23.0±1.4	12.0±0.0	12.0±0.0
	200	–	–	22.0±0.0	20.0±0.0	–	11.5±0.7	–	12.0±0.0	18.5±0.7	–	–
	300	–	–	19.0±0.0	17.5±0.7	–	–	–	12.0±1.4	15.5±0.7	–	–
	400	–	–	19.0±0.0	19.5±0.7	–	–	–	–	18.5±0.7	–	–
	500	–	–	19.0±0.0	19.5±0.7	–	–	–	–	18.0±0.0	–	–
	600	–	–	19.5±0.7	18.5±2.1	–	–	–	–	19.0±0.0	–	–
	700	–	–	21.0±1.4	20.5±2.1	–	–	–	–	19.0±0.0	–	–
	800	–	–	17.5±0.7	16.5±0.7	–	–	–	–	14.5±0,7	–	–
	900	–	–	18.0±0.0	16.5±0.7	–	–	–	–	15.5±0.7	–	–
	1000	–	–	17.5±0.7	16.5±0.7	–	–	–	–	15.0±1.4	–	–

cont. table 2

1	2	3	4	5	6	7	8	9	10	11	12	13
CSK 501	0	–	–	26.5±0.7	24.5±0.7	–	15.5±0.7	–	16.0±0.0	25.5±0.7	–	–
	100	–	–	26.5±0.7	23.0±1.4	–	17.5±0.7	–	15.0±0.0	25.0±0.0	–	–
	200	–	–	20.0±0.0	19.0±0.0	–	13.0±1.4	–	–	20.0±0.0	–	–
	300	–	–	16.5±0.7	15.0±0.0	–	–	–	–	16.5±0.7	–	–
	400	–	–	16.5±0.7	13.5±0.7	–	–	–	–	16.5±0.7	–	–
	500	–	–	15.5±0.7	13.0±1.4	–	–	–	–	15.5±2.1	–	–
	600	–	–	17.0±0.0	13.5±0.7	–	–	–	–	16.0±0.0	–	–
	700	–	–	15.0±0.0	12.5±0.7	–	–	–	–	16.0±1.4	–	–
	800	–	–	16.5±0.7	14.0±1.4	–	–	–	–	16.5±0.7	–	–
	900	–	–	19.0±1.4	14.0±0.0	–	–	–	–	16.5±0.7	–	–
	1000	–	–	18.0±0.0	12.5±0.7	–	–	–	–	15.0±1.4	–	–

(–) no inhibition zone
format of values: mean ± standard deviation

The effect of pressurization on the organoleptic properties of yoghurt

The flavour-aroma bouquet of yoghurt is created by all milk components and compounds formed by enzymatic reactions carried out by bacteria. The flavour and aroma of yoghurt depend, most of all, on the contents of lactic acid and carbonyl compounds of which acetic aldehyde and diacetyl occur in the highest concentrations. The level of flavour-aroma substances is determined by specific properties of bacteria and such factors as: the quality and type of milk, the intensity of thermal treatment, the content of dry matter, the method and parameters of incubation as well as storage conditions (TAMINE, DEETH 1980).

Prior to the pressurization, the flavor of all the examined yoghurts was evaluated as pure, typical, and nicely refreshing, the differences were observed mainly in the intensity of acid flavour. Panelists evaluated the appearance of the curd as homogenous, firm with a slight whey leakage, and the consistency – as homogenous, smooth in cross-section, and with a porcelain sheen. The colour of all the yoghurts was white.

The pressurization at 100-300 MPa caused slight changes in the flavour and aroma of the yoghurts analyzed, whereas the pressure treatment at 400-1000 MPa resulted in negative changes in the flavour, consistency and appearance of most of the yoghurts under evaluation. The pressurization of the YC-X16 yoghurt evoked only negligible changes in the flavour-aroma attributes. After the pressure treatment, the flavour of that yoghurt remained pure, typical, slightly acid and refreshing.

The yoghurt produced with the use of the XC-X11 culture lost its yoghurt--like flavour; after the pressure treatment at 400-800 MPa it was described as insipid with a pungent after-taste.

The pressurization of yoghurts obtained with the use of MYE 92 and MYE 95 cultures resulted in the deterioration of their flavour-aroma attributes. The panelists evaluated those yoghurts as insipid and devoid of a typical yoghurt-like flavour. The MYE 95 yoghurt pressurized at 700-800 MPa was characterized by a pungent after-taste.

The flavour-aroma attributes of the yoghurt produced with the use of CSK 501 culture were observed to change to a little extent upon the pressure treatment. The flavour of that yoghurt was typical, slightly acid, refreshing and without unfamiliar after-tastes, whereas its curd remained homogenous, firm and without whey flow.

The consistency of the pressurized yoghurts became more and more watery along with pressure increase.

Conclusions

1. The quantitative composition of microflora of the yoghurts examined was diversified depending on the type of starter culture used for their production. Pressure treatment above 300 MPa resulted in total reduction of the number of *Lactobacillus delbrueckii* ssp. *bulgaricus*. In contrast, *Streptoccocus thermophilus* appeared to be more resistant to the pressure treatment – at the investigated pressure range up to 1000 MPa no complete inactivation of that strain was observed.

2. The pressurization evoked a slight drop in the acidity of the yoghurts – by 0.025-0.080 pH units.

3. The antibacterial properties of the yoghurts were observed to be significantly deteriorated upon the pressure treatment The antibacterial activity of yoghurt cultures examined was also determined by the type of starter culture used.

4. The appearance, consistency, flavour and aroma of all the yoghurts analyzed were evaluated as typical of that product. After pressurization at pressure values higher than 400 MPa, the consistency and flavour of the yoghurts deteriorated, except for the yoghurt produced from the CSK 501 culture.

5. The obtained results indicate that the method of high pressures can be successfully used in yoghurt preservation. However, the inoculum microorganisms should be selected.

Translated by JOANNA JENSEN

Accepted for print 7.01.2008

References

ANCOS de B., PILAR CANO M., GÓMEZ R. 2000. *Characteristics of stirred low-fat yoghurt as affected by high pressure*. Int. Dairy J., 10: 105-111.

BIELECKA M., MELAN K., RUSZKIEWICZ A. 1982. *Inhibition of pathogenic and technologically undesirable microflora by Lactobacillus delbrueckii ssp. bulgaricus* and *Streptococcus thermophilus during production and storage of yogurt*. XXI Int. Dairy Congr. Moscow, p. 281.

BIROLLO G.A., REINHEIMER J.A., VINDEROLA C.G. 2000. *Viability of lactic acid microflora in different types of yoghurt*. Food Research Int., 33: 799-805.

BUTTRISS J. 1997. *Nutritional properties of fermented milk products*. Int. J. Dairy Technol., 50(1): 21-27.

FAO/WHO, Proposed Draft Standard A-11 for Fermented Milks (Alinorm 01/11/2000).

GODKOV A.V. 1986. *Startes as a means of controlling contra-acid bacteria.* Proceedings XXII Int. Dairy Congress, Hague, p. 86-93.

GOULD G.W., *The microb as a ligh pressure target* 1990. [In:] *High Pressure Processing of Foods*, Ledward D.A., Johnson D.E., Ernshaw R.G., and Hasting A.P. (Eds.), Nothingham University Press, Loughborough, U.K., p. 27.

HITE B.H. 1899. *The effects of pressure in the preservation of milk*. West Virginia Agric. Exp. Stat. Bull., 58: 15-21.

HOOVER D.G., METRICK C., PAPINEAU A.M., FARKAS D.F., KNORR D. 1989. *Biological effects of high hydrostatic pressure on food microorganisms*. Food Technol., 43: 99-107.

HOLT P.R. 2003. *Do fermented dairy products offer any protection against colon cancer*? Nutritopics, Danone Vitapole, 26: 13.

HUPPERTZ T., KELLY A.L., FOX P.F. 2002. *Effects of high-pressure on constituents and properties of milk*. Int. Dairy J., 12: 561-572.

IDF (International Dairy Federation). 1988. *Fermented milks: Sciences and technology*. Bulletin of the IDF, 227, Brussels.

JANKOWSKA A., KRASOWSKA M., REPS A., PROSZEK A. 2001. *Effect of high pressure on some properties of Lactic acid bacteria present in kefir*. Med. Fac. Landbouww. Univ. Gent, 66/3b, 503-509.

JOHNSTON D.E., AUSTIN B.A., MURPHY R.J. 1992. *Effect of high hydrostatic pressure on milk*. Milchwissenschaft, 47(12): 760-763.

JOHNSTON D.E. 1995. *High Pressure effects on milk and meat*. [In:] *High Pressure Processing of Foods*. Eds. Ledward D.A., Johnson D.E., Ernshaw R.G., Hasting A. P. Nothingham University Press, Loughborough, U.K., p. 99.

KOŁAKOWSKI P., REPS A., BABUCHOWSKI A. 1998. *Characteristics of pressurized ripened cheeses*. Pol. J. Food Nutr. Sci., 7(48): 473-483.

KNORR D. 1993. *Effects of high-hydrostatic-pressure processes on food safety and quality*. Food Technol., 47: 156-161.

KRUK A., KIEŁCZEWSKA K., REPS A. 1999. *Effect of High Pressure on Heat- and Ethanol Stability of Milk*. XXXVII EHPRG Meeting of the European High Pressure Research Group Montpellier, France, Abstract Book, p. 405.

LANCIOTTI R., VANNINI L., PITTIA P., GUERZONI M.E. 2004. *Suitability of high-dynamic-pressure-treated milk for the production of yoghurt*. Food Microbiology, 21: 753-760.

LOURENS-HATTINGH A., VILJOEN B.C. 2001. *Yogurt as a probiotic carrier food*. Int. Dairy J., 11: 1-17.

LUDWIG H. 2002. *Effect of high pressure on bacteria and fungi. Advances in High Presure Bioscience and Biotechnology*. Ed. R. Winter, II, 259-265.

MARTINI M., BOLLWEY G.L., LEVIT M.D., SOVAIANO D.A. 1987. *Lactose digestion by yogurt β-galactosidase: influence of pH and microbial cell integrity*. American Journal of Clinical Nutrition, 45: 432-436.

Mleko i przetwory mleczarskie. Napoje mleczne fermentowane. PN-83/A-86061.

REPS A., WARMIŃSKA-RADYKO I., DAJNOWIEC F. 1999. *Effect of high hydrostatic pressures on yoghurt*. [In:] *Advances in High Pressure Bioscience and Biotechnology*. Ed. H. Ludwig, Springer-Ferlag, pp. 453-456.

REPS A., WARMIŃSKA-RADYKO I., KRZYŻEWSKA A., TOMASIK J. 2001. *Effect of high pressures on Streptococcus salivarius ssp. thermophilus*. Milchwissenschaft., 56: 131-133.

SCHREDER K., BUCHHEIM W., MORR C.V. 1997. *High pressure effects on the colloid calcium phosphate and the structural integrity of micellar casein in milk*. Forschungsbeitrage/Research Paper, 41: 133-138.

TAMIME A.Y., DEETH H.C. 1980. *Yogurt: technology and biochemistry*. J. Food Protect. 43(112): 939-977.

TANAKA T., HATANAKA K. 1992. *Application of hydrostatic pressure to yoghurt to prevent its after--acidification*. Nippon Shokuhin Kogyo Gakkaishi, 39: 173-177.

POLISH JOURNAL OF NATURAL SCIENCES
Abbrev.: Pol. J. Natur. Sc., Vol 23(2): 496-505, Y. 2008

DOI 10.2478/v10020-008-0038-7

THERMOCHEMILUMINESCENCE OF SELECTED VEGETABLE OILS

***Halina Pieńkowska*[1], *Ryszard Zadernowski*[2], *Sylwester Czaplicki*[2], *Piotr Rolewski*[3]**

[1] Chair of Agricultural Engineering and Natural Raw Materials
[2] Chair of Food Plant Chemistry and Processing
University of Warmia and Mazury in Olsztyn
[3] Chair of Physics
University of Agriculture in Poznań

K e y w o r d s: chemiluminescence, vegetable oils, activation energy, borage, blue weed, evening primrose, oxidation.

A b s t r a c t

Vegetable oils, upon heating in the dark from room temperature to 373-393 K undergo oxidation according to the radical mechanism. A certain amount of chemical energy is used to cause excitation of the reaction products. Deactivation of the excited molecules through emission of chemiluminescence (CL) has been reported for the following vegetable oils: rapeseed oil, primrose oil, borage oil and blueweed seed oil. By employing the empirical Arrhenius law, the activation energy for blueweed seed oil was calculated.

TERMOCHEMILUMINESCENCJA W WYBRANYCH OLEJACH ROŚLINNYCH

***Halina Pieńkowska*[1], *Ryszard Zadernowski*[2], *Sylwester Czaplicki*[2], *Piotr Rolewski*[3]**

[1] Katedra Inżynierii Rolniczej i Surowców Naturalnych
[2] Katedra Przetwórstwa i Chemii Surowców Roślinnych
Uniwersytet Warmińsko-Mazurski w Olsztynie
[3] Katedra Fizyki
Akademia Rolnicza w Poznaniu

S ł o w a k l u c z o w e: chemiluminescencja, oleje roślinne, energia aktywacji, ogórecznik, żmijowiec, wiesiołek, utlenianie.

Address: Halina Pieńkowska, University of Warmia and Mazury, ul. Okrzei 1a, 10-256 Olsztyn, Poland, phone: (089) 526 63 80), e-mail: halszka@uwm.edu.pl

A b s t r a k t

Ogrzewanie olejów od temperatury pokojowej do 373-393 K powoduje ich utlenianie. Część energii chemicznej przekształca się w energię wzbudzenia produktów reakcji. Dezaktywacja wzbudzonych cząsteczek poprzez emisję promieniowania chemiluminescencji (CL) została zarejestrowana dla następujących olejów roślinnych: rzepakowego, wiesiołkowego, ogórecznikowego i oleju z nasion żmijowca. Stosując empiryczne prawo Arrheniusa, obliczono energię aktywacji reakcji dla oleju z nasion żmijowca.

Introduction

Chemiluminescence is the radiation emitted by molecules in an excited state as a result of exoergic reactions. A significant amount of the energy accompanying the chemical reaction (ΔH enthalpy) is used for electronic excitation of the product (P*), which emits a photon $h\nu$, under the condition that:

$$\Delta H + E_{akt} \geq h\nu \tag{1}$$

where:
ΔH – enthalpy,
E_{akt} – activation energy.

Chemiluminescence is generated in radical chain reactions and in the red-ox reactions taking place according to the radical mechanism. Usually the emitters are the organic compounds rich in the easily excitable π-electron clouds, e.g. aldehydes, ketones and molecular oxygen (SŁAWIŃSKI 1989).

The total yield of chemiluminescence (CL) depends on the chemical yield of the excited product (P*), the quantum yield of excitation and the quantum yield of luminescence. The total yield of chemiluminescence (ΦCL), the intensity of chemiluminescence (I) and the rate constant of the reaction of formation of excited molecules (w) are related by:

$$I = \Phi_{CL} \cdot w \tag{2}$$

where:
I – intensity of total CL
Φ_{CL} – total yield of CL
w – reaction rate constant.

The excited product (P*) can pass the excessive energy to other molecules characterised by a high yield of fluorescence, e.g. to chlorophyll, this process

is referred to as the activated or sensitized chemiluminescence (SŁAWIŃSKI 1989, SŁAWIŃSKA et al. 1998, PIEŃKOWSKA 2003). There is a high probability of the energy transfer from the primary products of excitation (P*) onto the energy acceptor (A), according to the equation:

$$^{1(3)}P^* + {}^1A \xrightarrow{k_{ET}} {}^1P + {}^1A^* \xrightarrow{k_{FA}} {}^1A + h\nu_A \qquad (3)$$

where:

$^{1(3)}$P* – the product in the excited singlet or triplet state,
^1A – energy acceptor in the singlet state (e.g. chlorophyll),
k_{ET} – the rate constant of energy transfer according to the resonance Förster mechanism or exchange Dexter mechanism,
k_{FA} – the rate constant of luminescence from acceptor A,
$h\nu_A$ – the emitted quantum of luminescence characterised by depending on &the spectral properties of the acceptor.

To date, the potential of the phenomenon of thermochemiluminescence has not been exhausted in the technology and analysis of vegetable oils. The study reported in this paper has been undertaken to contribute to a detailed recognition of the oxidation processes leading to sensitized chemiluminescence in vegetable oils upon heating.

Material and Methods

The material studied were vegetable oils pressed from the seeds of primrose (*Oenothera paradoxa H.*), borage (*Borago officinalis),* rape (*Brassica napus L.*) and blueweed (*Echium plantagineum)*. The seeds of the above plants were extruded on an expeller of the Komet type, model CA/59, made by JBG Monforts. The oils after pressing are referred to as pressed oils. The process of oil bleaching is described in PIEŃKOWSKA et al. (2005), the oils after bleaching are referred to as bleached oils. The composition of fatty acids in the oils studied was determined by gas chromatography an a Fisons 8000 gas chromatograph coupled with a flame ionization detector (FID) (Carlo Erba, Italy). Separation was performed on J&W ScientificDB-225 (30 m x 0.25 mm x 0.15 μm) capillary column at 190°C (ZADERNOWSKI, SOSULSKI 1978). The total amount of the unsaponifiable substance was determined according to the Polish Norm (*Oleje*... PN-ISO 3596-2/1994). The carotenoids and β-carotene were determined by the method proposed by RUTKOWSKA (1981), the chlorophyll dyes were determined by the method proposed by NIEWIADOMSKI et al.

(1965). The content of pheophytin was established according to the procedure proposed by NIEWIADOMSKI, BRATKOWSKA (1970). The colour of the oils was determined spectrophotometrically according to Polish Norm (*Oleje i tłuszcze*... PN-A 86934/1996). The peroxide number was determined according to Polish Norm (*Oleje i tłuszcze*... PN-ISO 3960/1996) and expressed in Lea units. The intensity of chemiluminescence was recorded by a photomultiplier P12FQ51(sensitive in the spectral range 300-700 nm), 1200V, separated by a flow-through water filter from the heated sample of oil. The samples of oils were heated in the dark, in the air atmosphere from room temperature to a target temperature of measurement. The temperature of measurement was maintained by a thermostat. Temperature was measured to the accuracy of 1 K.

Results and Discussion

Analytical results

The oils selected for the study differed in the content of the unsaturated fatty acids and unsaponifiable substances. The contents of different fatty acids in the oils studied is given in Table 1.

Table 1

Percentage of fatty acid in the oils from the studied seeds

Fatty acid $C_{m:n}$	Evening primrose	Rapeseeds	Borage	Echium
$C_{16:0}$	5.81 ± 0.09	4.44 ± 0.02	11.04 ± 0.09	7.75 ± 0.09
$C_{16:1}$	0.08 ± 0.03	0.22 ± 0.09	0.47 ± 0.03	0.15 ± 0.03
$C_{18:0}$	1.71 ± 0.02	1.11 ± 0.02	4.83 ± 0.03	4.23 ± 0.03
$C_{18:1}$	5.43 ± 0.08	62.63 ± 0.02	18.97 ± 0.05	16.04 ± 0.05
$C_{18:2}$	76.08 ± 0.05	20.62 ± 0.09	35.80 ± 0.08	15.52 ± 0.05
$C_{18:3}$	0.15 ± 0.05	9.01 ± 0.02	trace	31.75 ± 0.08
γ-$C_{18:3}$	9.51 ± 0.03	–	21.18 ± 0.04	10.70 ± 0.02
$C_{18:4}$	–	–	–	12.61 ± 0.02
$C_{20:0}$	0.20 ± 0.05	0.38 ± 0.05	0.31 ± 0.03	–
$C_{20:1}$	0.09 ± 0.01	1.77 ± 0.01	4.17 ± 0.29	–
$C_{20:2}$	0.06 ± 0.01	trace	trace	–
$C_{22:0}$	0.16 ± 0.02	trace	0.19 ± 0.02	–
$C_{22:1}$	–	0.16 ± 0.01	2.31 ± 0.16	0.71 ± 0.05

m – number of carbon atoms; n – number of double bonds
x ± SD (SD – standard deviation at 95% confidence level for $n = 3$)

Apart from triacylglycerides, partial acylglycerides and phospholipids, the pressed raw oils contain small amounts of other substances known as the unsaponifiable substances (SIKORSKI 1994, STOŁYHWO 1992). The main

components of the unsaponifiable substances are: carotenoids, polyphenols, tocopherols, sterols and sterol esters. The majority of these substances show antioxidant properties (Table 2).

Table 2

Selected unsaponifiable substances occurring in the oils studied

Components	Rapeseed	Evening primrose	Borage	Blueweed
Unsaponifiable substances (%)	1.20 ± 0.30	0.90 ± 0.20	0.81±0.20	1.09 ± 0.30
Carotenoids (mg/100 g oil)	0.428 ± 0.02	0.418 ± 0.05	0.235 ± 0.02	0.210 ± 0.02
β-carotene (mg/100 g oil)	0.295 ± 0.02	tr.	tr.	tr.

The oils studied satisfied the requirements of fresh vegetable oils (Table 3) specified by FAO/WHO, according to which the maximum admissible peroxide number of edible and pharmaceutical oils should not exceed 5 Lea units.

Table 3

The oil colour, content of pheophytin and the peroxide number in the oils studied

Type of oil	Colour	Pheophytin (mg/100 g)		Peroxide value
		a	*b*	
Rapeseed	1200 ± 10	0.662 ± 0.005	0.190 ± 0.005	1.33 ± 0.005
Blueweed	1530 ± 80	0.940 ± 0.005	0.120 ± 0.005	1.55 ± 0.005
Evening primrose	340 ± 60	0.120 ± 0.005	0.021 ± 0.005	2.00 ± 0.006
Borage	414 ± 60	0.312 ± 0.005	–	2.15 ± 0.006

It has been shown that under the effect of light, the chlorophyll contained in the oils undergoes decomposition to pheophytin, Table 3, see NIEWIADOMSKI, BRATKOWSKA (1970) and USUKI et al. (1984). It is generally assumed that the presence of chlorophyll dyes in the oils has a profound effect on their stability as the dyes are involved in the process of fatty acids oxidation.

Chemiluminescence

Chemiluminescence of the oils studied, induced by thermal energy and described by the kinetic curves $I = f(t)$, in Figure 1, Figure 2, Figure 3, provides data on the concentration of the excited molecules undergoing deactivation and the rate of their formation and decay. The intensity of CL reaches

a maximum in the red and near infrared, and it decreases as a result of introduction of the inhibitors of oxidation by free radicals (antioxidants) (SŁAWIŃSKI 1989).

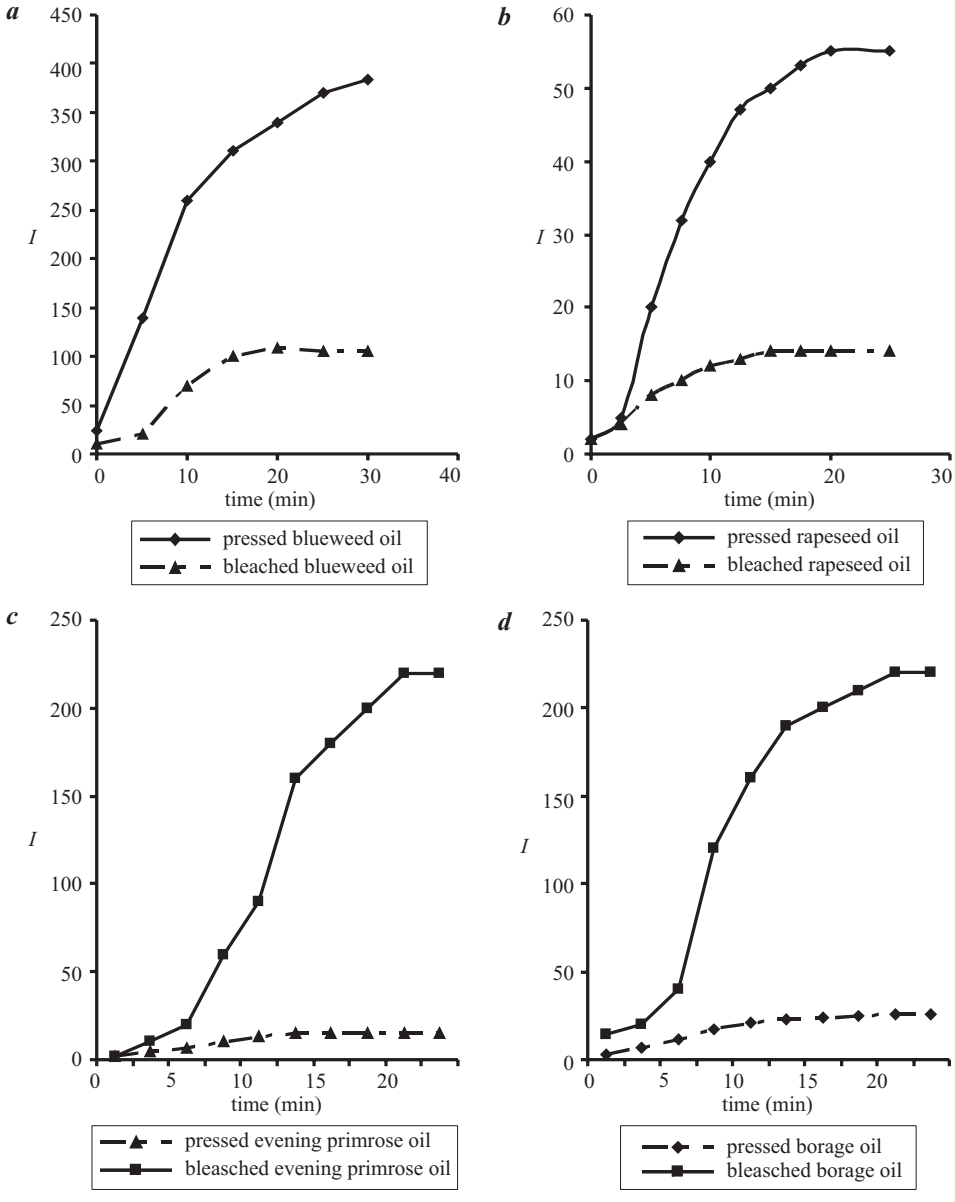


Fig. 1. Kinetic curves of I = *f*(*t*) illustrating the time changes of the chemiluminescence intensity (I) of pressed and bleached oils measured at 383 K: *a* – blueweed oil, *b* – rapeseed oil, *c* – evening primrose oil, *d* – borage oil

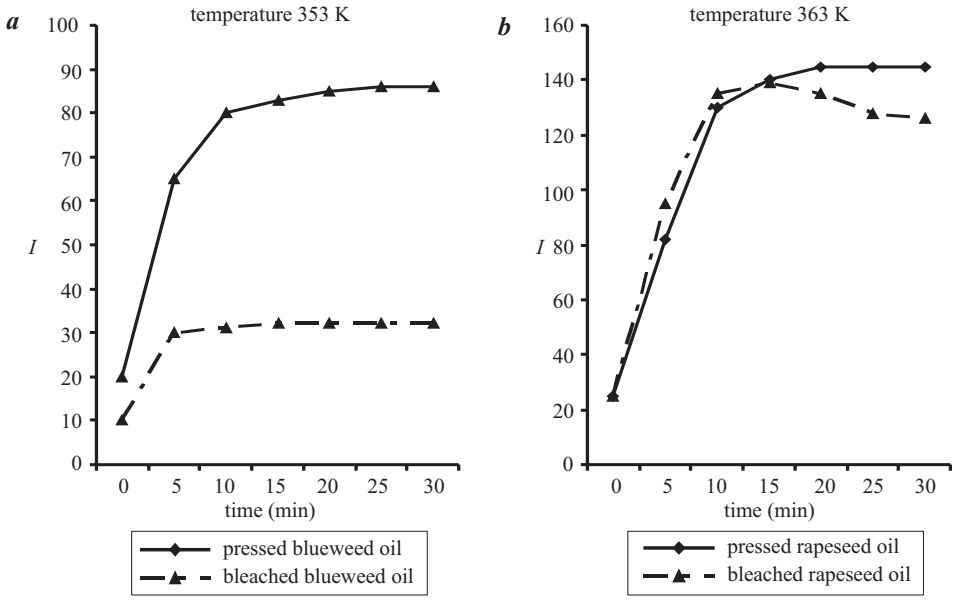


Fig. 2. The kinetic curves I = *f*(*t*) of CL of pressed and bleached blueweed oil, recorded at selected temperatures

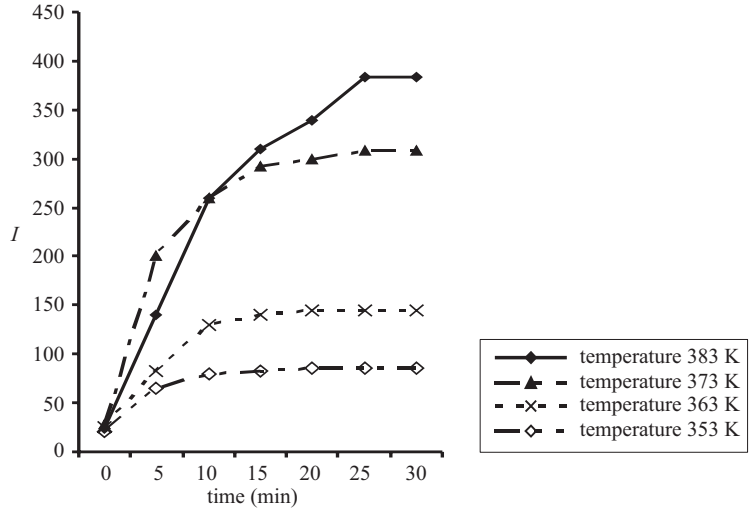


Fig. 3. The kinetic curves of CL of pressed blueweed oil recorded at four temperatures

The CL recorded is a consequence of oxidation of unsaturated fatty acids. The energy used for electronic excitation is the difference between the energy of bonds in the products of decomposition (dismutation) of aldehydes, ketones, molecular oxygen and that of the weak bonds of the intermediary peroxide complex (SŁAWIŃSKA et al. 1998). Some oils

contain chlorophyll compounds, showing efficient fluorescence, acting as acceptors of the energy of the electron excitation (PIEŃKOWSKA 2003, PIEŃKOWSKA et al. 2005, HANYŻ et al. 2006). In pressed oils containing considerable amounts of chlorophyll dyes, chemiluminescence has been observed to be enhanced (Figure 1a,b) and Table 3.

The amount of pheophytin in the oils pressed from mature seeds of primrose oil and borage oil is low (Table 3) and hence the intensity of chemiluminescence of bleached oils (Figure 1c, d) is much higher than that of pressed oils. The intensity of CL depends on the presence and activity of the reaction inhibitors, radical sweepers or luminescence quenchers. The intensity of CL of pressed blueweed seed oil (Figure 1b) recorded after 20 minutes of heating was six times higher than in the rapeseed pressed oil as the latter contains considerable amounts of carotene compounds (Table 2) that quench radicals. The intensity of CL of bleached oils depends on the presence of oxidised species of unsaturated fatty acids. The intensity of CL of bleached blueweed oil was 10 times higher than that of rapeseed oil as a consequence of differences in the contents of different unsaturated fatty acids in these oils. The evening primrose oil contained about 3.5 times greater linoleic acid (C18:2) than the rapeseed oil and about 5 times more of this acid than the blueweed oil. The content of α- linolenic acid (C18:3) in the rapeseed oil was of the same order as that of γ-linolenic (γ-C18:3) in the evening primrose oil. Greater amounts of these acids occur in the borage oil and blueweed oil (Table 1).

The kinetic curves $I = f(t)$, at selected temperatures (Figure 2, Figure 3) were recorded in pressed and bleached blueweed oil. With the temperature increasing from 353 K to 383 K, the rate of oxidation increased (w), leading to an increase in the CL intensity according to eq. (2).

The activation energy was calculated in a good approximation on the basis of the empirical Arrhenius law. The maximum intensity of CL I_{max} was recorded at least at three temperatures.

Figure 4 presents ln I_{max} CL versus reversed temperature in Kelvin scale. The activation energy was calculated from the following formula, eq. (4):

$$E_{akt} = \frac{\ln I_2 - \ln I_1}{\frac{1}{T_1} - \frac{1}{T_2}} R \tag{4}$$

where:

R – the gas constant,

I_1, I_2 – intensity of CL at temperatures T_1 and T_2.

The activation energy of the pressed blueweed oil was estimated as 66 kJ mol^{-1}, while that of bleached blueweed oil as about 143 kJ mol^{-1}.

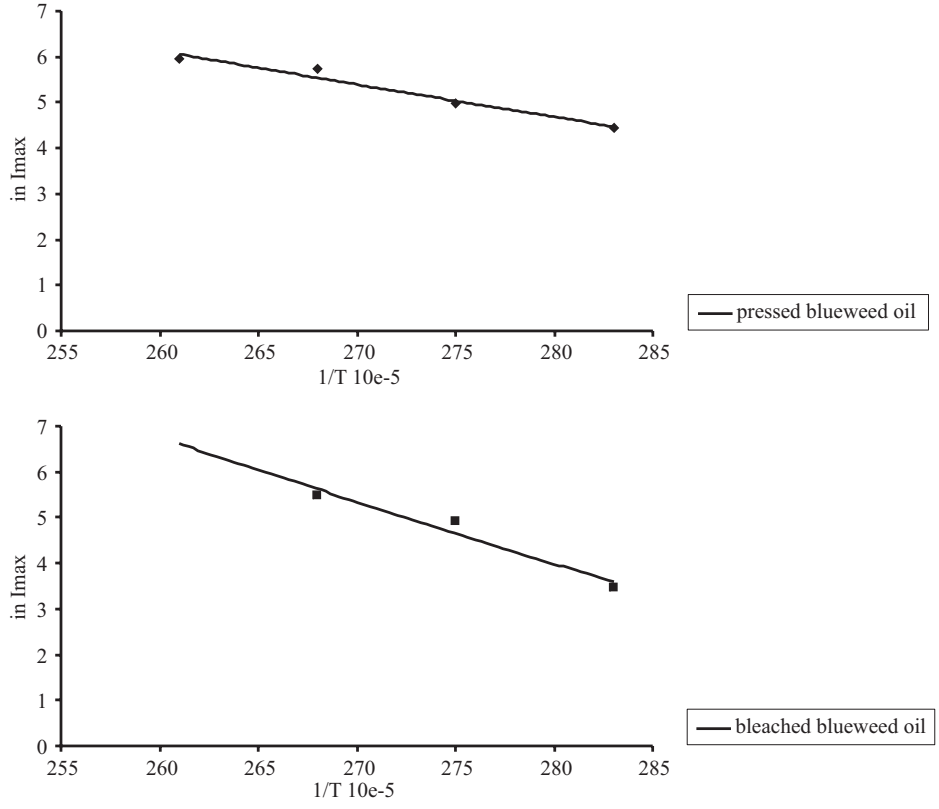


Fig. 4. The empirical Arrhenius Law dependence of maximum LC intensity of the pressed blueweed oil at four temperatures and bleached blueweed oil at three temperatures

Conclusions

1. The intensity of thermochemiluminescence of vegetable oil depends on the amount of chlorophyll dyes in the oils.

2. The activation energy calculated for the pressed and bleached blueweed oil is a measure of the oxidative stability of the oils and the proposed method of its estimation does not require the use of chemical reagents.

3. The proposed method of the activation energy estimation is a convenient way of characterisation of the effectiveness of the antioxidants added to vegetable oils.

Translated by JOANNA JENSEN

Accepted for print 14.09.2007

References

HANYŻ I., PIEŃKOWSKA H., DUDKOWIAK A., FRĄCKOWIAK D. 2006. *The photochemical stability of oil from Evening Primrose seeds*. Dyes and Pigments, 70(3): 177-184.

NIEWIADOMSKI H., BRATKOWSKA I. 1970. *The influence of the auto oxidation of rapeseed oil on the decomposition of pigments of chlorophylls group*. Zesz. Probl. Nauk. Rol., 91: 207-211.

NIEWIADOMSKI H. 1993. *Technologia tłuszczów jadalnych*. WNT, Warszawa.

NIEWIADOMSKI H., BRATKOWSKA I., MOSSAKOWSKA E. 1965. *Content chlorophylls and carotenes in rapeseed oil*. J. Am. Oil Chem. Soc., 42: 731-743.

PIEŃKOWSKA H. 2003. *Fizykochemiczne badania kinetyki utleniania olejów wiesiołkowego, ogórecznikowego i rzepakowego*. Monografie UWM, Olsztyn, nr 78.

PIEŃKOWSKA H., DUDKOWIAK A., MUSZYŃSKI D., FRĄCKOWIAK D. 2005. *The efficiency of the generation of photochemically active triplet states in oils containing various amount of pigments*. Dyes and Pigments, 64(2): 109-116.

Oleje i tłuszcze roślinne oraz zwierzęce. Oznaczanie substancji niezmydlających. Szybka metoda ekstrakcji heksanem. PN-ISO 3596-2/1994.

Oleje i tłuszcze roślinne oraz zwierzęce. Spektrofotometryczne oznaczanie barwy. PN-A 869 34/1995.

Oleje oraz tłuszcze zwierzęce. Oznaczanie liczby nadtlenkowej. PN- ISO 3960/1996.

RUTKOWSKA U. 1981. *Wybrane metody badania składu i wartości odżywczej olejów*. PZWL, Warszawa.

SIKORSKI E. 1994. *Chemiczne i funkcjonalne właściwości składników żywności*. WNT, Warszawa.

SŁAWIŃSKA D., GÓRSKI Z., SŁAWIŃSKI J., FORNAL J. 1998. *Chemiluminescence imaging of oxidative changes in plant food*. Pol. J. Food Nutr. Sci., 7(2): 67-72.

SŁAWIŃSKI J. 1989. *Metody badania słabych emisji fotonowych z układów biologicznych. in Biospektroskopia 3*. Red. J. Twardowski, PWN, Warszawa, 107-206.

STOŁYHWO A. 1992. *Analityczne kryteria jakości zdrowotnej oleju z nasion wiesiołka dziwnego (Oenothera paradoxa Hudziok). Zbiór prac I Sympozjum nt. Oleje z nasion wiesiołka w profilaktyce i terapii*. Łódz, 8-10.X. 1992, ss. 9-22.

USUKI R., ENDO J., KANEDA T. 1984. *Prooxidant activities of chlorophylls and pheophytins on the oxidation of edible oils*. Agric. Biol. Chem., 48: 991-994.

ZADERNOWSKI R., SOSULSKI F. 1978. *Composition of total lipids in rapeseed*. J. Am. Oil Chem. Soc., 55 (12): 870-872.

POLISH JOURNAL OF NATURAL SCIENCES
Abbrev.: Pol. J. Natur. Sc., Vol 23(2): 506-520, Y. 2008

DOI 10.2478/v10020-008-0039-6

CONSUMER OPINIONS ON THE INFORMATIONAL AND PROMOTIONAL FUNCTION OF UNIT PACKAGING OF DAIRY PRODUCTS

Katarzyna Staniewska, Helena Panfil-Kuncewicz, Bogusław Staniewski

Department of Dairy Science and Quality Management
University of Warmia and Mazury in Olsztyn

K e y w o r d s: packaging, labeling, dairy products, consumer research.

A b s t r a c t

The aim of the study was to determine the impact of packaging information and the promotional role of dairy product packaging on the consumers' decisions to purchase dairy products. To establish the above, a direct questionnaire survey involving 500 respondents was performed. It was found that the consumers focused mostly on the product's shelf-life / minimum durability period, the producer's trademark which is often identified with the specific brand as well as information on the product's ingredients and nutritive value. The consumers' propensity to make impulse purchases was confirmed by an observation that the buyers' attention is frequently drawn to packaging design which is often identified with product quality.

INFORMACYJNO-PROMOCYJNA FUNKCJA OPAKOWAŃ JEDNOSTKOWYCH WYROBÓW MLECZARSKICH W OPINII KONSUMENTÓW

Katarzyna Staniewska, Helena Panfil-Kuncewicz, Bogusław Staniewski

Katedra Mleczarstwa i Zarządzania Jakością
Uniwersytet Warmińsko-Mazurski w Olsztynie

S ł o w a k l u c z o w e: opakowanie, znakowanie, wyroby mleczarskie, badania konsumenckie.

Address: Helena Panfil-Kuncewicz, University of Warmia and Mazury in Olsztyn, ul. Oczapowskiego 7, 10-794 Olsztyn, Poland, phone: (089) 523 38 83, e-mail: panfil@uwm.edu.pl

A b s t r a k t

Celem badania było określenie wpływu poszczególnych informacji zamieszczonych na opakowaniach wyrobów mleczarskich oraz aspektu promocyjnego opakowań na decyzje dotyczące zakupu tych produktów. W badaniach, przeprowadzonych na grupie 500 respondentów, posłużono się kwestionariuszem ankiety bezpośredniej. Stwierdzono, że najistotniejsze dla konsumentów okazały się informacje o terminie przydatności do spożycia/ dacie minimalnej trwałości, nazwie producenta, często utożsamianej z konkretną marką, a także o składzie i wartości odżywczej produktów. Potwierdzeniem faktu, że konsumenci niekiedy dokonują zakupu impulsywnie, jest obserwacja, że bardzo często ich uwagę przykuwają cechy wzornicze opakowania, które kojarzą z jakością produktu.

Introduction

In view of the diversity of individual preferences which are often shaped by years of personal experience, it is very difficult to define consumer needs. The consumer's first contact with a packaged product is often the key factor in the purchasing decision because the buyer's perception of product packaging may substantially influence his/her decision to purchase a given item. The product's attractiveness can often be enhanced by modifying the packaging rather than the product itself.

Unit packaging, its labeling, size, shape and graphic design are often regarded as an "integral part of the product". It is up to the consumer to decide which product and in what packaging will conquer the market. For this reason, producers need to survey the consumers' needs and preferences in this area to be able to swiftly respond to their expectations (CICHOŃ, UCHEREK 2000, GÓRSKA-WARSEWICZ 2001, KUŚMIERCZYK 2000, MCKENNA 2005, SMYCZEK 2003a and b).

In view of the above, the aim of the study was to determine the impact of packaging information and the promotional role of dairy product packaging on the consumers' decisions to purchase dairy products.

Methods

The survey was carried out in the second half of 2005 and it involved a group of 500 clients of selected retail outlets within chains of supermarkets in Olsztyn. The survey was conducted based on a direct questionnaire comprising two parts. In the first part, the respondents were asked to evaluate the impact of packaging information and the promotional role of dairy product packaging on their decisions to buy dairy products. The second part featured questions on the respondents' sex, age, occupation, place of residence and monthly income per person in the household. The questionnaire comprised a set of closed questions.

Respondent characteristics

The characteristic features of the surveyed group are presented in Table 1. Despite the fact that women slightly outnumbered men in the respondent group, the age structure of the surveyed female and male populations was similar. The majority of respondents were aged from 21 to 40. When the surveyed population was analyzed in view of the occupational criterion, the researchers found that the vast majority of respondents were white-collar workers who had a 44.4% share of the surveyed group. Students (22.6%) and blue-collar workers (19%) were less well represented, while old age and disability pensioners and the unemployed (14%) had the smallest share of the respondent group.

Table 1

Respondent characteristics

Respondents		Population	(%)
Sex	female	277	55.4
	male	223	44.6
	total	**500**	**100.0**
Age in years	up to 20	38	7.6
	21-40	244	48.8
	41-60	186	37.2
	above 60	32	6.4
	total	**500**	**100.0**
Occupation	pupi/student	113	22.6
	blue-collar worker	95	19.0
	white-collar worker	222	44.4
	old age/disability pensioner	58	11.6
	unemployed	12	2.4
	total	**500**	**100.0**
Place of permanent residence	rural area	74	14.8
	city < 50 000	38	7.6
	city 50 000-100 000	49	9.8
	city > 100 000	339	67.8
	total	**500**	**100.0**
Monthly income per person	under PLN 300	37	7.4
	PLN 301-900ą122	24.4	
	PLN 601-900	139	27.8
	PLN 901-1200	83	16.6
	above PLN 1200	119	23.8
	total	**500**	**100.0**

The vast majority of respondents resided in cities with a population higher than 100 000 (67.8%). A smaller percentage share was reported in respect of residents from smaller cities (17.4%), while only around 15% of the polled subjects resided in rural areas. This configuration of the respondent structure, subject to the place of permanent residence, was nearly identical for both sexes.

The largest group of respondents, accounting for nearly 60% of the entire surveyed population, comprised persons generating monthly incomes of up to PLN 900 per person in the household.

Results and Discussion

Consumer opinions on the information displayed on the unit packaging of dairy products

When selecting food products, consumers are increasingly likely to be guided by the information displayed on product packaging which facilitates their choice of a given brand. Label information enables consumers to compare the product's value and properties against its price. The information displayed on the packaging prevents the consumer from selecting products and ingredients which could cause an allergic reaction or which do not meet the buyer's personal preferences (SZPONAR, WIERZEJSKA 2004). According to research carried out by PANFIL-KUNCEWICZ et al. (2007), the information displayed on the packaging of dairy products was an important and a very important factor which affected the purchasing decisions of nearly 30% of the surveyed respondents (of a total population of 500 persons). Around 20% respondents disregarded the above information, while the remaining consumers paid attention to label data only occasionally.

The researchers concluded that information on the product's shelf-life / minimum durability period (Figure 1) is the most important factor for the consumers. Nearly 90% of the respondents always or very often account for the above information when making their decision to buy a specific product. Similar results were reported by GÓRNA (2000) and ŚWIDA and KULIŃSKI (2002) who found that the information on the product's shelf-life was the most important factor shaping the consumers' opinions. The importance of this criterion is demonstrated by the fact that only 2.2% of the surveyed respondents declared that their decision to buy a dairy product was never or very rarely contingent on the product's shelf-life.

The majority of dairy products are characterized by a short shelf-life, which is why the related information is particularly important to guarantee the consumers' safety. Yet the information on the product's durability is often printed illegibly on the packaging, thus preventing the consumer from reading the relevant data and discouraging them from making the purchase.

Survey results, verified with the use of the χ^2 test, showed a significant relationship between the importance of the product's shelf-life/ minimum durability period and the consumers' sex. Nearly 80% of the surveyed female

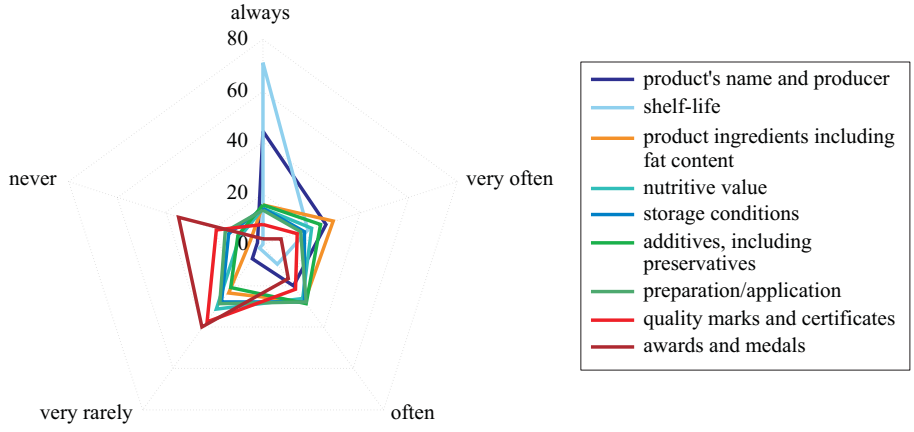


Fig. 1. Information on the packaging of dairy products in the opinion of the surveyed consumers (% indications)

population declared that they always paid attention to the above information. The population of men who claimed to always check the product's shelf-life was nearly 20% lower (Table 2, Figure 2).

In a market with a high number of competing dairy producers, customers are increasingly likely to focus on products offered by a given company. Many consumers emphasize the importance of the product brand and the trust

Table 2

Impact of information displayed on unit packaging of dairy products on the consumers' decisions to buy dairy products subject to: sex, age, place of permanent residence, occupation and monthly income per person (value of χ^2 test)

Specification	Sex	Age	Place of permanent residence	Occupation	Monthly income per person
Product's name and producer	3.10	30.24*	23.94	11.68	8.68
Shelf-life / minimum durability period	16.67*	22.19	8.08	16.53	17.37
Product ingredients, including fat content	23.52*	21.44	12.91	16.37	18.54
Nutritive value	33.56*	7.94	140.05*	28.29	11.15
Storage conditions	15.84*	15.04	13.79	8.51	6.23
Additives, including preservatives	24.72*	20.87	16.39	14.79	18.51
Preparation/application	11.23	23.51	10.57	12.92	13.19
Quality marks and certificates	17.72*	24.96	8.51	19.07	12.12
Awards and medals	6.21	32.54*	12.79	24.73	7.68

* – significant value at $\alpha = 0.01$

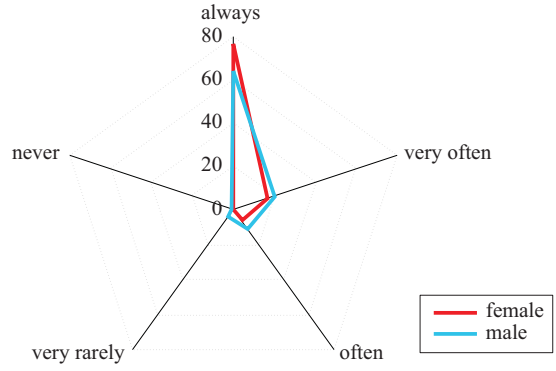


Fig. 2. Information on the shelf-life of dairy products in the opinion of the surveyed consumers, subject to respondents' sex

vested in the brand proprietor. Information on the brand and the producer's trademark was the second most important factor determining the consumers' purchasing decisions. Around 70% of the respondents claimed that they always or very often accounted for the above criterion (Figure 1).

Following an analysis of the impact of the respondents' sex on their interest in the information displayed on product packaging, the researchers found that women were more likely to focus on the brand and the producer when making their purchasing decisions. The only statistically significant dependency was that between the consumers' interest in the above information and the age of the surveyed respondents (Table 2). The data presented in Figure 3 indicates that the importance of packaging information increases proportionally with the consumers' age.

The third most significant criterion which attracted the consumers' attention was the information regarding the product's ingredients, including fat

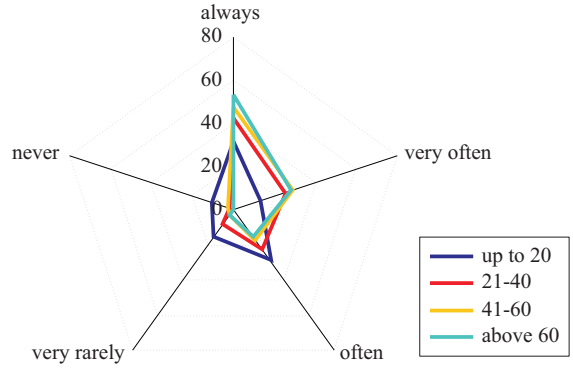


Fig. 3. Information on the product's name and supplier of dairy products in the opinion of the surveyed consumers, subject to the respondents' age

content (Figure 4). As shown by the obtained results, female respondents considered the above information to be more significant than the male population. Fat content and the specification of product ingredients proved to be as important in the hierarchy of data displayed on dairy product packaging as indicated in the research of ŚWIDA and KULIŃSKI (2002).

In view of current nutrition trends which support the consumption of natural, minimally processed food products, the authors of the study were of the opinion that the importance of information on product additives, including preservatives, should also be investigated. The above criterion ranked high in the hierarchy of the most important data displayed on the packaging of dairy products. More than 68% of the respondents claimed that they paid attention to this information "very often" and "often" (Figure 1).

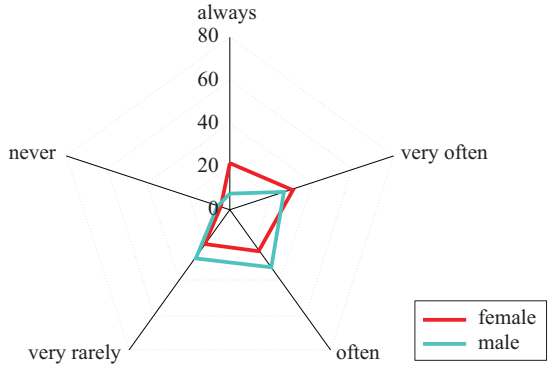


Fig. 4. Information on the ingredients of dairy products, including fact content, in the opinion of the surveyed consumers, subject to the respondents' sex

Consumers have a growing demand for reliable nutritional information to be able to choose a diet which meets their individual needs, a fact which explains their growing interest in label data on the nutritive value of the consumed products. The results of the study indicate that nutrition data is the fourth most important criterion which determines the respondents' choice of a given dairy product.

Information on the nutritive value of dairy products was correlated to the respondents' sex and place of residence. The number of female respondents who claimed to investigate the above data at least often exceeded the number of the surveyed males by more than 20%. Information on the product's nutritive value was examined very often or even upon every purchase by 42.6% of the surveyed respondents. The above claim was made by 64.9% of subjects who reside in rural areas. It should be noted, however, that persons residing in rural areas or respondents with a rural background accounted for only 14.8% of the entire surveyed population (Figure 5a and Figure 5b). According

to the findings of DRICHOUTIS et al. (2005), packaging information on the product's nutritive value is more likely to attract the attention of persons with greater knowledge of healthy nutrition standards and the nutritive value of food products.

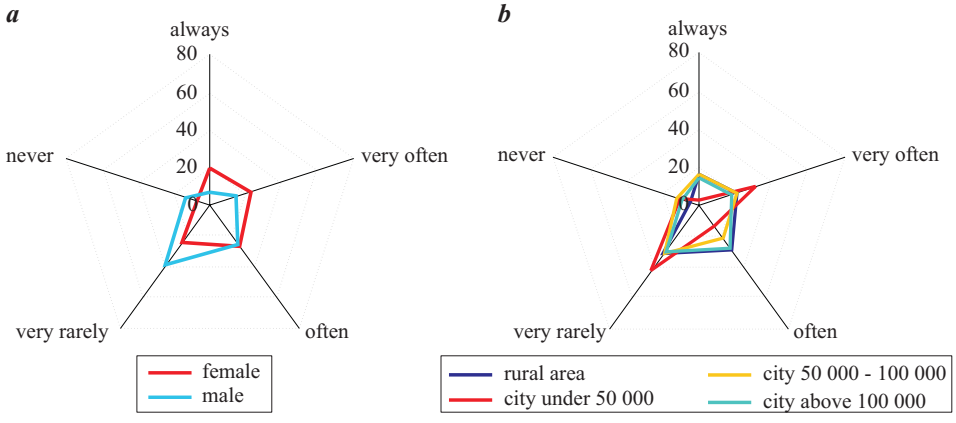


Fig. 5. Information on the nutritive value of dairy products in the opinion of the surveyed consumers: *a* – subject to the respondents' sex, *b* – subject to the respondents' place of residence

The remaining categories of packaging information (storage conditions, method of product preparation/application, quality marks, certificates, awards and medals given to the product) proved to be less significant in the opinion of the surveyed respondents (Figure 1).

Consumer opinions on the external appearance of product packaging

The external appearance of packaging is one of the most important factors which differentiate the consumers' perception and evaluation of the product's qualitative features. When shopping, consumers pick the product from the shelf based on the appearance of its packaging and only then do they examine the remaining product qualities. For this reason, the product's attractiveness is more likely to be determined by a change in packaging design than an improvement in the product's qualities, and the progress in packaging technology is often accompanied by an improvement of the product itself. In this sense, packaging becomes the product's equivalent partner. In view of the broad range of products available on the market, the individual features which contribute to the appearance of external packaging should be designed to ensure that the product stands out from

the remaining items and breaks the monotony of display (*Food Labelling* 2000b, GÓRNA 2000, UCHEREK 2001, SZYMCZAK, ANKIEL-HOMA 2002A, SZYMCZAK, ANKIEL-HOMA 2002b, ZALEWSKI 2001).

In this study, the respondents were polled about the significance of particular elements which contribute to the external appearance of dairy product packaging. Those elements were inclusive of the shape, color and graphic design of packaging. The shape of packaging which, until recently, was designed by the manufacturers in line with traditional guidelines, proved to be the most important criterion in product selection. More than 43% of the polled subjects claimed that the shape of packaging was a very important or an important factor determining their decision to buy a given dairy product. Therefore, contemporary packaging whose shape matches the consumers' expectations has to be functional, i.e. easy to use, it has to enable the consumer to fully utilize the product, it should be easy to store both in the retail outlet and at home, e.g. in a refrigerator. Survey results, verified with the use of the χ^2 test, showed a significant relationship between the influence of the respondents' age and place of residence on their perception of packaging shape as an important factor (Table 3). The shape and functionality of packaging was regarded as the most important criterion by nearly 49% respondents in the 41-60 age group. For 34.2% respondents aged up to 20, packaging shape was not an important criterion determining their choice of dairy product. The Color is an integral part of packaging and it fulfils several functions in marketing communication. The main functions are: capturing attention, creating psychological and symbolic conformity, enhancing brand memorization, increasing contrast, improving esthetic attractiveness, facilitating identification – i.e. differentiating the brand from competitive products, and determining the character of the product and the brand. In packaging design, color is applied to evoke the desired physiological and psychological reactions. Scientists have discovered that color plays a distinctive and permanent role in prompting the consumers' specific feelings and reactions.

Table 3

Influence of external appearance of unit packaging of dairy products on the consumers' decisions to buy dairy products subject to: sex, age, place of permanent residence, occupation and monthly income per person (value of χ^2 test)

Specification	Sex	Age	Place of permanent residence	Occupation	Monthly income per person
Shape	3.37	36.84*	49.41*	34.92	16.77
Color scheme	11.02	57.38*	43.88*	45.58*	10.96
Graphic design	5.41	69.21*	33.91*	34.98	21.36

* – significant value at $\alpha = 0.01$

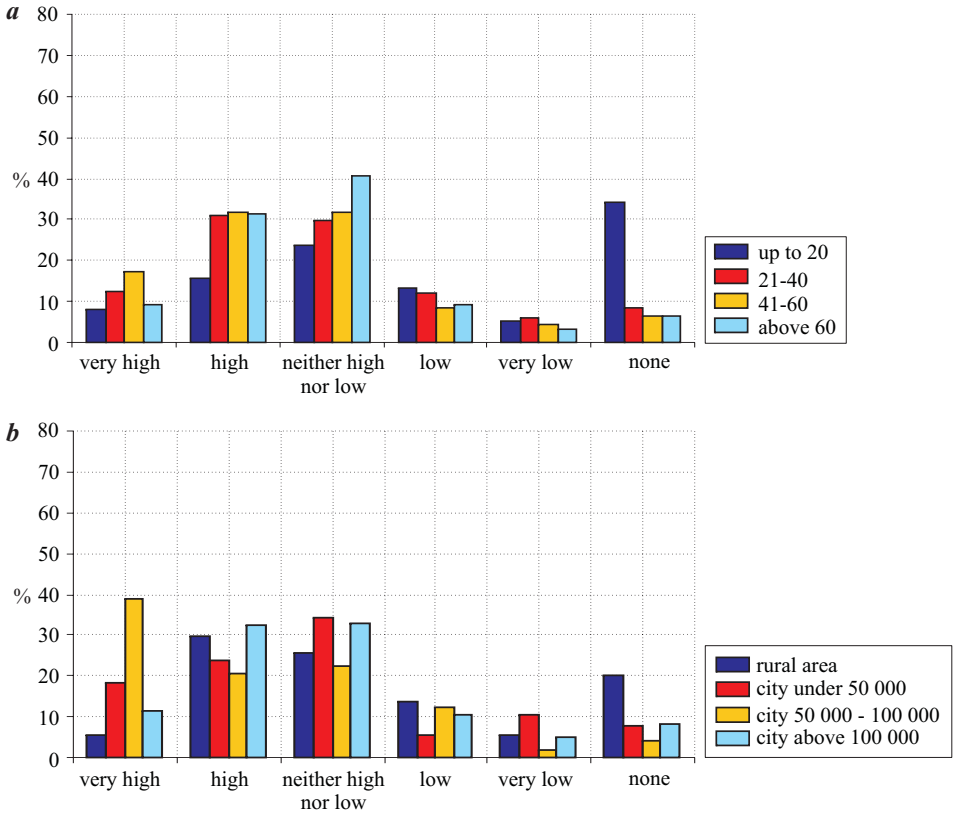


Fig. 6. Influence of packaging shape on the consumers' choice of dairy products: *a* – subject to the respondents' age, *b* – subject to the respondents' place of residence

Color can be used to emphasize the product's positive qualities such as purity, freshness or mildness.

Yet according of 60.4% of the surveyed respondents, the color scheme of packaging does not play a significant role in promoting dairy products. The significance of color in determining consumer attitudes towards products was largely dependent on the respondents' age, place of residence and occupation (Table 3). The highest positive perception of color as an important feature of the external appearance of product packaging was demonstrated by respondents in the 21-40 and 41-60 age groups. Nearly 40% of respondents aged above 60 had an indifferent attitude to the color of product packaging. Similarly to their perception of packaging shape, the youngest group of respondents claimed that the color scheme of packaging did not affect their choice of a given dairy product (Figure 7).

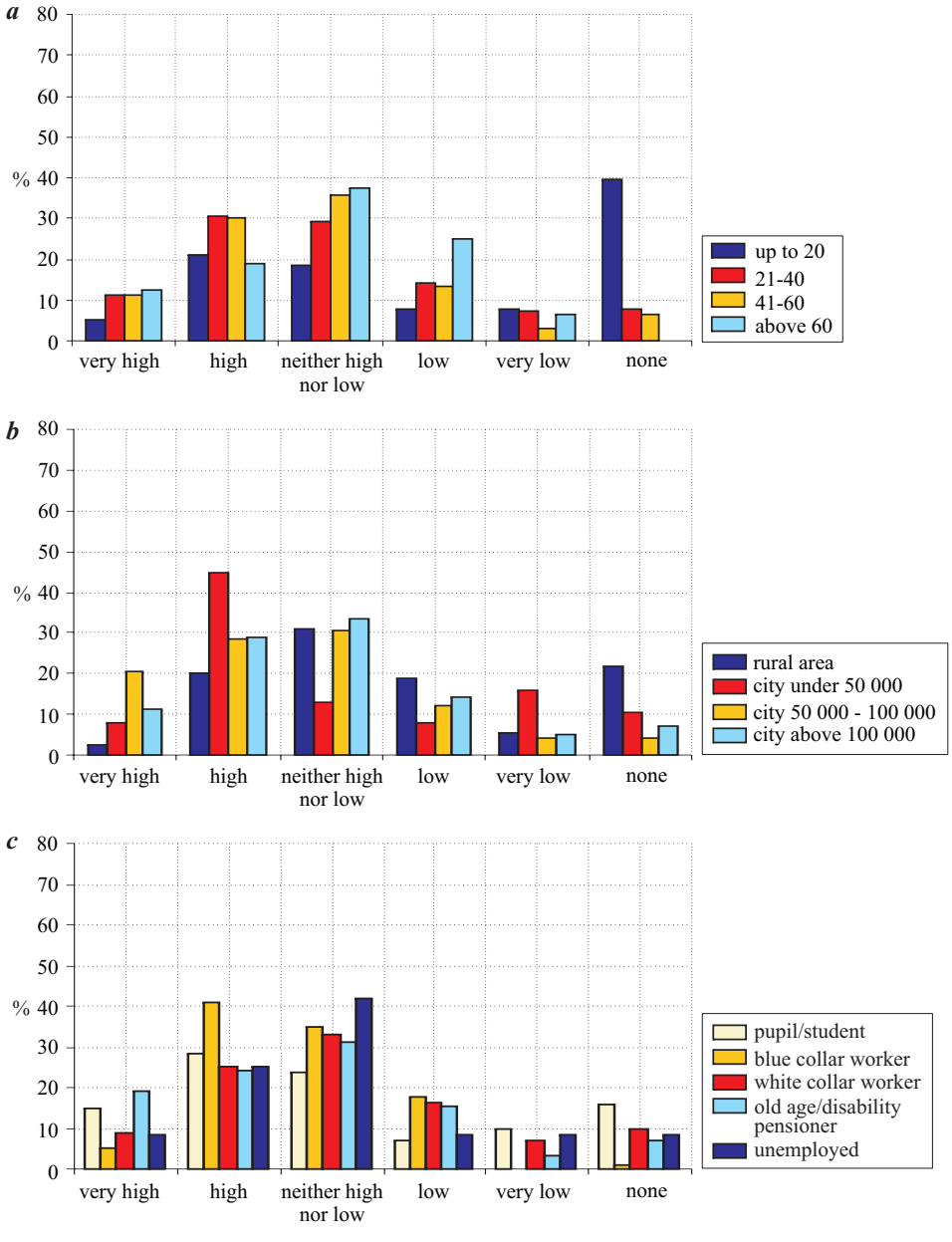


Fig. 7. Influence of packaging color on the consumers' choice of dairy products: *a* – subject to the respondents' age, *b* – subject to the respondents' place of residence, *c* – subject to the respondents' occupation

An even higher percentage of the surveyed respondents (65.4% indications) did not pay attention to the graphic design of product packaging. A statistically significant relationship was determined between graphic design and the respondents' age and place of residence (Table 4). Only a small fraction of respondents aged above 60 and respondents up to 20 years of age considered graphic design to be an important feature of external packaging (Figure 8a and Figure 8b).

Table 4

Significance of ecological properties of packaging as recognised by the consumers subject to: sex, age, place of permanent residence, occupation and monthly income per person (value of χ^2 test)

Sex	Age	Place of permanent residence	Occupation	Monthly income per person
0.25	1.37	3.28	4.20	1.95

* – significant value at $\alpha = 0.01$

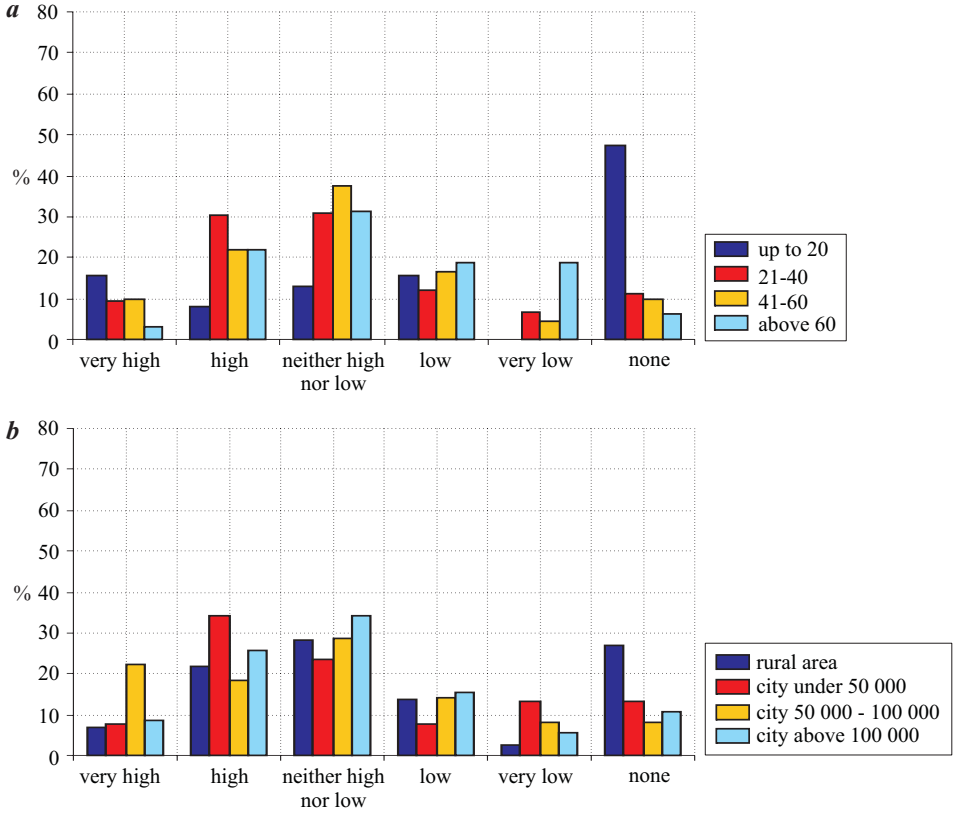


Fig. 8. Influence of the graphic design of packaging on the consumers' choice of dairy products: *a* – subject to the respondents' age, *b* – subject to the respondents' place of residence

Yet the focus on attractive graphic design of product packaging, which comprises print size, color, contrast, typography and the spatial layout of the displayed information, largely contributes to the legibility of product data and seems vital in view of the forecasts of the Central Statistical Office, the Government Population Council and the Committee on Demographic Studies. According to statistical forecasts, the Polish population in the 60/65+ age group will grow by 2030. Analysts are expecting a further decrease in mortality rates and a continued increase in life expectancy. In view of the above data, the producers need to shift their focus to the needs of this privileged population group. Older consumers experience a gradual deterioration of their sensory perception, such as sight or hearing, and stand an increased risk of certain diseases such as arthritis. Those ailments gradually impair their ability to effectively cope with daily life duties. On the other hand, those shortcomings incite them to remain independent and tackle daily problems by encountering as few obstacles as possible (*Food Labelling* 2000a, *Mały Rocznik Statystyczny...* 2005, SZUKALSKI 2003).

According to the Royal National Institute of the Blind, there are around 970 000 people in Great Britain who could be registered as blind or partially blind. 90% of people in that group are over 60 years of age which clearly indicates that the impairment of sight is an inseparable feature of the ageing process. Industrial producers should accommodate the needs of this group of consumers by, among others, enhancing the legibility of label information. According to estimates, around 45% of the population could benefit from more legible information on product packaging (*Food labelling*. 2000a).

Consumer opinions on the ecological properties of packaging

Whereas the functionality (usable value) and attractive external appearance (commercial value) play an important role in the purchase of food products, consumers are increasingly likely to pay attention to the ecological value of products and their packaging. This trend is particularly visible in countries where the consumers' quantitative needs have already been satisfied. In view of the significance of packaging information in the purchasing decisions made by the consumers, it becomes obvious that environmental policy also relies on packaging as a carrier of important information (WITCZAK 2003).

The above trend is not yet clearly manifested in Poland, mainly due to low average incomes and low environmental awareness of Polish consumers. Yet in view of growing environmental pollution, the popularity of products which satisfy the consumers' qualitative requirements and which are sold in environ-

mentally-friendly packaging is steadily on the rise. The above theory is supported by the results of this survey which are indicative of the respondents' growing interest in the ecological properties of packaging. This importance of this aspect was recognized by nearly 80% of the surveyed population regardless of sex, age, place of residence, occupation or income (Table 4).

By focusing on the environmentally-friendly aspects of packaging upon purchase, consumers are indirectly contributing to a reduction in the packaging materials' harmful impact on the environment. The producers monitor the consumers' environmentally-conscious choices and adapt their technology to manufacture products which are safe and friendly to the environment. In this context, packaging is an important carrier of information and it is used in environmental policy to raise the ecological awareness of both the consumers and the producers (SZYMCZAK, URBANIAK 2000).

Conclusions

The results of the survey indicate that dairy product consumers who pay attention to product labels read the information displayed on the packaging.

The most important factors shaping the consumers' choice of product were the shelf-life / minimum durability period, the producer's name which is often identified with a given brand, as well as information on the product's ingredients and nutritive value.

The consumers' propensity to make impulse purchases was confirmed by an observation that their choice of product is frequently determined by the shape and graphic design of the packaging.

It can be concluded that food producers should continue to improve their product labeling standards while ensuring attractive packaging design for their products. The information displayed on product packaging should be an important source of knowledge on nutrition and should contribute to an improvement in the consumers' nutritional awareness. The above requirements pose a challenge for food producers – as indicated by the results of this study, there are very few consumers who are not aware of or not interested in the significance of information displayed on the packaging of dairy products.

Translated by ALEKSANDRA POPRAWSKA

Accepted for print 20.08.2007

References

CICHOŃ Z., UCHEREK M. 2000. *Konsumenckie i laboratoryjne modele ocen opakowań bezpośrednich do produktów spożywczych*. Przem. Spoż., 8:27-28.

DRICHOUTIS A.C., LAZARIDIS P., NAYGA R.M. Jr. 2005. *Who is looking for nutritional food labelling?* Eurochoices, 4(1): 18-23.

Food Labelling. 2000a. *Problems of labelling for blind and visually impaired people.* Abington Hall, Cambridge 6AH, UK, Woodhead Publishing Ltd, pp. 233-236.

Food Labelling. 2000b. *Special issues in food labelling. Good design*. Abington Hall, Cambridge 6AH, UK, Woodhead Publishing Ltd, 205-211.

GÓRNA J. 2000. *Wpływ opakowania na zakupy artykułów nabiałowych*. Prz. Mlecz., 1: 21-24.

GÓRSKA-WARSEWICZ H. 2001. *Opakowania w strukturze zakupów produktów żywnościowych*. Przemysł Spożywczy, 8: 95-96.

KUŚMIERCZYK K. 2000. *Konsumenci produktów mlecznych*. Prz. Mlecz., 3: 71-74.

Mały Rocznik Statystyczny Polski. 2005. BIP GUS, Warszawa, <http://www.stat.gov.pl/>.

MCKENNA T. 2005. *Markets evolve with consumer preferences*. Dairying. 3 December, pp. 64-65.

PANFIL-KUNCEWICZ H., STANIEWSKA K., STANIEWSKI B. 2007. *Factors that simulate the consumer behaviour while purchasing the dairy products.* Pol. J. Nat. Sci. (in printing).

SMYCZEK S. 2003a. *Modele zachowań rynkowych konsumentów, cz. 1.* Marketing i rynek, 1: 14-19.

SMYCZEK S. 2003b. *Modele zachowań rynkowych konsumentów, cz. 2.* Marketing i rynek, 2: 14-19.

SZPONAR L., WIERZEJSKA R. 2004. *Znakowanie żywności jako narzędzie ochrony zdrowia konsumenta*. Przem. Spoż., 12(12): 14-16.

SZUKALSKI P. 2003. *Osoby bardzo stare w Polsce i w krajach Unii Europejskiej – przeszłość, teraźniejszość*. Prace Instytutu Ekonometrii i Statystyki UŁ.

SZYMCZAK J., ANKIEL-HOMA M. 2002a. *Koncepcja projektowania opakowania jednostkowego dla produktu wprowadzanego na rynek (na przykładzie żelu pod prysznic)*. Opakowanie, 2: 12-14.

SZYMCZAK J., ANKIEL-HOMA M. 2002b. *Warstwa wizualna opakowań jednostkowych kreatorem wizerunku wyrobów kosmetycznych*. Opakowanie, 2:17-19.

SZYMCZAK J., URBANIAK M. 2000. *Ekologiczne oznakowanie opakowań jednostkowych jako czynnik wpływający na decyzje zakupowe*. Opakowanie, 11: 14-16.

ŚWIDA J., KULIŃSKI A. 2002. *Opakowania produktów mleczarskich w opinii konsumentów*. Żywność, 3(22): 164-190.

UCHEREK M. 2001. *Charakterystyka cech jakościowych opakowań jednostkowych*. Opakowanie, 2: 18-20.

WITCZAK J. 2003. *Jakość żywności jako czynnik wpływający na decyzje nabywcze konsumentów*. Marketing i rynek, 8: 14-18.

ZALEWSKI R. I. 2001. *Preferencje nieokreślone w konsumenckich badaniach żywności*. Przem. Spoż., 10: 20-23.

POLISH JOURNAL OF NATURAL SCIENCES
Abbrev.: Pol. J. Natur. Sc., Vol 23(2): 521-530, Y. 2008

DOI 10.2478/v10020-008-0040-0

EFFICIENCY OF SUCROSE CRYSTALLIZATION FROM SUGARBEET MAGMA AFTER SONICATION

Dariusz M. Stasiak, Zbigniew J. Dolatowski

Chair of Meat Technology and Food Quality
University of Life Sciences in Lublin

K e y w o r d s: ultrasound, sonication, sugarbeet magma, crystallization, sucrose.

A b s t r a c t

The aim of the present study was to determine the effects of ultrasound waves on sucrose crystallization process from sugarbeet magma. The low frequency (40 kHz) batch ultrasonic device working at power density 35 W dm^{-3} was used. The viscosity, conductivity, dry substance content, sugar content, purity and pH of sugarbeet magma and molasses was determined after sonication. The higher effectiveness of nucleation process and mass transfer in complex solution such as sugarbeet magma were stated. The viscosity of the magma increases proportionally and conductivity decreases proportionally to time of sonication. The crystallization level in sonicated magma increased logarithmically with sonication time, and the increment was 27% after 20 min ultrasound treatment. Sugar content in molasses is decreased about 1.5 and purity of molasses is decreased by 0.7% after 20 min sonication. Sucrose crystallization in complex solutions like sugarbeet magma can be controlled by ultrasound and ensures higher process efficiency.

EFEKTYWNOŚĆ KRYSTALIZACJI CUKRU BURACZANEGO Z CUKRZYCY PODDANEJ SONIKACJI

Dariusz M. Stasiak, Zbigniew J. Dolatowski

Katedra Technologii Mięsa i Zarządzania Jakością
Uniwersytet Przyrodniczy w Lublinie

S ł o w a k l u c z o w e: ultradźwięki, sonikacja, cukrzyca, krystalizacja, cukier.

Address: Dariusz M. Stasiak, University of Life Sciences, ul. Skromna 8, 20-704 Lublin, Poland, phone: +48(81) 462 33 42, e-mail: dariusz.stasiak@ar.lublin.pl

Abstrakt

Celem badań była ocena wpływu fal ultradźwiękowych na proces krystalizacji cukru buraczanego z cukrzycy. Sonikację prowadzono za pomocą urządzenia wyposażonego w przetworniki o niskiej częstotliwości (40 kHz) i zapewniającego uzyskanie gęstości mocy 35 W dm^{-3}. Stwierdzono, że zmieniła się lepkość, kondunktancja, zawartość suchej substancji i cukru, a także czystość oraz pH cukrzycy i melasy. W szczególności zaobserwowano wyższą efektywność zarodkowania oraz wymiany masy w cukrzycy. Lepkość cukrzycy wzrastała, a kondunktancja malała proporcjonalnie do czasu trwania sonikacji. Stopień krystalizacji wzrastał logarytmicznie (o 27% po 20 min) wraz z długością czasu oddziaływania ultradźwięków. W wyniku sonikacji cukrzycy, zawartość cukru w melasie obniżyła się o ok. 1,5, a jego czystość wzrosła o ok. 0,7%.

Wykazano wzrost efektywności krystalizacji cukru buraczanego w cukrzycy poddanej sonikacji. Przebieg procesu może być sterowany za pomocą ultradźwięków.

Introduction

Ultrasonic irradiation and cavitation in liquid and solid-liquid systems can produce a number of unique chemical and physical effects like facilitate mass transfer and reactant diffusion. Many scientists have proved, the ultrasound is useful in crystallization processes (VAN HOOK, FRULLA 1952, POWEY, MASON 1998, MULLIN 2001, SUSLICK 2001, MCCAUSLAND, CAINS 2003, NAPIERAŁA et al. 2003). It serves a number of roles in the initiation of seeding and subsequent crystal formation and growth. The control of the nucleation and crystallization is very difficult due to an interaction of external parameters such as temperature, pressure, composition of substance. Sonication can standardize the process and enhance the nucleation and a rate of crystal growth in nucleation sites in the medium. This may be due to microflows ultrasonically induced in liquids and mainly by the physical phenomenon called cavitation. At implosion stage, the cavitation bubbles generate great pressure and temperature gradients accompanied by complex physicochemical phenomena. A higher rate of mass transfer in solid-liquid system and the disruption of seeds (nuclei) increase the number of nuclei present in solution and enhance crystallization. Power ultrasound usually operates at frequencies below 50 kHz but above the audible range. The higher frequencies of ultrasound are applied to investigations of material properties and are unusable to cause changes in sonicated substances (MASON, LORIMER 2002, ŚLIWIŃSKI 2001). The installation with ultrasonically assisted crystallization (sonocrystallization) was achieved successfully in the production of crystalline drugs in chemistry. The ultrasound can be generated on a number of methods. The most significant are mechanically and electrically powered transducers (generators). The introduction of ultrasonic waves into working chamber is the next source of difficulties in effective application on large scale in industry (HU et al. 2006).

Sucrose crystallization is one of the final stages of the process for producing sugar from beets. Sugar crystallization consists in creating the crystallizing nuclei and gradual increasing their weight due to spontaneous transfer of sucrose molecules from liquid to solid phase. Mainly inter and intramolecular forces between different particles of solutions take part. Invoking sucrose solution nucleation requires combining of several hundred sucrose molecules. Physicochemical properties of magma and conditions of evaporation affect the nucleation and crystallization of sucrose. Nucleation can be controlled by the level of supersaturation, seeding, and impulses of energy. A lot of processes in food industry, especially sucrose crystallization may be controlled by ultrasonic waves energy (McCLEMENTS 1995, MASON et al. 1996, POVEY, MASON 1998, MASON, LORIMER 2002, KACZMARSKI, LEWICKI 2005).

The aim of presented study is to verify the effectiveness of ultrasonically induced sugar crystallization in sugarbeet magma. The authors wanted to establish how ultrasound treatment changes the technological parameters of the magma, sugar and molasses and how modifies the crystallization process.

Theoretical consideration

Sucrose and its solutions are probably one of the most studied substance by food scientists, since it plays an important role as an ingredient in many technological processes. The pure solutions are investigated mainly. The crystallization of sugar from unpurified aqueous solution (such as sugarbeet magma) is conventionally difficult, because solutions are highly concentrated and syrups are very viscous. Additionally, non-sugar substances presented in the magma counteract to crystallization. The scope of sonocrystallisation depends on properties of solutions, operating conditions and characteristic of ultrasound source too. McCAUSLAND, CAINS (2003) stated the power densities of the scaled-up systems is about 70-80 W dm^{-3}, which is well in excess of the cavitation threshold for most liquids. Generally, the expected power is below 35 W dm^{-3} (ROBERTS 1993, McCLEMENTS 1995, POVEY, MASON 1998, MASON, LORIMER 2002). The ultrasound induces nucleation and narrows the width of metastable zone (McCAUSLAND, CAINS 2005). Due to higher mobility of the sucrose molecules, the frequency of collisions between associations is more probable. Studies revealed that mass transfer intensifies in field of ultrasound. Molecules by gaining additional kinetic energy faster penetrate the solution layer barrier that separates sucrose molecules. The temperature increase associated with energy dissipation favors the decrease of solution viscosity and the increase of molecule's energy. Implosions of cavitation bubbles may locally produce temperatures over 2000°C and pressure of 50 MPa. Under such conditions, molecules may be moving at speed much over 100 m s^{-1} (SUSLICK

2001). Therefore, treatment of sugarbeet magma with ultrasounds increases the probability of fluctuations and collisions of sucrose molecules and in effect, a higher number of stable agglomerate arises.

Materials and Methods

Preliminary preparation of samples

The sugarbeet magma (mixture of sugar syrup and sugar crystals produced during sugar refining) was collected from a site in sugar refinery just before crystallizers (Figure 1). Samples separated from magma stream were divided into parts, from which one was the control (non-treated with ultrasound), and others were treated with ultrasound for 1, 5, 10 or 20 min respectively with the laboratory stand (Figure 2). The samples were stored 24 hours at 20 ± 2°C.

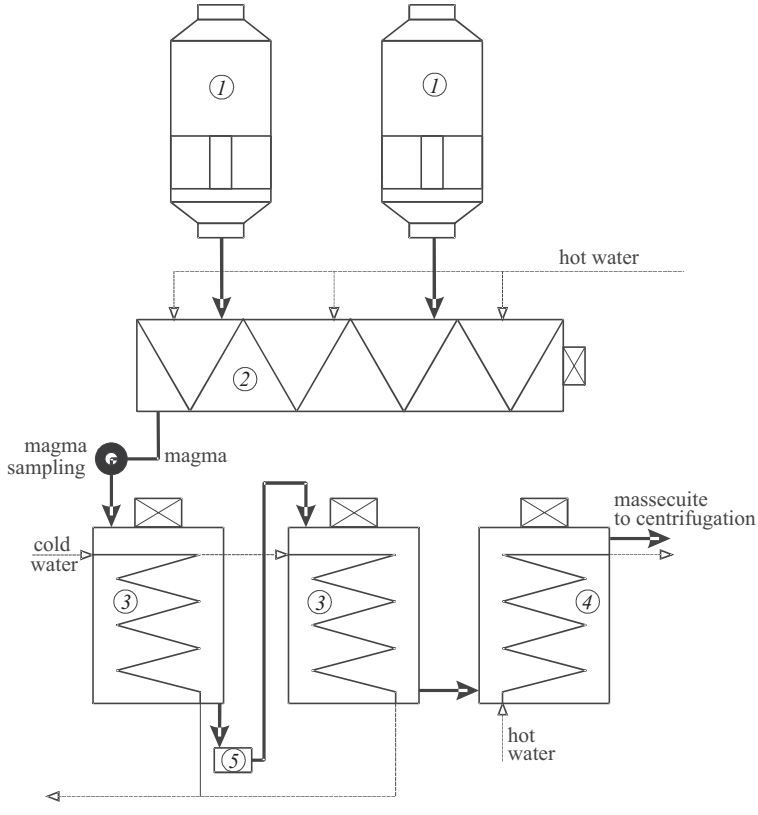


Fig. 1. Schematic diagram of sugar rafinery crystallization unit: *1* – vacuum pan, *2* – stirrer, *3* – cooling crystallizer, *4* – heating crystallizer, *5* – pump of magma (massecuite)

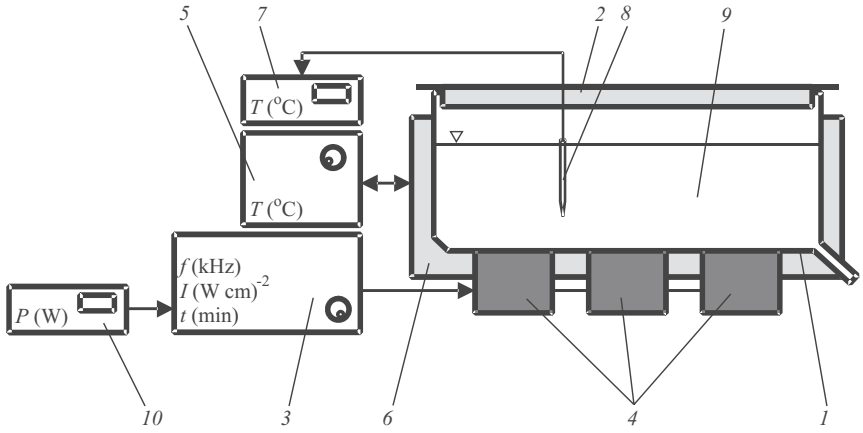


Fig. 2. Schematic diagram of sonication stand: *1* – vessel, *2* – cover, *3* – generator, *4* – transmitter, *5* – thermostat, *6* – thermo-coat, *7* – thermometer, *8* – thermocouple, *9* – sonication chamber, *10* – wattmeter

Sonication method

Sonication was performed using a batch ultrasound device 5 dm^3 capacity (Figure 2). Three piezoelectric emitters mounted below the thin-walled stainless steel work chamber bottom generated 40 kHz ultrasonic waves at intensity of transducer about 2 W cm^{-2} and power density of device about 35 W dm^{-3}. Temperature of sonicated sample in vessel was stabilized with electric thermo-coat. The working chamber was covered.

Measurements

Temperature of sonicated samples was recorded by MPI-L (Metronic Instruments) thermometer. Accuracy of measurement at used temperatures was ± 1°C. Viscosity was determined using viscosimeter (model RV, Brookfield) with SSA adapter with spindle No 21 (n = 3.33 s^{-1}). Measurement was conducted at 65°C. The temperature was ensured by a water jacket connected to thermostat (model 9112, Polyscience). Conductance and acidity were measured using pH/conductometer (model CPC-501, Elmetron) with temperature compensation. Conductance measurements at 63 ± 2°C were made using conductometric electrode (model CD-2, Hydromet), and pH level – using electrode (model ERH-111, Hydromet) at 20 ± 1°C.

Crystallization level was calculated as a ratio of crystallizate weight to initial sucrose weight in the magma. Crystallizate was separated from the

solution using vacuum filter and dried at room temperature (20 ± 2°C). The crystalizate was observed under the optical microscope at magnification ×25 and ×50. Sugar content (CK) in solutions was measured polarimetrically. Aliquots of 30 ± 0.01 g of the solution and distilled water were weighed and placed in laboratory extractor. Extractor was heated for 15 min at 72°C and forcefully shaken every 5 min. Then, extractor was cooled with water to 20°C. Sample of 26 ± 0.01 g of solution from the extractor was mixed with 10 cm^3 of Harles's I liquid, 10 cm^3 of Harles's II liquid and mixed again. The solution's volume was adjusted to 200 cm^3 with distilled water and mixed again. The mixture was separated on double filter. Real sugar concentration was read from the scale of saccharimeter (Polamat). Content of dry substance (SS) in solutions was measured using refractometer with °Bx scale. Purity (CZ) of sugar and molasses expressed as the per cent of sugar amount in dry substance was calculated from a formula $CZ = 100 \cdot CK / SS$, where: CZ – sugar purity, CK – sugar content, SS – apparent content of dry substance.

The experiment was carried out in 10 repetitions. The data were analyzed by statistic computer software. The significance of mean differences (LSD), regression and 95% confidence intervals were calculated. Graphs were plotted using mean values.

Results and Discussion

The conductivity and viscosity of the magma characterize the state of crystallization process (NIKIEL 1996). Studies upon the apparent viscosity of the magma revealed that it increases proportionally to the time of sonication. The gain of viscosity after 20 min of treatment with ultrasound was about 25 mPas (Figure 3). Simultaneously the sonication decreases conductance of the magma almost by 0.6 mS cm^{-1}. The observed changes of viscosity and conductivity of the magma show, that ultrasound initialize and stimulate the nucleation of crystallization in comparison with the unsonicated magma. The changes of viscosity (ΔVisc) and conductance (ΔCond) of sonicated samples in comparison with control one are described by the linear equation (Figure 3) at high value of coefficient of determination ($R^2 > 0.77$).

Results of observation under the optical microscope revealed that the number of crystals in sonicated sample was higher compared to the control (Figure 4). The difference (ΔSK) between of amount of separated crystalizate from sonicated and unsonicated magma (Figure 5) is considerable. Difference of crystallization level (ΔSK) increases with the sonication time (t), and the dependence is described by logarithmic equation with a high coefficient of determination $R^2 = 0.94$ (Figure 5). The highest rise (27%) of crystallization

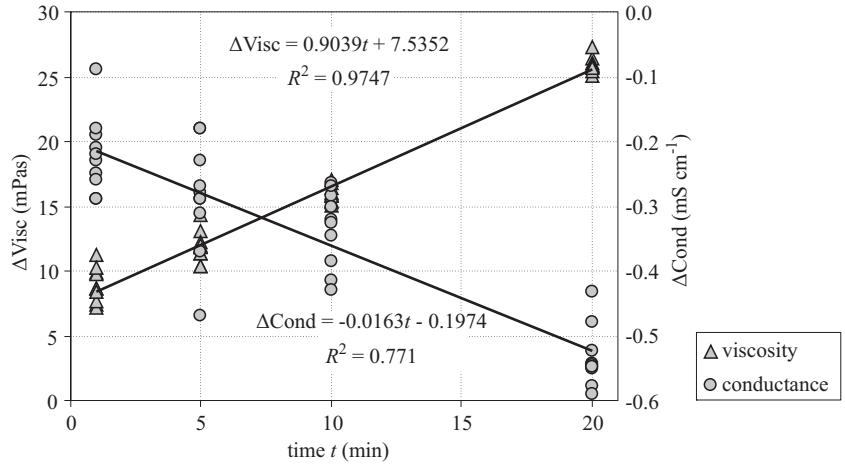


Fig. 3. Changes of viscosity (ΔVisc) and conductance (ΔCond) of sugarbeet magma after sonication

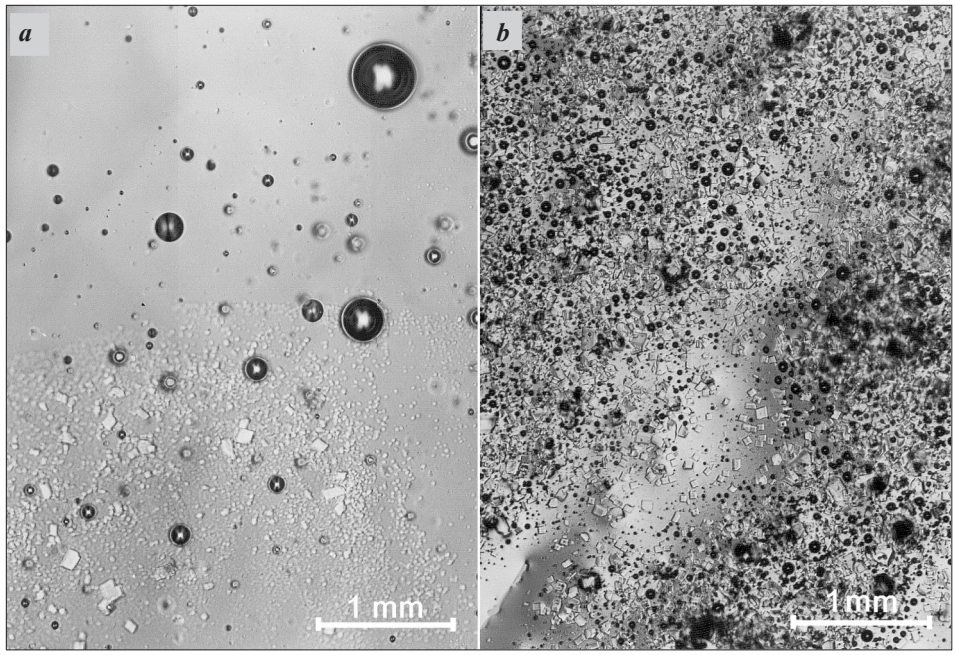


Fig. 4. Optical micrographs of sucrose crystals obtained without (a) or with (b) ultrasound

level was achieved after maximum (20 min) sonication time. Increase in in the crystallizate mass in magma after sonication resulted from more efficient nucleation and higher mass transfer rate between solid and a solution. Results indicated that nucleation and mass transfer rate may be controlled using ultrasound.

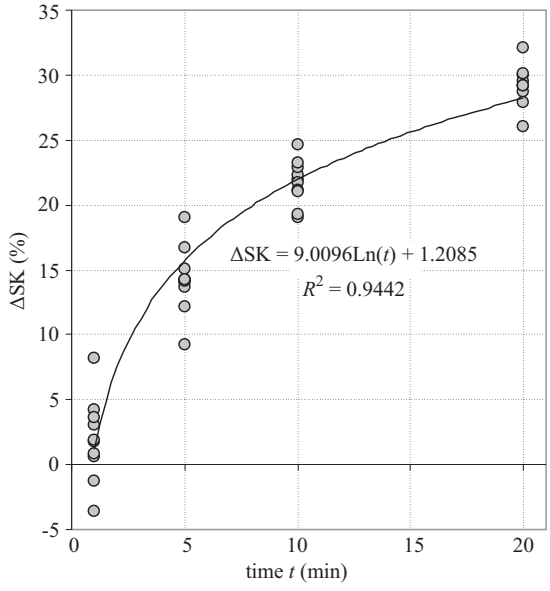


Fig. 5. Changes of crystallization level (ΔSK) after sonication

Dry substance content (SS) in the magma is altered after sonication. Directly after 20 min treatment, content of dry substance in magma (Table 1) increases by 0.57°Bx compared to the control (t = 0 min). The content in the molasses decreases by 1.15°Bx after 20 min sonication. After 24 hours storage the differences between sonicated and control samples are higher (0.9 after 20 min). Sugar content (CK) in molasses is decreased by 1.5 directly after 20 min sonication and by 0.8 after 24 hours storage of sample. Acidity of magma and molasses ranged from pH 8.2 to pH 8.5. Directly after sonication, pH of magma lightly decreased by about 0.1 and after 24 hours – by next 0.1. Regardless the change in apparent dry substance and sugar contents, magma purity after sonication was about 75.2%. Sonication of magma affected the purity (CZ) of molasses (Table 1). Reduction of molasses purity was 0.7% after 20 min sonication. On the basis of presented data and GRUSZECKA work (1993) it may be supposed that sonication invoked changes in some non-sugars properties, namely the decrease of colloid viscosity and reduction of their molasses--forming abilities. NIKIEL (1996) reported that a decrease of purity (CZ) of the molasses by about 1% may increase the efficiency of crystalline sugar by about 0.07-0.08%. The most obvious change of purity (over 0.8%) was observed after 20 min of treatment. In 24 hours after sonication, the purity of molasses decreased by about 1.5%.

Table 1

Dry substance content, sugar content, purity and pH of sugarbeet magma and molasses

Material	Storage (h)	Sonication (min)	Dry substance (°Bx)	Sugar content	Purity (%)	pH
Magma	0	0	92.52±0.12	69.57±0.26	75.19±0.28	8.46±0.04
		1	92.66±0.08	69.66±0.20	75.18±0.21	8.45±0.03
		5	92.80±0.07	69.76±0.21	75.17±0.20	8.44±0.02
		10	92.84±0.14	70.08±0.26	75.49±0.29	8.50±0.02
		20	93.09±0.12	69.95±0.37	75.15±0.37	8.43±0.01
	24	0	92.70±0.16	69.74±0.16	75.20±0.23	8.38±0.06
		1	92.76±0.11	69.75±0.15	75.17±0.17	8.39±0.04
		5	92.82±0.08	69.76±0.19	75.15±0.16	8.40±0.03
		10	92.94±0.14	69.78±0.33	75.09±0.32	8.41±0.02
		20	93.47±0.30	70.24±0.45	75.15±0.35	8.21±0.09
Molasses	0	0	89.51±0.61	56.93±0.49	63.58±0.50	8.48±0.03
		1	89.22±0.46	56.55±0.40	63.37±0.47	8.47±0.02
		5	88.93±0.39	56.18±0.40	63.17±0.50	8.45±0.02
		10	88.92±0.56	56.14±0.56	63.15±0.39	8.47±0.02
		20	88.36±0.56	55.44±0.64	62.76±0.67	8.43±0.01
	24	0	89.53±1.05	55.51±0.85	62.00±0.47	8.35±0.06
		1	89.45±0.85	55.42±0.70	61.95±0.34	8.37±0.04
		5	89.37±0.75	55.33±0.57	61.90±0.23	8.39±0.03
		10	89.21±0.89	55.15±0.50	61.81±0.20	8.42±0.03
		20	89.25±0.44	54.70±0.48	61.27±0.48	8.27±0.05

Conclusions

The obtained experimental data shows differences between physicochemical properties of sonicated and unsonicated sugarbeet magma and molasses. The viscosity increases and conductance decreases proportionally to time of sonication as a result of more efficient nucleation in ultrasound field. A longer time of sonication increases the number of nuclei formed, so that the size and shape of crystal product can be customized.

Crystallization of sugar from multicomponent solutions like the magma is improved by sonication. The great number of crystals and higher mass transfer rate are the main effects of ultrasound treatment. The dependence of time and sugar crystallization level is described by logarithmic equation.

Magma sonication decreases the sugar loses to molasses about 1-1.5%. So, thanks to the application of ultrasound, the efficiency of sugar refination is greater and the productivity of sugarbeet can by higher. Especially the ultrasonic equipment can be easy to operate, needs little maintenance and has a good automised performance.

Translated by AUTHORS

Accepted for print 15.10.2007

References

GRUSZECKA H. 1992. *Wyniki zastosowania ciekłych preparatów obniżających lepkość cukrzyc*. Gazeta Cukrownicza, 9(100): 168-169.

HOOK A. VAN, FRULLA F. 1952. *Nucleation in Sucrose Solutions*. Ind. Eng. Chem. 44(6): 1305-1308.

HU A., ZHENG J., QIU T. 2006. *Industrail experiments for the application of ultrasound on scale control in the Chinese sugar industry*. Ultrasonic Sonochemistry, 13: 329-333.

KACZMARSKI L.K., LEWICKI P.P. 2005. *Zastosowanie technik ultradźwiękowych w przetwarzaniu żywności*. Przemysł Spożywczy, 9(59): 34-36.

MASON T.J., LORIMER J.P. 2002. *Applied Sonochemistry*. *Uses of Power Ultrasound in Chemistry and Processing*. Wiley-VCH Verlag. Weinheim, pp. 4-21, 53-54.

MASON T.J., PANIWNYK L., LORIMER J.P. 1996. *The uses of ultrasound in food technology*. Ultrasonics Sonochemistry, 3(3): S253-S260.

MCCAUSLAND L., CAINS P. 2003. *Ultrasound to make crystals*. Chem. Ind., 5: 15-17.

MCCLEMENTS D.J. 1995. *Advances in the application of ultrasound in food analysis and processing*. Trends Food Sci. Technol., 6(9): 293-299.

MULLIN J.W. 2001. *Crystallization. Elsevier Butterworth-Heinemann*. Oxford, pp. 181, 462.

NAPIERAŁA D.M., BISKUPSKI P., PLENCLER G.B. 2003. *Observations of the crystallization of glucose from aqueous solution*. Acta Agrophysica, 2(2): 257-366.

NIKIEL S. 1996. *Cukrownictwo*. WSiP, Warszawa.

POVEY M.J.W., MASON T.J. 1998. *Ultrasound in Food Processing*. Blackie Academic & Professional, London, pp. 115-126.

ROBERTS R.T. 1993. *High intensity ultrasonics in food processing*. Chem. Ind., 15(2): 119-121.

SUSLICK K.S. 2001. *Sonochemistry and Sonoluminescence*. [In:] *Encyclopedia of Physical Science and Technology*. Ed. R. Mayers. San Diego: Academic Press, 17: 363-376.

ŚLIWIŃSKI A. 2001. *Ultradźwięki i ich zastosowania*. WNT. Warszawa, pp. 182-184.

POLISH JOURNAL OF NATURAL SCIENCES
Abbrev.: Pol. J. Natur. Sc., Vol 23(2): 531-539, Y. 2008

DOI 10.2478/v10020-008-0041-z

FELINE CRYPTOCOCCOSIS

***Anna Wiśniewska*[1], *Tadeusz Rotkiewicz*[1], *Andrzej Pomianowski*[2]**

[1] Chair of Pathology and Pharmacology
[2] Chair of Clinical Sciences
University of Warmia and Mazury in Olsztyn

K e y w o r d s: cryptococcosis, cats.

A b s t r a c t

This paper reports on the pathogenic significance of fungi from the genus Cryptococcus to cats. In Poland, diseases induced by those fungi occur sporadically, hence their diagnosis is difficult. Two cases of cryptococcosis in cats were described. Histopathological analyses found the occurrence of blastospores, 4.68 μ (± 1.39) in diameter, in numerous internal organs of the cats, including brain, lungs, liver, kidneys, and spleen. However, the highest number of blastospores was observed in the mucous membrane of nasal cavity, nasal sinuses, trachea and lungs. Microscopic analyses of smears prepared from the upper respiratory tract exudate stained with PAS (according to McManus), HE and mucicarmine were found to be useful in disease diagnosis.

KRYPTOKOKOZA KOTÓW

***Anna Wiśniewska*[1], *Tadeusz Rotkiewicz*[1], *Andrzej Pomianowski*[2]**

[1] Katedra Patologii i Farmakologii
[2] Katedra Nauk Klinicznych
Uniwersytet Warmińsko-Mazurski w Olsztynie

S ł o w a k l u c z o w e: kryptokokoza, koty.

A b s t r a k t

W pracy opisano chorobotwórcze działanie grzybów z rodzaju *Cryptococcus* u kotów. W Polsce choroba występuje sporadycznie i dlatego sprawia duże trudności rozpoznawcze. Opisano dwa przypadki kliniczne kryprosporidiozy kotów. Badania histopatologiczne licznych narządów

Address: Tadeusz Rotkiewicz, University of Warmia and Mazury, ul. Michała Oczapowskiego 13, 10-718 Olsztyn, Poland, phone: (089) 523 34 58, e-mail: tadeusz.rotkiewicz@uwm.edu.pl

wewnętrznych, w tym mózgu, płuc, wątroby, nerek i śledziony, wykazały obecność blastospor o średnicy 4.68 μ (± 1.39). Największą ich liczbę stwierdzono w śluzie błony śluzowej jamy nosowej, zatok okołonosowych, tchawicy oraz płuc. Badanie śluzu z błon śluzowych górnych dróg oddechowych oraz barwienie preparatów hematoksyliną i eozyną, metodą PAS wg McManusa oraz mucikarminem okazały się najbardziej przydatne do diagnostyki choroby.

Cryptococcosis (also referred to as Torulosis or European Blastomycosis) occurs in a number of animal species as well as in humans (AL-DOORY 1971, DICKSON, MEYER 1970, GELATT et al. 1973). In cats, it occurs as organ mycosis (BEMIS et al. 2000, MALIK et al. 1992). Its etiological factors are *Cryptococcus neoformans*, a fungi belonging to the kingdom *Fungi*, phylum *Basidimycota*, subphylum *Basidimycotina*, order *Sporidiales*, family *Sporidiobolaceae*, and genus *Filobasidiella* (*Cryptococcus*). They occur in soil, especially that fertilized with pigeon feces (Stephen et al. 2002), in dust and many plants. There have been 37 species of cryptococci isolated, among others, *C. albidus, C. hominis, C. laurentii, C. terreus, C. uniguttulans, C. luteous, C. gastricus, C. neoformans* and others detected most often. Most of the species of these fungi are non-pathogenic saprophytes. *C. neoformans* is a pathogenic fungi that occurs in 4 (A, B, C, D) or 5 serotypes (A, B, C, D, AD) (O'BRIEN et al. 2004). Fungi produce spherical or oval blastospores 3.5-7.0 μm in diameter, the shape of which resembles soap bubbles covered with a polysaccharide envelope protecting them against drying. Blastospores are resistant to the action of environmental factors and under favorable conditions of humidity and once non-exposed to UV radiation they are capable of surviving for up to 2 years (BOOTHE 2001, BUCHANAN, MURPHY 1998). In an animal organism, blastospores produce a gel-like envelope that protects them against phagocytosis by neutrophils, monocytes and tissue macrophags, which additionally enables lysis of host cells (KOZEL et al. 1984, KOZEL, MASTROIANNI 1976). Blastospores produce an enzyme – phenolic oxidase – that participates in the production of melanin (O'BRIEN et al. 2004), which is claimed to prevent the harmful effects of hydroxyl radicals formed at the inflammation site on blastospores (O'BRIEN et al. 2004). Of a similar protective significance to these fungi may be superoxide dismutase (SOD), which has been detected in cultures of *C. neoformans* run at a temperature of 37°C (JACOBSON et al. 1994). These fungi produce proteases that destroy plasma and skin proteins, e.g. collagen (BRUESKE 1986, MULLER, SETHI 1972, SALKOWSKI, BALISH 1991). In addition, CHEN et al. (1997) demonstrated that most strains of *C. neoformans* produce phospholipases which damage cellular membranes and thus facilitate the penetration of fungi to different organs.

Direct infection of man or animals from an infected animal is hardly probable, therefore, infections are sporadic and refer to single animals. Cases

of cryptococcosis in animals are reported most often on areas with a warm and temperate climate (BOOTHE 2001). It seems that the significance of this disease is also increasing in Poland, which is linked with dissemination of FIV (feline immunodeficiency virus) and FeLV (feline leukemia virus) infections in cats (HOSIE et al. 1989, KITA, FRYMUS 2003) as well as with frequent incidence of airways inflammation, neoplastic disease and resistance disorders. The highest incidence is observed in the case of multi-breed crosses, as well as Persian and Siamese cats (O'BRIEN et al. 2004). In infected cats, there are usually benign anemia, eosinophilia and monocytosis observed, as well as a very noticeable decrease in the number of leucocytes (BROWN, ROGERS 1995). WALKER et al. (1996) additionally reported on a decrease in the number of CD4+ lymphocytes and a lower ratio CD4:CD8, whereas HENDERSON et al. (1986), and SALVIN, SMITH (1961) on disturbances in the regulatory function of T cells.

In cats, the disease usually proceeds as a chronic ailment. Clinical examinations demonstrate: anorexia, enlargement of peripheral lymph nodes, chronic cough accompanied by mucopurulent or mucopurulent with blood nasal exudate, as well as renal failure and bone softening, whereas less frequently – an accumulation of dry secretion in the nasal cavity that impairs breathing. In ca. 10% of cats there are disturbances of vision and nervous symptoms. Cats fall into coma and neither eat nor drink, which results in a considerable loss of body weight and the appearance of subcutaneous tissue odema, especially in the area of nose, mouth and eyes.

A common method of cryptococcosis diagnosis is a microscopic analysis of a nasal exudate smear or skin lesions. An analysis of smears stained with mucicarmine, PAS acc. to the method of McManus and silver-plated acc. to the method of Gomori with metanamine demonstrates the presence of blastospores, which enables morphological determination of fungi. Attention should also be paid, however, to the possibility of obtaining a negative result of cytological analysis in the case of a small number of blastospores. In such a case, a precipitation method is recommended to enable the identification of a capsular antigen of cryptococci in blood plasma. In cryptococcosis diagnosis, high usability has been demonstrated for the ELISA method that allows to determine the level of plasma antibodies. Several ELISA analyses have confirmed the development of this disease in cats. Investigations by FLATLAND et al. (1990) have demonstrated that in cats there might occur a high titre of antibodies maintained for months or years, irrespective of clinical symptoms of cryptococcosis. The final diagnosis of the disease should be based on a histopathological analyses of specimens of altered tissues, culture of fungi and microscopic analyses of smears.

Cryptococci are susceptible to a number of therapeutics. It is claimed that a drug of choice is amphotericin B (MEDLEAU et al. 1990), though

its effectiveness is low, especially in the treatment of cryptococcic meningitis, as it does not overcome the blood-brain barrier and has a side effect in the form of nephrotoxicity, leading to azotemia. Despite these faults, amphotericin B is often applied subcutaneously (MALIK et al. 1996). Another medicine used in the treatment of cryptococcosis is ketoconazole, a derivative of imidazole (GERDS-GROGAN, DAYRELL-HART 1997). Therapy is also carried out with itraconazole and fluconazole (CRAIG et al. 1994, FAGGI et al. 1999, MALIK et al. 1992, BOOTHE 2001, MEDLEAU et al. 1990) as well as by means of a combined therapy with amphotericin B and imidazole (BOOTHE 2001). Prognosis is worse when a neural form of the disease occurs or when it is accompanied by FIV or FeLV infection.

Description of cases.

The first case referred to a male cat, 9 years old, cross-breed, with frequent sneezing and nasal seromucous exudate. The cat was dejected, its general condition was very bad, it displayed symptoms of malnutrition and dehydration. A clinical examination indicated catarrhal inflammation of nasal mucosa, odema of subcutaneous tissue of nose dorsum, sneezing and impaired breathing. Its body temperature was decreased. The cat was not treated and soon died. Autopsy demonstrated cachexy, paleness of mucous membranes, odema of the subcutaneous tissue most noticeable in the nasal and submandibular areas as well as deformation of mandibular bone and turbinated bones. Mucosa of the nasal cavity, perinasal sinuses and trachea was affected by acute catarrhal inflammation. Numerous foci of catarrhal inflammation were also detected in lungs. Visceral cavities were characterized by an increased content of serous fluid. Parenchymatous degeneration was observed in liver, kidneys and cardiac muscle, whereas liver and kidneys were additionally affected by hypostatic congestions. Peripharyngeal lymph nodes and mesenteric lymph nodes as well as spleen were enlarged and hyperemic. Cerebral meninges were hyperemic and with gelatinoid infiltrations. The brain was hyperemic, and an increased content of serous fluid was observed in its lateral ventricles.

During autopsy, smears were prepared from exudate occurring on mucosa of nasal cavity, mouth, trachea and bronchi, as well as from fluid occurring in body cavities and lateral ventricles of the brain. They were stained with HE, PAS acc. to McManus and mucicarmine. In addition specimens of lungs, liver, peripharyngeal and mesenteric lymph nodes, spleen, kidneys, cardiac muscle, skeletal muscles, skin and eye were collected for histopathological analyses. The preparations were stained with HE, PAS acc. to McManus and mucicarmine with the paraffin method.

In smears and histopathological preparations, there were numerous blastospores 4.68 μ (±1.39) in diameter observed. The blastospore diameters were determined using a Digital Image Analysis (LUCIA 3,52a software,

Panasonic digital camera and Carl Zeiss Jena microscope). Other authors measured the diameter of blastospores in the range of 3.5 to 7.0 microns (BUCHANAN, MURPHY 1998). The surface of the nasal cavity mucosa was covered with mucocellular exudate with numerous blastospores (Figure 1).

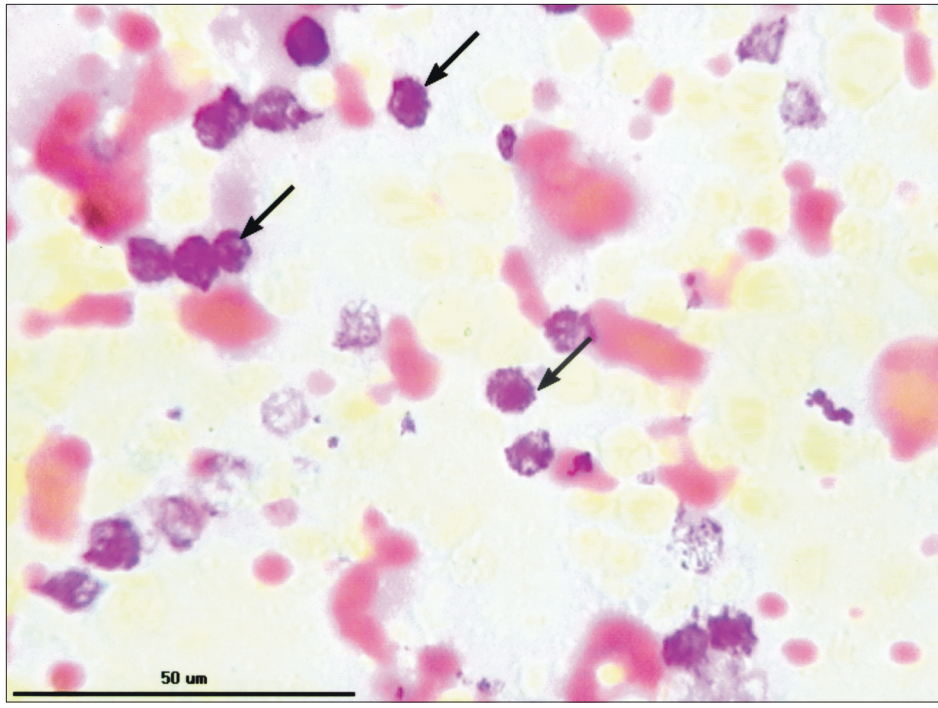


Fig. 1. Smear stained with PAS acc. to MacManus – arrows show cryptococci blastospores stained in purple

The histopathological examination of internal organs serves for the detection of granulomas formed of histiocytes, giant cells, single plasmatic cells and cryptococci (BEMIS et al. 2000, FOSTER et al. 2001). In the described case, no granulomas were detected in the cat's cells, whereas all of its organs had lymphocytes infiltrations and single blastospores. In the lungs, clusters of histiocytic cells, leucocytes with a segmented nucleus and a considerable amount of seromucous exudate were observed at the site of cryptococci occurrence. In the brain, there were single blastospores (Figure 2), surrounded by clusters of glia cells. Lymph nodes were characterized by the occurrence of focal infiltrations of lymphocytes, atrophy or necrosis of proliferation centers in follicles and the presence of serous exudates. In kidneys, apart from

single blastospores, there were observed: vacuolar, hyaline degeneration and necrosis of tubule epithelium cells. In the liver, there were multiple clusters of blastospores surrounded with dead hepatic cells (Figure 3). In the case of skin, no cryptococci were detected, but odema of subcutaneous tissue was confirmed. No morphological changes were either observed in the eyes, however, in a study on cats suffering from cryptococcosis, GIONFRIDDO (2000) found inflammatory processes affecting eyelids and internal tissues of eye in all infected animals.

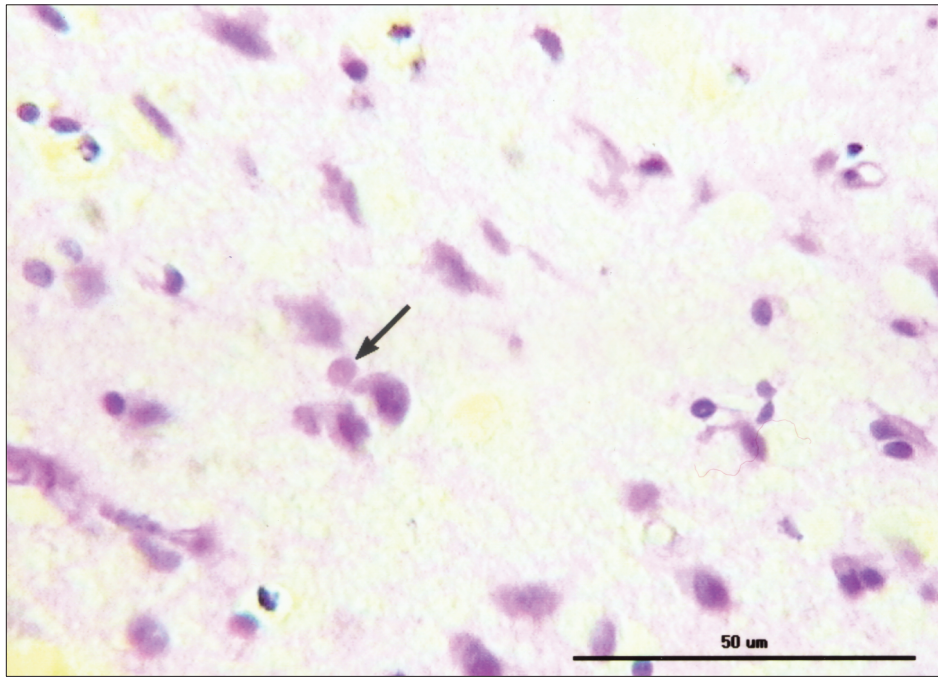


Fig. 2. Presence of a single blastospore (arrow) in brain. Staining with PAS acc. to McManus

The second case referred to a male cat, of European breed, aged 5 years and weighing 4 kg. Neurological examination demonstrated apathy, motor astigmatism, proprioceptive dysesthesia in all limbs, and paraparesis. Unequal pupillary dilation (anisocoria) and bilateral lack of reaction to threats were also observed. In addition, the examination demonstrated dysfunction of the V and XII pair of cranial nerves, consisting in paralysis of mandible and difficulties in tongue retraction. Results of hematological analyses demonstrated an increased hematocrit values (0.55). In turn, microscopic analysis of cerebrospinal

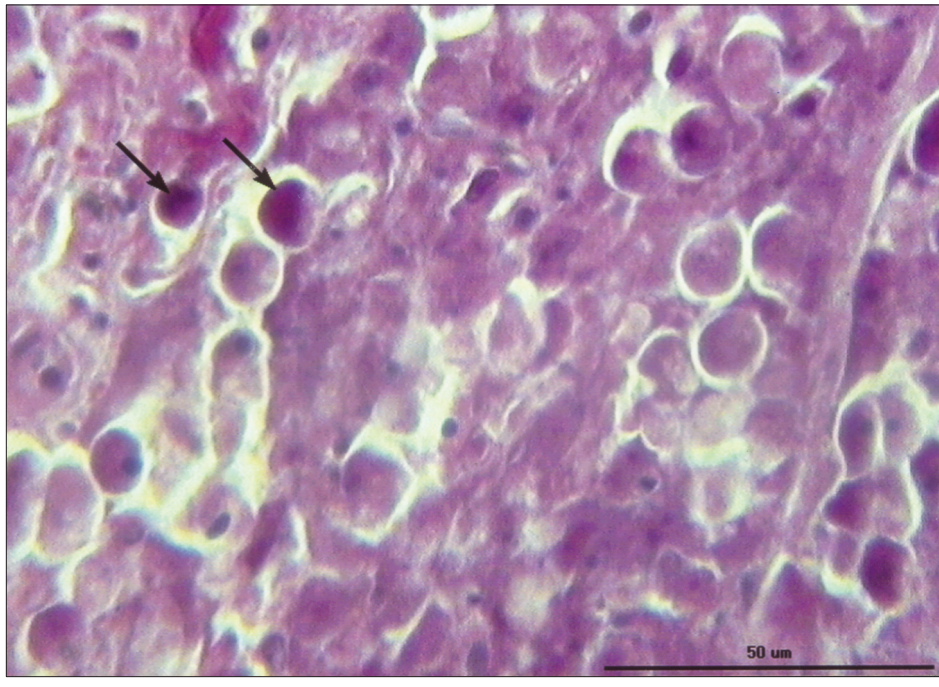


Fig. 3. Presence of numerous blastospores (arrows) in liver. Preparation stained with mucicarmine

fluid, collected from subarachnoid cisterna magna, showed pleocytosis in the number of 210 cells/μl. Mononuclear cells accounted for 40%, polynuclear cells (neutrophil granulocytes) for 50%, and eosinophilic cells for 10% of the population. In a smear of cerebrospinal fluid after fixing with 70% methyl alcohol and staining with India ink, analyses demonstrated the presence of cells of *Cryptococcus neoformans* fungus with a typical polysaccharide envelope (Figure 4). A therapy was undertaken that consisted in oral administration (two times a day) of fluconazole in a dose of 15 mg/kg bw. After 7 days of the therapy, due to a lack of any improvement and at the owner's request, the cat was euthanized. No consent was given for autopsy.

The presented cases of feline cryptococcosis point to, perhaps, a higher incidence of this disease in Polish cats than it is commonly claimed. Clinical practice indicates that many cats are treated as diagnosed with 'cat rhinitis', the therapy of which is unsuccessful most likely due to an incorrect diagnosis. Clinical symptoms of cryptococcosis are changeable, which impairs its diagnosis. In a study on cats suffering from cryptococcosis, O'BRIEN et al. (2004) observed pathological symptoms linked only with the nasal cavity in 40% of the animals as well as a more frequent occurrence of inflammations at atypical

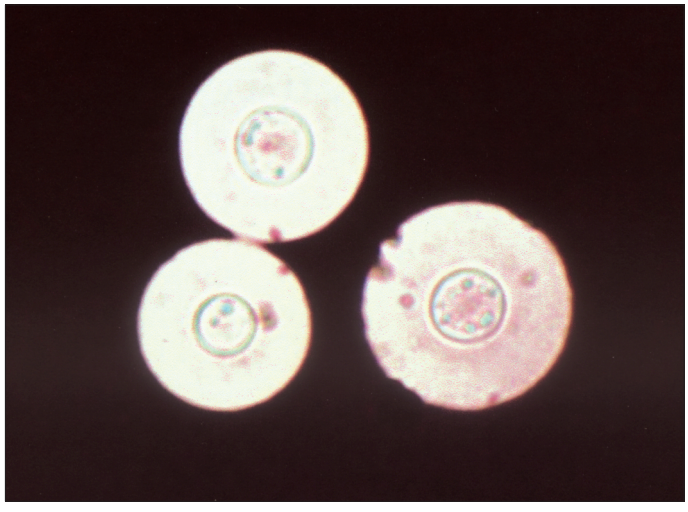


Fig. 4. Presence of blastospores in cerebrospinal fluid. Preparation stained with India ink. Magnification 720 x

sites, e.g. in connective tissues around joints, in bones or salivary glands, while reported no changes in other organs.

The above cases of cryptococcosis deserve to be described as they indicate that this disease occurs in Polish cats and may pose serious problems in diagnosis and therapy.

Translated by JOANNA JENSEN

Accepted for print 5.10.2007

References

AL-DOORY Y. 1971. *Bibliography of cryptococcosis.* Mycopathol. Mycol. Appl., 45: 1-60.

BEMIS D.A., KRAHWINKEL D.J., BOWMAN L.A., MONDON P., KWON-CHUNG K.J. 2000. *Temperature--sensitive strain of Cryptococcus neoformans producing hyphal elements in a feline nasal granuloma.* J. Clin. Microbiol., 38: 926-928.

BOOTHE D.M. 2001. *Small Animal Clinical Pharmacology and Therapeutics.* W.B. Saunders Company, Philadelphia.

BROWN M.R., ROGERS K.S. 1995. *Neutropenia in dogs and cats: a retrospective study of 261 cases.* Aust. Vet. J., 72: 93-97.

BRUESKE C.H. 1986. *Proteolitic activity of a clinical isolate of Cryptococcus neoformans.* J. Clin. Microbiol., 23: 631-633.

BUCHANAN K.L., MURPHY J. W. 1998. *What Makes Cryptococcus neoformans a pathogen?* Emerg. Infect. Dis., 4: 1-4.

CHEN S.C.A., MULLER M., ZHOU J.Z., WRIGHT L.C., SORRELL T.C. 1997. *Phospholipase activity in Cryptococcus neoformans: a new virulence factor?* J. Infect. Dis., 175: 414-420.

CHEN S.C.A., WRIGHT L.C., SANTANGELO R.T., MULLER M., MORAN V.R., KUCHEL P.W., SORRELL T.C. 1997. *Identification of extracellular phospholipase B, lysophospholipase, and acyltransferase produced by Cryptococcus neoformans.* Infect. Immun., 65: 405-411.

CRAIG A.C., RAMZAN I., MALIK R. 1994. *Pharmacokinetics of intravenous and oral fluconazole in cats.* Res. Vet. Sci., 57: 372-376.

DICKSON J., MEYER E.P. 1970. *Cryptococcosis in horses in Western Australia*. Aust. Vet. J., 46: 558.

FAGGI E., GARGANI G., PIZZIRANI C., PIZZIRANI S., SAPONETTO N. 1999. *Cryptococcosis in domestic mammals*. J. Am. Vet. Med. Assoc., 215: 1301-1305.

FLATLAND B., GREENE R.T., LAPPIN M.R. 1990. *Clinical and serologic evaluation of cats with cryptococcosis*. J. Am. Vet. Med. Assoc., 196: 1470-1473.

FOSTER S.F., CHARLES J.A., PARKER G., KROCKENBERGER M., CHURCHER R.M., MALIK R. 2001. *Cerebral cryptococcal granuloma in a cat.* J. Fel. Med. Surg., 3: 37-44.

GELATT K.M., MCGILL L.D., PERMAN V. 1973. *Ocular and systemic cryptosporidiosis in a dog.* J. Am. Vet. Med. Assoc., 162: 370-375.

GERDS-GROGAN S., DAYRELL-HART B. 1997. *Feline cryptococcosis: a retrospective evaluation.* J. Am. Anim. Hosp. Assoc., 33: 118-122.

GIONFRIDDO J.R. 2000. *Feline systemic fungal infections*. Vet. Clin. North Am. Small Anim. Pract., 30: 1029-1050.

HENDERSON D.K., KAN V.L., BENNETT J.E. 1986. *Tolerance to cryptococcal polysaccharide in cured cryptococcosis patients: faire of antibody secretion in vitro.* Clin. Exp. Immunol., 65: 639-646.

HOSIE M.J., ROBERTSON C., JARRETT O. 1989. *Prevalence of feline leukemia virus and antibodies to feline immunodeficiency virus in cats in the United Kingdom.* Vet. Rec., 125: 293-297.

JACOBSON E.S., JENKINS N.D., TODD J.M. 1994. *Relationship between superoxide dismutase and melanin in a pathogenic fungus.* Infect. Immun., 62: 4085-4086.

KITA P., FRYMUS T. 2003. *Występowanie białaczki kotów w Warszawie i okolicach*. Medycyna Wet., 59: 217-220.

KOZEL T.R., HIGHISON B., STRATTON C.J. 1984. *Localization on encapsulated Cryptococcus neoformans of serum components opsonic for phagocytosis by macrophages and neutrophils*. Infect. Immun., 43: 574-579.

KOZEL T.R., MASTROIANNI R.P. 1976. *Inhibition of phagocytosis by cryptococcal polisacharide: dissociation of the attachment and ingestion phase of phagocytosis.* Infect. Immun., 14: 62-67.

MALIK R., CRAIG A.J., WIGNEY D.I., MARTIN P., LOVE D.N. 1996. *Combination chemotherapy of canine and feline cryptococcosis using subcutaneously administered amphotericin B.* Aust. Vet. J., 73: 124-128.

MALIK R., MARTIN P., WIGNEY D.I., CHURCH D.B., BRADLEY W., BELLIENGER C.R., LAMB W.A., BARRS V.R., FOSTER S., HEMSLEY S., CANFIELD P.J., LOVE D.N. 1997. *Nosopharyngeal cryptococcosis.* Aust. Vet. J., 75: 484-489.

MALIK R., WIGNEY D.I., MUIR D.B., GREGORY D.J., LOVE D.N. 1992. *Cryptococcosis in cats: clinical and mycological assessment of 29 cases and evaluation of treatment using orally administered fluconazole.* J. Med. Vet. Mycol., 30: 133-144.

MEDLEAU L., GREENE C.E., RAKICH P.M. 1990. *Evaluation of ketaconazole and itraconazzole for treatment of disseminated cryptococcosis in cats.* Am. J. Vet. Res., 51: 1454-1458.

MEDLEAU L., MARKS M.A., BROWN J., BORGES W.L. 1990. *Clinical evaluation of a cryptococcal antogen latex agglutination test for diagnosis of cryptococcosis in cats.* J. Am. Vet. Med. Assoc., 196: 1470-1473.

MULLER H.E., SETHI K.K. 1972. *Proteolitic activity of Cryptococcus neoformans against human plasma proteins.* Med. Microbiol. Immunol. (Berlin), 158: 129-134.

O'BRIEN C.R., KROCKENBERGER M.B., WIGNEY D.I., MARTIN P., MALIK R. 2004. *Retrospective study of feline and canine cryptococcosis in Australia from 1981 to 2000: 195 cases*. Med. Mycol., 42: 449-460.

SALKOWSKI C.A., BALISH E. 1991. *Cutaneous cryptococcosis in athymic and beige-athymic mice.* Infect. Immun., 59: 1785-1789.

SALVIN S.B., SMITH R.F. 1961. *An antigen for detection of hypersensitivity to Cryptococcus neoformans.* Proc. Soc. Exp. Biol. Med., 108: 498-501.

STEPHEN C., LESTER S., BLACK W., FYFE M., RAVERTY S. 2002. *Multispecies outbreak of cryptococcosis on southern Vancover Island, British Columbia.* Can. Vet. J., 43: 792-794.

WALKER C., MALIK R., CANFIELD P.J. 1996. *Analysis of leucocytes and lymphocyte subsets in cats with naturally-occurring cryptococcosis but differing feline immunodeficiency virus status* J. Am. Vet. Med. Assoc., 209: 1110-1113.