

**PARTICULARITIES OF *BLUMERIA GRAMINIS* F.SP. *HORDEI* POPULATION IN SALASPILS****Iloņa Arāja, Isaak Rashal**

Arāja I., Rashal I. 2001. Particularities of *Blumeria graminis* f.sp. *hordei* population in Salaspils. *Acta Biol. Univ. Daugavp.*, 1 (2): 65 - 68.

Investigation of the Latvian population have been done since 1970ies. All together frequencies of 25 virulence genes were evaluated in those years. All virulences can be divided to the two different sets. To the first one belong virulence genes *Va6*, *Va7*, *Va9*, *Va12*, *Vat*, *Vk*, *VLa*, *Vh* that have high frequencies (45-100%). To the other group belong virulence genes *Va1*, *Va3*, *Va13*, *Va23*, *VMe* which have low or gradually increasing frequencies (0-53%). Low or medium frequencies observed also for virulence genes corresponding to resistance genes of test differentials 'Meltan' (0-8.5%), 'SI-1' (0-6.4%), 'Steffi' (12.9-31.9%) and 'Goldie' (1.4-2.1%). There did not detected any *mlo* virulent isolate during all investigation period in Latvia. In Latvia both factors – selection pressure of host resistance genes and spreading by wind are important. It is possible that spreading by wind in previous years was more important factor that caused appearance of new virulence In Latvia.

Key words: *Blumeria graminis* f.sp. *hordei*, barley, resistance, virulence

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**Introduction**

Diversity in a pathogen population is important aspect of biodiversity. Knowledge about structure and changes in pathogen populations is also very important in connection with diversity of the corresponding host. From one hand, pathogen is a pest for host but, from other hand, it is one of the most important stimuli to maintain diversity in host species, including diversity of cultivated plants.

Barley powdery mildew (*Blumeria graminis* f.sp. *hordei*) is one of the most wide spread barley pathogen. Information about genetic structure of population of this fungus is important to maintain and/or create new corresponding genetic diversity in barley varieties. Therefore it is very important to monitor a pathogen population regularly.

Investigation of the Latvian population of barley powdery mildew have been done since 1970ies, samples have been taken mainly in Salaspils location (Rashal, Tuerapina 1987, 1996, 1997; Rashal et al. 2000). All together frequencies of 25 virulence genes were evalu-

ated in those years.

In this article changes of virulence genes frequencies in 1995-2000 years for Salaspils location are described and overview of results of 1970-80ies years are given.

**Materials and methods**

Samples in sporulation (1995, 1996, 1999 years data) and cleistocarp (1998, 2000 years data) phase were collected from leaves of trap plants (universally susceptible variety 'Otra'). Monopustule isolates were tested on detached leaves placed on agar with 0.004% benzimidazol. For the virulence test a common set of differentials (Table 1) were used. Varieties 'SI-1', 'Steffi' and 'Goldie' included into the test differentials list since 1999. Inoculation was done by microinoculation technique. Infection type was scored according Torp et al. (1978). Mean virulences from several samples are presented. The most frequent pathotypes are calculated for virulence genes *Va1*, *Va3*, *Va6*, *Va7*, *Va12*, *Va13*, *Vk*, *VLa* (for 1995, 1998-1999 years data).

Table 1. List of differentials used for detection of virulence genes of *Blumeria graminis* f.sp. *hordei* in Latvia since 1995

Differential	Main resistance genes
P-01	Mla1
P-02	Mla3
P-03	Mla6
P-04B	Mla7
P-08B	Mla9
P-10	Mla12
P-11	Mla13
P-13	Mla23
P-17	Mlk
P-19	Mlp
P-20	Mlat
P-22	mlo5
P-23	MlLa
P-24	Mlh
Meltan	Mla13, Ml(Im9), Ml(Hu4)
Jarek	MlLa, Ml(Kr)
SI-1 *	Ml(SI1)
Steffi *	Ml(St1), Ml(St2)
Goldie *	Mla12, MlLa, U

\* used since 1999

## Results

All virulences can be divided to the two different sets. To the first one belong virulence genes *Va6*, *Va7*, *Va9*, *Va12*, *Vat*, *Vk*, *VLa*, *Vh* that have high frequencies (45-100%) in 1995-2000 (Table 2).

To the other group belong virulence genes *Va1*, *Va3*, *Va13*, *Va23*, *VMe* (Table 2) which have low or gradually increasing frequencies (0-53%). Low or medium frequencies observed also for virulence genes corresponding to resistance genes of test differentials 'Meltan' (0-8.5%), 'SI-1' (0-6.4%), 'Steffi' (13-32%) and 'Goldie' (1.4-2.1%). Not any *mlo5* virulent isolate was found during the all years of investigation.

Frequencies of pathotypes calculated for 1995, 1998, 1999 years (Table 3). In 1995 there were 18 pathotypes. The most frequent three pathotypes are: *Va6*, *Va7*, *Vk*, *VLa* – 23.4%; *Va6*, *Va7*, *Va9*, *Va12*, *Vk*, *VLa* – 19.5%; *Va6*, *Va7*, *Va9*, *Vk*, *VLa* – 14.3%. In 1998, 70 pathotypes detected, all presented with low frequency. The two most frequent (>3.5%) are: *Va6*, *Vk* – 4.6% and *Va6*, *Va12*, *Vk* – 3.7%. In 1999, 29 pathotypes, the most frequent are: *Va6*, *Va12*, *Vk*, *VLa* – 10.6%, *Va1*, *Va6*, *Va7*, *Va9*, *Va12*, *Vk*, *VLa* – 8.5%, *Va6*, *Va12*, *Vk* – 8.5%. The all most frequent pathotypes contained virulence genes *Va6* and *Vk*.

Table 2. Virulence frequencies in Salaspils barley powdery mildew population in 1995-2000

Virulence	Virulence frequencies				
	1995	1996	1998	1999	2000
Va1	2.6	3.9	17.6	19.1	32.9
Va3	28.6	3.9	28.7	25.5	38.6
Va6	100	83.7	75.0	89.4	68.6
Va7	100	95.9	62.0	59.6	75.7
Va9	72.7	95.9	32.4	46.8	64.3
Va12	46.8	72.5	63.0	78.7	64.3
Va13	10.4	13.7	25.0	23.4	52.9
Va23	0	0	7.4	6.4	10
Vk	98.7	100	58.3	93.6	62.9
Vp	1.3	0	69.4	91.5	72.9
Vat	16.9	62.7	18.5	66.0	21.4
Vmlo5	0	0	0	0	0
VLa	83.1	62.7	26.9	72.3	51.4
Vh	92.2	100	95.4	89.4	80
Va13, V(Im9), V(Hu4)	1.3	0	0.9	8.5	0
VLa, V(Kr)	45.5	42.9	13.9	55.3	38.6
V(SI1)	-	-	-	6.4	0
V(St1), V(St2)	-	-	-	31.9	12.9
Va12, VLa, V(U)	-	-	-	2.1	1.4
Number of isolates	77	75	108	47	70

Particularities of *Blumeria graminis f.sp. hordei* population in Salaspils

Table 3. The most frequent pathotypes in 1995, 1998-1999, Salaspils, Latvia (calculated for genes *Va1*, *Va3*, *Va6*, *Va7*, *Va9*, *Va12*, *Va13*, *Vk*, *VLa*)

Pathotype	Frequency, %		
	1995 (S)	1998 (C)	1999 (S)
Va6, Va7, Va9, Va12, Vk, VLa	19.5	0	2.1
Va6, Va7, Va9, Vk, VLa	14.3	0	0
Va6, Va7, Vk, VLa	23.4	0.9	4.3
Va6, Va12, Vk, VLa	0	0.9	10.6
va6, Va12, Vk	0	3.7	8.5
Va6, Vk	0	4.6	2.1
Number of pathotypes	18	70	29
Complexity	5.2	3.9	5.0
Number of isolates	77	108	47

S – sporulation phase

C – cleistocarp phase

### Discussion

High frequencies of virulences *Va6*, *Va7*, *Va9*, *Va12*, *Va1*, *Vk*, *VLa*, *Vh* observed not only in 90ies but also in 70-80ies. High frequencies of *Va7*, *Va9* and *Vg* can be explained by the intensive growing of varieties with corresponding resistance genes during 1970ies-1980ies in Latvia. Other virulence genes had a high frequency without corresponding resistance genes in varieties grown in Latvia (Rashal et al. 1996, 1997, 2000). In 1995-1999 some decreasing of frequencies were observed for virulences *Va6*, *Va7* and *Va9*: from 100% to 89%, 100% to 60% and 73% to 47% respectively. In Latvia, variety 'Nadja' containing gene *Mla7* was intensively grown during 70-80ies. 'Nadja' removed from Latvian fields in 1985-1986 but clear tendency of decreasing of *Va7* frequency has been observed only since 1995-1996.

The first isolates with virulence *Va1* appeared in 1988 (Rashal, Tuerapina 1997). Few isolates with *Va3* observed in 1972 and no *Va3* detected in 1973-1974. Since 1981, *Va3* have been present every year with low to medium frequency. In Latvia *Va13* virulence have been tested since 1987, since this year *Va13* have detected with low to medium frequency. In last five years there were observed increasing of frequencies of *Va1*, *Va3*, *Va13*. In Salaspils, in 1995-2000 frequencies of these genes changed from 2.6% to 32.9%, 28.6% to 38.6% and 10.4% to 52.9% respectively. Frequencies of these virulence genes are increasing in most Western and Central European countries (Howmüller et al. 2000).

Rather low virulence frequencies of *Va1*, *Va3*, *Va13* in previous years can be explained by the absence of corresponding resistance genes in barley grown in Latvia. Variety 'Sencis' with *Mla13* was released in 1996 and still is not grown widely. The most probably that these virulence genes (*Va1*, *Va2*, *Va13*) came to Latvia by a wind spread from Western Europe where corresponding resistance genes were introduced in commercial varieties and are already overcome in some European countries. Therefore there is a high probability that these resistance genes will lose effectiveness in Latvia in some years. Test differentials 'Meltan', 'SI-1', 'Steffi', 'Goldie' each contains two or more resistance genes (Table 1). Only virulence matching 'Steffi' was rather high (32%). 'Meltan' showed effective resistance all years of investigation (1995-1999). Not any *mlo5* virulent isolate was found in Latvia during the all time of investigation.

The highest number of pathotypes detected in sample collected in cleistocarp phase in 1998. It is possible that at sexual stage virulence gene combinations are more diverse than at asexual (sporulation) phase.

To increase diversity of mildew resistance in Latvian barley varieties investigation of wild barley *Hordeum vulgare ssp. spontaneum* as mildew resistance donors have been started in 1998. According experiments in laboratory and three years field tests results 21 perspective mildew resistant accessions were selected which are proposed for use in further investigations and in Latvian barley prebreeding programs.

## Conclusions

Due of spreading of powdery mildew spores by wind, isolates with new virulence can appeared in a region without presence of corresponding host variety. In Latvia both factors – selection pressure of host resistance genes and spreading by wind are important. It is possible that spreading by wind in previous years was more important factor that caused appearance of new virulence In Latvia, because of for long time Latvian barley varieties had no specific mildew resistance genes. Those appeared first in the 1980ies. In Latvian barley varieties recognised genes *Mla7*, *Mla9*, *Mla13*, *Mlg*, *Mlk*, *Ml(Ru3)*, *MLLa*, *Ml(1192)* (Tueryapina et al., 1997). From these genes only *Mla13* is rather effective. Not any *mlo5* virulent isolate was found in Latvia during all the years of investigation and this resistance is still effective. Survey results show necessity for increasing of powdery mildew resistance source diversity in Latvian barley.

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**PARTICULARITIES OF THE GENETIC STRUCTURE OF *BLUMERIA GRAMINIS* F.SP. *HORDEI* POPULATION IN THE LATGALE REGION****Inese Kokina, Isaak Rashal**

Kokina I., Rashal I. 2001. Particularities of the genetic structure of *Blumeria graminis* f.sp. *hordei* population in the Latgale region. *Acta Biol. Univ. Daugavp.*, 1 (2): 69 - 72.

In 1995-2000 spores of *Blumeria graminis* f.sp. *hordei* had been sampled near Daugavpils. Virulences Va6, Va7, Va9, Va12, Va23, Vat, Vk, Vh, Vla had a high level of frequencies in all years of the investigation independently from the host plant genotype and from the pathogen stage of life cycle. Virulences Va1, Va3, Va13 were low but there are clear tendency to increase of this virulences in the last years. Only some sporadic isolates, which overcome S11, 'Goldie', 'Steffi', 'Jarek' and 'Meltan' resistance genes, were found in Daugavpils in 1995-2000. Not any isolate virulent to mlo gene was found.

Key words: powdery mildew, virulence, barley, resistance

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**Introduction**

The economical value of cereals for Latvia can not be overestimated. Barley is the main cereal there. Barley powdery mildew caused by *Blumeria graminis* f.sp. *hordei* is one of the widespread barley disease in Latvia. Knowledge of genetic structure of the pathogen population is necessary to choose the best strategy for the resistance breeding. Long-term and geographically wide monitoring of this pathogen in Europe led to interesting both theoretical and practical conclusions (Limpert 1987, 1996; Hovmüller et al. 2000). Evaluation of the virulences of *Blumeria graminis* f.sp. was done in Latvia since 1981. Investigations were made mainly in Salaspils located in the central part of Latvia (Rashal, Tueryapina 1997).

In the Latgale region climate and agricultural conditions differ remarkably therefore a local population of the pathogen can have important particularities. For this reason, since 1995 we started regular observation of pathogen population virulences in the Latgale region. In this paper data for 1995-2000 are presented.

**Materials and methods**

Spores of *Blumeria graminis* f.sp. *hordei* had been sampled near Daugavpils. In 1995 samples both in sporulation and cleistocarp phase were collected from leaves of barley lines M66R, SR-1R, Risq5678R and in 1996-2000 from leaves of universally susceptible variety 'Otra'. Trap plants were grown on experimental plot located at least 5 km from nearest commercial barley fields.

Monopustule isolates were tested for virulence by a set of differentials (Table 1) on detached leaves placed on agar with 0.004 % benzimidazol. Inoculation was done by microinoculation technique. Each isolate was designated by infection type using a 0-4 scale (Torp et al., 1978) under laboratory conditions, (0-3 - avirulence, 4 - virulence). Frequencies of the virulence genes on different stages of pathogen development in different years were calculated.

**Results and discussion**

Virulences in 1995 are presented in Table 2. All tested

Table 1 List of differentials used for detection of virulence genes of *Blumeria graminis* f.sp. *hordei* population in the Latgale region in 1995-2000

Differentials	Main resistance genes
P01	Mla1
P02	Mla3
P03	Mla6
P04B	Mla7
P08B	Mla9
P10	Mla12
P11	Mla13
P13*	Mla23
P17	Mlk
P20*	Mlat
P22*	mlo5
P23	MILa
P24*	Mlh
Jarek**	MI(1192)
Meltan***	MI(Me)
Steffi****	MI(St)
Goldie****	MIGo
SI1****	MI(SI1)

- \* - used only 1995-1998  
 \*\* - used only 1996-1998  
 \*\*\* - included in the set since 1996  
 \*\*\*\* - included in the set since 1999

pathogen virulences can be divided in two sets. The first one contained genes that have high frequencies and the second set included genes with low and low-medium frequencies. The virulences Va7, Va9, Va12, Vk, Vla, Vh were recorded at frequencies of at least 90-100 % in all cases independent from host plant genotype. Va23 had the same level of virulence with one exception, high virulence exceeded 50-60 % had also Va6 and Vat. There were not detected Va1, Va3, and Va13 virulences in 1995.

In 1996-2000 the division of virulences to the two sets remain the same. Most of them occurred with high frequencies: Va6, Va7, Va9, Va12, Va23, Vat, Vh, Vk, Vla (Table 3). Those high virulence frequencies were independent from pathogen stage of life cycle and the date of sampling. It means that corresponding resistance genes (Mla6, Mla7, Mla9, Mla12, Mla23, Mlat, Mlh, Mlk, Mlla,) are ineffective in the Latgale region. According data from another part of Latvia (Salaspils) mentioned genes are ineffective there too (Rashal et al., 2000). These genes were lost they effectiveness also in other parts of Europe (Hovmüller et al. 2000).

Three race specific resistance genes Mla1, Mla3, Mla13 were effective during the period observed. In the first years of the observation, there was no detected virulence against these genes (Table 4). During recent years, however, clear tendency was observed to increase frequencies of virulences Va1, Va3, Va13 in the Latgale

Table 2. Virulence genes in the Latgale population of *Blumeria graminis* f.sp. *hordei* depending of host genotype

Virulence	Host		
	M66R	Risø5678R	SR-1R
Va1	0.0	0.0	0.0
Va3	0.0	0.0	0.0
Va6	63.3	53.5	63.8
Va7	98.5	100	93.0
Va9	92.9	100	97.2
Va12	87.3	100	90.2
Va13	0.0	0.0	0.0
Va23	95.7	33.8	80.5
Vat	57.7	52.1	68.0
Vh	94.3	100	94.4
Vk	97.1	100	98.6
Vla	94.3	100	95.8
Number of tested isolates	71	71	72

Particularities of the genetic structure of *Blumeria graminis* f.sp. *hordei* population in the Latgale region

Table 3. Virulencies with high frequencies in the Latgale population of *B. graminis* f.sp. *hordei* in 1996-2000

Sample			Number of tested isolates	Virulence frequencies, %								
Year	Date	Phase		Va6	Va7	Va9	Va12	Vk	Vla	Va23	Vat	Vh
1996	15.07.	sp	75	97.3	98.6	93.3	25.3	98.6	93.3	50.6	70.6	98.6
	18.08.	cl	122	83.6	96.7	90.1	57.3	96.7	96.7	58.2	72.9	95.9
1997	28.06.	sp	200	93.0	92.5	80.0	52.5	95.0	95.0	42.5	49.0	89.5
	15.07.	sp	189	95.2	96.8	61.3	66.6	97.3	96.8	44.4	52.3	80.9
1998	04.08.	cl	150	98.0	97.3	72.6	68.0	99.3	98.0	-	-	97.3
	15.07.	sp	79	96.2	98.7	100	89.8	100	100	67.0	100	96.2
	28.07.	cl	125	92.0	92.9	88.0	80.8	93.6	87.2	48.0	88.8	91.2
	18.08.	cl	130	93.0	89.2	91.5	86.9	90.0	92.3	56.1	83.8	93.8
1999	01.07.	sp	116	93.9	91.3	94.8	85.3	87.0	94.8	-	-	-
	15.07.	sp	120	90.8	91.6	89.1	90.0	88.3	90.0	-	-	-
	06.08.	cl	116	87.0	86.2	84.4	82.7	85.3	76.7	-	-	-
2000	15.07.	sp	111	90.9	91.8	88.2	90.0	88.2	90.0	-	-	-
	07.08.	cl	114	89.4	90.3	86.8	82.4	88.6	84.2	-	-	-

region. Some isolates, which overcome 'Meltan' resistance, were found these years (Table 4).

In 1999 additional resistance genes from new resistant varieties 'Steffi', 'Goldie' and a barley line SII were also included in the Latvian set of differentials. In Table 5 are presented virulences against those resistance genes in 1999-2000. Only some sporadic isolates were found, which overcome SII, 'Goldie' and 'Steffi' resistance genes, what means that corresponding virulence genes VSI, Vgo, V(St) have were low frequency in the Latgale region. There were a tendency

to increase of a frequency of V(St) virulence in cleistocarp phase of the pathogen in comparison with sporulation phase.

214 isolates collected in 1995 from barley lines M66R, Risq5678R, SR-1R and 1070 isolates collected in 1996-1998 from the susceptible barley variety 'Otra' were tested for Mlo virulence. Only separate mildew colonies (infection type 0/4) were detected on leaf segments of the corresponding test line, but not any Mlo-virulent isolate was found during all years of investigation. Presented data proves that Mlo resistance was

Table 4. Virulencies with low frequencies in the Latgale population of *B. graminis* f.sp. *hordei* in 1996-2000

Sample			Number of tested isolates	Virulence frequencies, %			
Year	Date	Phase		Va1	Va3	Va13	Vme
1996	15.07.	sp.	75	0.0	0.0	0.0	-
	18.08.	cl.	122	0.0	0.0	0.0	0.0
1997	28.06.	sp.	200	0.0	0.0	0.0	0.0
	15.07.	sp.	189	0.0	0.0	0.0	12.1
1998	04.08.	cl.	150	0.0	0.0	0.0	0.0
	15.07.	sp.	79	0.0	0.0	0.0	0.0
	28.07.	cl.	125	0.0	14.4	0.0	0.0
	18.08.	cl.	130	0.0	10.0	2.3	2.3
1999	01.07.	sp.	116	1.7	1.7	3.4	6.9
	15.07.	sp.	120	1.6	6.6	4.1	1.6
	06.08.	cl.	116	2.5	5.1	6.0	0.8
2000	15.07.	sp.	111	2.7	1.8	4.5	6.3
	07.08.	cl.	114	3.5	4.3	3.5	4.3

Table 5. Virulences to additional set of resistance genes in the Latgale population of *B. graminis* f.sp. *hordei* in 1999-2000

Sample			Number of tested isolates	Virulence frequencies, %		
Year	Data	Phase		VSI	VSt	Vgo
1999	01.07.	sp	116	0.0	0.0	11.2
	15.07.	sp	120	1.6	0.8	0.8
	06.08.	cl	116	3.4	2.5	3.4
2000	15.07.	sp	111	2.7	0.0	1.8
	07.08.	cl	114	0.0	2.6	6.1

completely effective in the Latgale region. This type of resistance is still used very little in Latvia (less than 1% of total barley area) and in the other Baltic States (Rashal et al., 2000). Till now not any isolate which overcome Mlo resistance was found also in any other European country nevertheless of the very wide exploiting of this gene.

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## RESTORING MORPHOLOGICAL CHARACTERISTICS OF THE LATVIAN CUCUMBER (*CUCUMIS SATIVUS* L.) VARIETY 'DINDOŅA ZAĻĒ ĶEKARU'

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Lepse L., Baumane M., Rashal I. 2001. Restoring morphological characteristics of the Latvian cucumber (*Cucumis sativus* L.) variety 'Dindoņa zaļie ķekaru'. *Acta Biol. Univ. Daugavp.*, 1 (2): 73 - 76.

Due to the high variety pollution the old cucumber variety 'Dindoņa Zaļie Ķekaru' was contaminated and become a population with a broad spectrum of phenotypic and genotypic diversity. Inbreeding was started for variety homogenisation and dividing in morphologically and genotypically distinct lines. Evaluation of lines for morphological characters was done for each generation. Both by inbreeding and sib-crossing with the subsequent selection rather rapid morphological adequacy of plants to the original description of the cucumber variety 'Dindoņa Zaļie Ķekaru' was reached. Some lines with the good adequacy were established. Inbreeding led to more rapid homogenisation in comparison with the sib-crossing.

Key words: *Cucumis sativus* (L.), morphological characters, genetic diversity

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### Introduction

According to the Convention of the Biological Diversity, which is ratified also by Latvia, each country is responsible for maintenance of genetic diversity of the local origin. It includes also genetic resources of cultivated plants as important part of the agrobiodiversity.

In 1994 inventorying of vegetable genetic resources of the Latvian origin was started (Rashal, Weibull 1997). It was stated that since 1960ies the number of maintained vegetable varieties of the Latvian origin decreased dramatically and seeds of many local varieties are no longer available (Lepse 1998).

The Latvian cucumber variety 'Dindoņa Zaļie Ķekaru' was bred in 1930-1950ies by Pēteris Dindonis as a cultivar for growing in the open field or in hotbeds. It was bred by open pollination of three cultivars 'Borovskiy', 'Muromskiy' and 'Kulenkampa'. After selection over a long period the cultivar was officially registered in 1961. It was characterised as a mid-late

(55-65 days) with a long yielding period, fruits are 15 cm long, 5 cm in a diameter and have weight 150-200 g. The flesh is juicy, compact and aromatic. The fruit colour is green - dark green, light striped till the 1/3 of fruit. Fruit is covered with warts. Plant main vine is up to 120 cm, side vines weak developed. Fruits mainly located on the main vine. Fruits develop in clusters, what is reflected in the name of variety - "ķekari" in Latvian means "clusters". This was the main typical trait of 'Dindoņa Zaļie Ķekaru' (Dindonis et al. 1957). Due to improper seed propagation the variety was contaminated and become a population with a broad spectrum of phenotypic and genotypic diversity. In 1994 was done evaluation of commercially available population for morphological characters. Only 3 % of plants answered to the original description of the variety 'Dindoņa Zaļie Ķekaru' for morphological traits. These plants were selected as parents for creating of inbred lines with the aim to renew the old cultivar. In 1997 the sib-lines selection was also started from the population material.

Table 1. Distribution of plants of separate lines and families by the fruit development in clusters in subsequent generations

Inbred-line	I <sub>3</sub> , 1998			I <sub>4</sub> , 1999			I <sub>5</sub> , 2000		
	0-1	1,5-2	2,5-3	0-1	1,5-2	2,5-3	0-1	2	3
1	78,4	19,6	2,0	75,0	25,0	0,0	32,4	26,5	41,2
2	80,9	10,6	8,5	9,1	18,2	72,7	0,0	24,3	75,7
3	78,9	13,2	7,9	51,1	10,6	38,3	21,6	21,6	56,8
4	93,3	6,7	0,0	93,8	3,1	3,1	18,4	28,9	52,6
5	94,3	5,7	0,0	100,0	0,0	0,0	-	-	-
6	85,5	10,5	3,9	97,8	0,0	2,2	72,5	25,0	2,5
7	100,0	0,0	0,0	100,0	0,0	0,0	46,3	41,5	12,2
8	88,7	7,5	3,8	82,1	15,4	2,6	45,2	21,4	33,3
Sib-line	S <sub>1</sub> , 1998			S <sub>2</sub> , 1999			S <sub>3</sub> , 2000		
	0-1	1,5-2	2,5-3	0-1	1,5-2	2,5-3	0-1	2	3
3	0,0	8,3	91,7	85,4	8,3	6,3	7,7	10,3	82,1
5	83,3	12,5	4,2	74,2	22,6	3,2	26,8	24,4	48,8
6	86,7	13,3	0,0	77,8	17,8	4,4	7,7	0,0	92,3
11	70,8	25,0	4,2	76,2	14,3	9,5	8,6	8,6	82,9
15	82,8	6,9	10,3	85,1	12,8	2,1	14,7	14,7	70,6
20	90,2	7,3	2,4	62,8	25,6	11,6	0,0	18,2	81,8
21	71,9	15,6	12,5	52,6	26,3	21,1	5,4	27,0	67,6

The objective of the present study is to trace of changes of morphological characters of the variety 'Dindoņa Zāļie Ķekaru' after crossing and selection in some subsequent generations.

### Material and methods

Cucumber seedlings were grown in the greenhouse in plastic pots and planted out in open field conditions at the beginning of June, space between rows 1 m, between plants in row 0.4 m. All lines were planted in 3 replications, random arrangement. On average there were 15-60 plants planted from each line. Harvest and evaluation were done until the first autumn frosts (beginning of September).

Most important morphological and agronomic traits of inbred lines and sib-lines were described in every generation. Morphological parameters such as flowering type, clusters, fruit skin surface, skin colour, taste and also a general adequacy to the original variety description were evaluated by 3 point system (0 - trait not correspond to variety, 3 - trait correspond to the variety 'Dindoņa Zāļie Ķekaru'). Plants which corresponded to the variety only partly (consisted some of

morphological traits) are scored between 0 and 3, depending on the amount of typical traits. 0 evaluated plants, which were not typical or had only one typical trait. The yield was harvested once - twice per week, weighted, sorted, and registered for the evaluation of yield (kg m<sup>-2</sup>) and yielding dynamic.

Every year plants most correspond to the main traits of the variety 'Dindoņa Zāļie Ķekaru', were selected for inbreeding. Male and female flowers were isolated by cheese-cloth isolators before flowering. Blossom flowers were pollinated within one plant for inbred lines. Isolators were placed back and kept until the fruit is set. Mature seeds were harvested, after 40 days. The same pollination system was used for producing of sib-lines. For this case both female and male flowers were chosen from the different plants within one line, which most correspondent to the variety.

Multidimensional analysis of all morphological data obtained from the plants of I<sub>3</sub> and S<sub>3</sub> generations was performed. Cluster analysis based on the unweighted pair-group method with arithmetic average (UPGMA) was applied, and corresponding dendrogram was generated.

**Results and discussion**

Results obtained from morphological data analysis reflect the dynamic of homogzygotisation of each line during inbreeding and sib-selection. Among the evaluated traits the fast-moving increasing of plants scored by 3 points was stated for fruit development in the clusters (clustering) (Table 1). This character is the most critical in the selection of plants for inbreeding and sib-mating. Tendency for increasing of plants with clustered fruits was common for all the lines but impetuosity of this process was different. Amount of plants with clustered fruits in I<sub>3</sub> generation (1998) was different. Some of lines even had not any plants with clustered fruits (inbred lines 4 I<sub>3</sub> and 5 I<sub>3</sub>, and sib-lines 6 S<sub>1</sub> and 7 S<sub>1</sub>). In three years of inbreeding the amount of plants with clustered fruits increased from zero up to 52% (4 I<sub>3</sub>). The highest amount of plants evaluated by 3 points was registered in the inbred line 2 I<sub>3</sub>. The most rapid increasing of amount of clustered fruits was found for the line 11 S<sub>3</sub> (4% in 1998 and 92% in 2000). The same tendency was found also for the general adequacy to the original description of the variety (Table 2). It can be seen that for some of lines the adequacy was improved very drastic (inbred lines 2, 3, 4, most

of sib-lines). In the same time adequacy was not increased in some inbred lines (1, 6, 7). This fact is a consequence of genetic variation in source plants. It can be concluded also that at least the clustering is a character with rather simple inheritance that allows rapid selection of needed plants by the phenotype.

Genotypic differences between lines are well seen also from the dendrogram (Fig. 1). Inbred lines are remarkably genetically distinct what reflects more rapid homogzygotisation in comparison with the sib-lines, which remain more diverse in-line variation.

**Conclusions**

Both by inbreeding and sib-crossing with the subsequent selection rather rapid morphological adequacy of plants to the original description of the cucumber variety 'Dindona Zāļe Ķekaru' was reached. Some lines with the good adequacy were established. Inbreedisation led to more rapid homogzygotisation in comparison with the sib-crossing.

Table 2. Distribution of plants of separate lines and families by the general adequacy to the original description of the variety in subsequent generations

Inbred-line	I <sub>3</sub> , 1998			I <sub>4</sub> , 1999			I <sub>5</sub> , 2000		
	0-1	1,5-2	2,5-3	0-1	1,5-2	2,5-3	0-1	2	3
1	60,8	21,6	17,6	75,0	12,5	12,5	85,3	2,9	11,8
2	70,2	21,3	8,5	36,4	18,2	45,5	16,2	16,2	67,6
3	78,9	15,8	5,3	72,3	0,0	27,7	24,3	29,7	45,9
4	83,3	13,3	3,3	90,6	3,1	6,3	50,0	7,9	42,1
5	88,6	8,6	2,9	100,0	0,0	0,0	-	-	-
6	86,8	9,2	3,9	95,7	2,2	2,2	100,0	0,0	0,0
7	100,0	0,0	0,0	100,0	0,0	0,0	65,9	29,3	4,9
8	90,6	9,4	0,0	84,6	7,7	7,7	47,6	33,3	19,0
Sib-line	S <sub>1</sub> , 1998			S <sub>2</sub> , 1999			S <sub>3</sub> , 2000		
	0-1	1,5-2	2,5-3	0-1	1,5-2	2,5-3	0-1	2	3
3	88,9	11,1	0,0	85,4	2,1	12,5	66,7	5,1	28,2
5	100,0	0,0	0,0	77,4	19,4	3,2	26,8	19,5	53,7
6	86,7	13,3	0,0	71,1	17,8	11,1	46,2	15,4	38,5
11	66,7	16,7	16,7	83,3	4,8	11,9	45,7	17,1	37,1
15	93,1	6,9	0,0	89,4	6,4	4,3	41,2	5,9	52,9
20	46,3	43,9	9,8	88,4	9,3	2,3	20,5	20,5	59,1
21	75,0	15,6	9,4	60,5	10,5	28,9	8,1	10,8	81,1

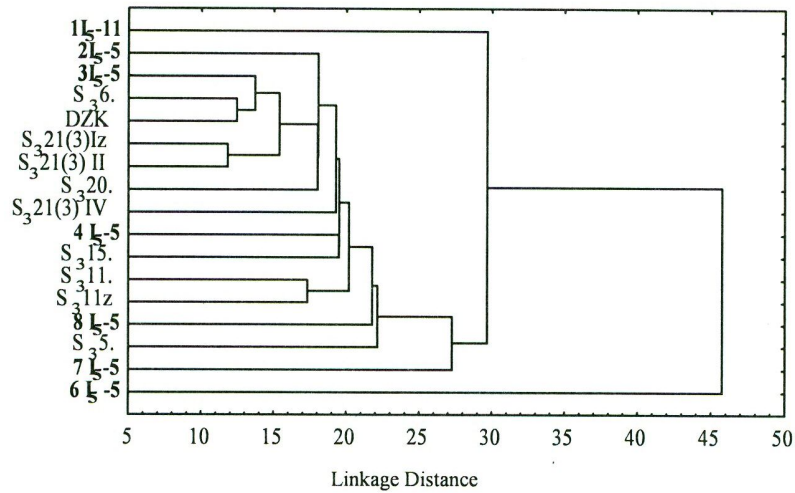


Fig. 1. Dendrogram of inbred- and sib-lings clustered by morphological characters

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## DISTRIBUTION OF HLA-DQ ALLELES ASSOCIATED WITH RISK OR PROTECTION FOR TYPE 1 DIABETES IN LATVIANS

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Bekmane U., Kovalchuk L., Eglīte E., Denisova A., Ilonen J., Sochnevs A. 2001. Distribution of HLA-DQ alleles associated with risk or protection for type 1 diabetes in Latvians. *Acta Biol. Univ. Daugavp.*, 1 (2): 77 - 79.

Numerous studies have indicated that the distribution of HLA (human leucocyte antigen) specificities is a stable marker and a powerful tool of population genetics which can be used to support anthropological, linguistic and ethnographical methods in unraveling historic relationships between ethnically distinct populations. At present association of HLA with various diseases is an undoubted fact, and that connection has often a specific population attribute. We studied the distribution of HLA alleles defining risk for type 1 diabetes in Latvians, 173 healthy native individuals from four ethnic-geographical regions of Latvia: Kurzeme, Zemgale, East Vidzeme and Latgale were examined. A method combining PCR amplification and subsequent with sequence specific hybridization was used to determine the HLA alleles. These alleles were: HLA-DQB1 \*02, \*0301, \*0302 and \*0602-03 as well as DQA1\*0201 and \*05 alleles in DQB1\*02 positive subjects. Significant heterogeneity was found in the distribution of the analyzed alleles among residents of the studied regions.

Key words: HLA (human leucocyte antigen), population, type 1 diabetes

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### Introduction

The HLA genetic complex plays a major role in the development of acquired immune response and homeostasis of the body. Specific alleles present in genotype define the formation of T cell repertoire during T cell maturation in thymus and affect the individual capacity to mount an immune response to various antigens and their specific epitopes. According to a major hypothesis the extended polymorphism of the HLA system is caused by natural selection because it provides maximal probability to mount defence against new infectious agents spread in the population environment.

Several investigators have also found that a number of diseases, especially those with autoimmune features are associated with specific HLA alleles and genotypes (Lecher 1994). Typing for HLA markers may be useful in the estimation of the probability of disease on-

set, its clinical course or efficiency of the treatment. There are often differences in disease associated HLA markers among various populations which may be informative in mapping the gene loci of primary association with the disease susceptibility, e.g. trans-racial studies of HLA class II association in type 1 diabetes were important in localisation of disease risk to HLA-DQ molecules (Fletcher et al. 1988; Todd et al. 1989). Autoimmune diseases occur in Caucasians, Blacks and Asians with a different incidence, which may be related to the background frequency of disease, associated alleles among these populations (Dorman et al. 1990). In some diseases, susceptible HLA alleles are closely related, but different and clinical manifestations may be different among ethnic groups (Nishimura et al. 1998).

The high polymorphism of HLA complex makes it extremely useful for population studies. By detecting specific HLA allele frequencies in different

populations, it is possible to identify ethnic groups and evaluate the genetic relationships among them. Several studies have demonstrated the sensitivity of HLA studies in detecting differences between various ethnic groups even among relatively homogeneous populations (Belchenko et al. 1994; Mattullo et al. 1995; Martinez-Laso et al. 1995). The historical events in Latvia have created population groups in various geographic regions, which can be separated based on anthropological markers, language dialects, different confession and cultural traditions. In this paper we analysed whether these population groups also differ for a set of HLA class II markers known to be associated with susceptibility or protection for type I diabetes

## Material and methods

### Population sampling

Blood samples were collected from 180 unrelated healthy individuals from the areas Kurzeme, Zemgale, East Vidzeme and Latgale (more specifically Līvāni). They were of both sexes, aged 18-60 and had parents in two generations born in the same areas. The geographic location of regions under study was selected according to the recommendation of the Institute of History of Latvia.

### HLA typing

PCR amplification and sequence specific hybridisation with lanthanide labelled probes was made as described earlier (Sjöroos et al. 1995; Sjöroos et al. 1998). The presence of HLA-DQB1\*02, \*0301, \*0302, \*0602 and \*0603 alleles was defined first, DQB1\*0602 and \*0603 were defined by the same sequence spe-

cific probe and could not be separated from each other, thus they are marked together as DQB1\*0602-03. Samples positive for DQB1\*02 allele were further tested for DQA1\*0201 and DQA1\*05 alleles to separate between DQA1\*0201-DQB1\*02 (DR7 associated) and DQA1\*0501-DQB1\*02 (DR3 associated) haplotypes.

### Statistical analysis

Chi-square statistics was used to look for heterogeneity among the frequencies of studied alleles and haplotypes.

## Results and discussion

The data presented in the Table 1. show that all of the studied HLA DQA1 and HLA DQB1 alleles were found in populations of Kurzeme, Zemgale, East Vidzeme and Latgale, but their frequencies were variable. Statistically significant heterogeneity was observed in the frequency of DQB1\*0602-03 ( $p=0.003$ ) allele and some evidence for heterogeneity was obtained also for DQB1\*0301 ( $p=0.05$ ) and DQB1\*0302 ( $p=0.04$ ). These differences were especially due to the low frequency of DQB1\*0602-03 in Zemgale compared to other regions whereas DQB1\*0301 was rare in Latgale and DQB1\*0302 especially in Kurzeme.

Frequencies of serologically defined HLA alleles were earlier compared between Latgale and Vidzeme populations and e.g. DR2 and DR5 found more often in Vidzeme population (Belchenko et al. 1994). The low frequency of DQB1\*0301 in Latgale is in accordance with the earlier result as DR5 is regularly associated with DQB1\*0301 in the same haplotype. DR2 was earlier also found differing between these two populations, but in the present study the DR2 as-

Table 1. Number of positive subjects for each studied allele in populations of various regions of Latvia

DQ alleles	Kurzeme		East Vidzeme		Zemgale		Latgale	
	n=48		n=48		n=35		n=42	
	N	%	N	%	N	%	N	%
DQA1*0201	5	10,4	10	20,8	7	20,0	4	9,5
DQA1*0501	5	10,4	7	14,6	11	31,4	5	11,9
DQB1*0301	16	33,3	23	47,9	17	48,6	8	19,0
DQB1*0602-3	19	39,6	27	56,3	9	25,7	27	64,3
DQB1*0201	10	20,8	14	29,2	16	45,7	11	26,2
DQB1*0302	3	6,3	3	6,3	5	14,3	6	14,3

sociated DQB1\*0602 was defined only together with DQB1\*0603 and their combined frequency was very similar between Latgale and Vidzeme.

The distribution of the HLA specificities defined in the present study demonstrate the significant differences in the genetic background of the analyzed populations in different geographical regions of Latvia. This finding emphasizes the need of accurate selection of control group when new studies of HLA and disease associations are planned. The distribution of allele frequencies of these diabetes risk or protection associated alleles do not suggest any simple concentration of type 1 diabetes susceptibility to some of the regions and controversial tendencies balancing each others were detected. For example although the protective allele DQB1\*0301 was rare in Latgale, the other protective allele DQB1\*0602-03 was there common.

The finding of significant differences with this small panel of class II markers suggest that HLA markers might be valuable tool when the early history of Latvian population is studied. A more complete scheme of allele definition would of course be valuable for such studies.

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**IMMUNE STATUS AND HLA PHENOTYPE IN RESIDENTS LIVING IN REGION WITH HIGHER ENVIRONMENTAL POLLUTION IN LATVIA****Olga Shitova, Arturs Sochnev, Helena Eglite, Liliya Kovalchuk, Maija Eglite, Mara Bake**

Shitova O., Sochnev A., Eglite H., Kovalchuk L., Eglite M., Bake M. 2001. Immune status and Phenotype in residents living in region with higher environmental pollution in Latvia. *Acta Biol. Univ. Daugavp.*, 1 (2): 80 - 85.

The aim of the study was to investigate the association between immunological indices and HLA-A1-B8 haplotype as a marker of certain diseases in residents living in the region with higher environmental chemical contaminants in Latvia. The peripheral blood of 180 persons was tested for the number of CD3, CD4, CD8, CD19, CD57 cells by indirect immunofluorescence test using monoclonal antibodies. Proliferative activity of lymphocyte was studied in the blasttransformation reaction (BTR) by the radiometric method with 3-H-thymidine. The phagocytic activity of neutrophils was tested by chemoluminescence method. The levels of immunoglobulins (IgA, IgG, IgM) in blood serum were determined by the radial immunodiffusion and IgE by immunoenzymatic assay. Class I HLA antigens were identified using microcytotoxicity test. It has been established that in the females carrying HLA-A1B8 haplotype and residing in the contaminated region the decrease in the number of CD3<sup>+</sup>, CD4<sup>+</sup> cells was accompanied by the suppression of lymphocyte response to polyclonal mitogen. There were significant reduction of phagocytosis and an increase in IgG and IgA levels. The findings characterise the associations between HLA antigens of the major histocompatibility complex and the immune status in residents of ecologically hazardous regions.

Key words: immune status, HLA antigens, haplotype, and environmental pollution.

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**Introduction**

Chemical pollutants have accumulated in the environment and food on a global basis, having damage wild-life population (Thornton 2000). Chemicals are now found in every person. The bioaccumulation of these compounds in some individuals can lead to a variety of metabolic and systemic dysfunction, in some cases outright disease states. The immune system is one of the most affected by this xenobiotic compounds (Crinnion 2000). Chronic exposure to environmental chemicals may cause profound changes in our immune system and result in a broad spectrum of different diseases: allergy (Mariethoz et al. 1999; Kemeny 2000; Saxon et al. 2000), autoimmunity (Bigazzi 1997; Rao et al. 1999), cancer (Griem et al. 1998; Spurny 1996), reproductive dysfunction (Sinawat 2000) and many other. Human Leukocyte Antigens (HLA) system plays

an important role in the regulation and control of immune response ( Vapalahti et al. 2001; Torres Galvan et al. 2000).

HLA are known to be "markers" of a number of diseases (Welsh 1981). In particular, HLA-A1B8 haplotype is associated with predisposition to several autoimmune diseases (Price et al. 1999; Schroeder et al. 1998).

Investigation of relationship between immune status and HLA phenotype is important to understand the immunopathogenic mechanisms involved and to identify human population at risk.

The aim of this study was to investigate the relationship between indices of the cellular, humoral and innate immunity with HLA-A1B8 haplotype in the sub-



jects exposed to environmental chemical pollution.

## Materials and methods

### Subjects

We studied two groups of HLA-A1B8 haplotype carriers: one group exposed to chemicals and a control group. The exposed group consisted of 18 females and 15 males aged 19 to 63 years (mean age 39 years) living in region of Latvia of high environmental chemical pollution. The control group included 15 males and 14 females aged 19 to 60 years (mean age 37 years) living in an unpolluted region of Latvia.

All subjects were interviewed using questionnaire, developed in the Institute of Immunology of Medical Academy of Latvia to ascertain their social conditions, previous illnesses, heredity, unhealthy habits, act. All subjects were examined by a group of physicians.

### Immunological and immunogenetic examination

All subjects gave their agreement to undergo venous blood test (10 ml) for immunological and immunogenetic checking. Their peripheral blood was tested for the number of lymphocyte subpopulations: CD3<sup>+</sup>, CD4<sup>+</sup>, CD8<sup>+</sup>, CD19<sup>+</sup>, CD16<sup>+</sup> cells by indirect immunofluorescence (Blann 1978); lymphocyte functional activity in the blasttransformation reaction (BTR) with polyclonal mitogens (Phytohemagglutinin (PHA-P) – Serva, Germany, 10 mg/ml, Pokeweed mitogen (PWM) - Sigma, USA, 1 mg/ml) by radiometric method with 3-H thymidine (Sochnev et al. 1988). Previously, these concentration of mitogens were found to be optimal for lymphocyte stimulation as determined by dose-response curves performed on healthy control subjects (Sochnev et al. 1988). Phagocytic activity of neutrophils by luminol-dependent chemoluminescence (Sochnev et al. 1987); levels of A, G and M immunoglobulins (IgA, IgG, IgM) by the radial immunodiffusion (Mancini et al. 1965); circulating immune complexes (CIC) with PEG-6000 (Haskova et al. 1978). Immunoglobulin E (IgE) levels were detected in exposed subjects only by immunoenzymatic assay in Diaplustest system.

The standart two-step microlymphocytotoxicity test (Ray 1978) was used to determine HLA-A, -B, -C antigens using 109 typing sera: for locus A 1, 2, 3, 9, 10, 11, 19, 24, 26, 28, 30, 32, 34; for locus B 5, 7, 8, 12, 13, 14, 15, 16, 17, 18, 21, 22, 27, 35, 38, 40, 60, 44, 51 and for locus C 1, 2, 3, 4, 6 specificities.

### Toxicological examination of Olaine's environment

The level of chemicals in the atmospherical air and in the soil of Olaine were determined by gas chromatography (Piotrowski 1977).

### Statistical analysis

All results are presented as mean + standart deviation. Values in the range of  $X \pm s$  were determined to represent the norm. The Student and Wilcoxon-Mann-Whitney tests were used to determine significant differences ( $p < 0.05$ ) between groups (Mann and Whitney 1947).

## Results

The atmospherical air and sewage were high contaminated with an array of chemicals. Isopropanol, aliphatic amine, formaldehyde, hydrochloric acid, nitric oxide, ammonia were determined as priority pollutants. The permissible concentrations (PC) of these chemicals were exceeded in 9% of the collected air samples. In the drinking water PC of manganese, arsenic and phenol were exceeded also.

All inspected subjects were recognised being practically healthy: they fulfilled their daily professional duties, had no health complaints, within the last three weeks had not suffered from any acute illnesses, chronic pathology observed in some subjects was in remission.

The results of all immunologically tested HLA-A1B8 haplotype carriers are summarised in Table 1 and Table 2.

There were significant differences ( $p < 0.05$ ) between female cases and control in percentage of lymphocyte, CD3<sup>+</sup>, CD4<sup>+</sup> cells, the level of PWM- induced lymphocytes proliferation, IgA and IgG levels and phagocytic activity of neutrophils (Table 1). There were significant differences ( $p < 0.05$ ) between male cases and control in percentage of CD3<sup>+</sup>, the level of spontaneous lymphocyte proliferation and concentration of CIC (Table 2). The details of the exposed subjects used in the analysis are shown in Table 3.

There was no difference in sex with regard to mean age in the exposed group. Significantly greater prevalence rates of current smokers, smoking duration years and number of daily smoked cigarettes were found in

Table 1. Immunological parameters in the females exposed to chemicals and control with -A1<sup>+</sup>B8<sup>+</sup>halotype

Parameters	Unit	Exposed females, A1 <sup>+</sup> B8 <sup>+</sup> M ± SD	Control, A1 <sup>+</sup> B8 <sup>+</sup> M ± SD
Lymphocytes	%	37.11 ± 2.42*#(n=9)	30.22 ± 1.86 (n=9)
CD3	%	66.11 ± 2.11*# (n=7)	75.14 ± 2.62 (n=7)
CD4	%	44.42 ± 0.77*# (n=7)	48.67 ± 1.56 (n=6)
CD8	%	25.79 ± 3.11 (n=7)	24.00 ± 2.33 (n=6)
CD4/CD8	%	1.63 ± 0.12 (n=6)	2.23 ± 0.38 (n=6)
CD19	%	20.63 ± 2.89 (n=6)	19.29 ± 1.82 (n=7)
CD16	%	24.81 ± 8.70 (n=7)	14.75 ± 3.70 (n=6)
BTR-PHA10	μg/ml	IS 65.74 ± 15.14 (n=6)	118.89 ± 26.30 (n=6)
BTR-PWM1	μg/ml	IS 14.57 ± 3.35# (n=6)	56.10 ± 16.14 (n=6)
IgA	G/l	2.51 ± 0.16*# (n=17)	1.69 ± 0.16 (n=8)
IgG	G/l	15.14 ± 0.65*(n=17)	12.06 ± 0.63 (n=8)
IgM	G/l	1.90 ± 0.24 (n=17)	1.69 ± 0.18 (n=8)
CIC	V	15.46 ± 1.21 (n=14)	12.70 ± 0.77 (n=8)
Chlsp	mV	15.40 ± 4.13 (n=7)	20.05 ± 3.24 (n=8)
ChlPh	mV	172.56 ± 31.92 *#(n=8)	363.45 ± 41.48 (n=8)

\* Significant at p<0.05 (the Student's criterion); # Significant at p<0.05 (Wilcoxon- Mann-Whitney's criterion)

Explanation to Table 1 to 2:

RBT IS -Blasttransformation reaction index of stimulation;  
Chlsp; ChlPh -chemoluminescence spontaneous and phagocytic;

males compared to females. The immunological indices in exposed females and males were compared too.

There were significant differences (p<0.05) between females and males in IgE levels (19,72 ± 3,34 kU/l and 135,65 ± 41,73 kU/l).

### Discussion

Our findings indicate that practically healthy HLA-A1B8 haplotype carriers exposed to environmental chemicals have a number of immune distinctions. The more interesting immunological data were obtained in females. It is known, that in a number of cases autoimmune pathologies develop predominantly in women (Derijk et al. 1991). We observed the decrease in the proportion of CD4<sup>+</sup> cells, the population which play a leading role in the regulation of immune response (Romagnani 1995). CD4<sup>+</sup> T-helpers are involved in the pathogenesis of hapten-induced diseases (Gocinski and Tigelaar 1990). CD4<sup>+</sup> cells play an important role in sensitisation to xenobiotics. CD4<sup>+</sup> cells can differentiate into Th1 (producing interferon gamma (IFNγ) and interleukin (IL)-2) or Th2 (producing IL-4, IL-5, IL-10, IL-13) lymphocytes. Some experimental models allowed to demonstrate a link between Th1-

or Th2-type responses and different hypersensitivity (Lebec et al. 1999). Modulation of Th cell responses is one means by which xenobiotics may cause immunotoxicity (Selgrade et al. 1997). In addition, Major Histocompatibility Complex (MHC) control of the mode of the immune response at the level of cytokine production is the a sophisticated way in which this occurs. The MHC/peptide complex, generated after antigen immunisation, indicates which cytokines production is induced and, thus determines the outcome of the immune response (Caruso et al. 1996, Caruso et al. 1997). In fact, peripheral blood mononuclear cells from HLA-B8,DR3-positive and negative individuals differ in their ability to produce IL-2, IL-5, and IFNγ on stimulation with PHA (Caruso et al. 1996). The past few years revealed that MHC-restricted hapten-specific T cell receptors in their majority react to haptenized peptides associated with the MHC peptide-binding groove (Martin et al. 1994; Kessler et al. 1999).

The changes in the number of CD4<sup>+</sup> cells were accompanied by the suppression of PWM-induced lymphocyte proliferative activity. The data on the suppression of mitogen-induced lymphocyte response in HLA-A1B8 positive healthy females were presented

Table 2. Immunological parameters in the males exposed to chemicals and control with HLA-A1\*B8+ halotype

Parameters	Unit	Exposed males, A1* B8+ M ± SD	Control, A1* B8+ M ± SD
Lymphocytes	%	35.75 ± 2.17 (n=8)	36.80 ± 4.31 (n=10)
CD3	%	64.48 ± 1.74*# (n=7)	74.20 ± 2.40 (n=10)
CD4	%	47.66 ± 5.91 (n=7)	47.63 ± 2.90 (n=8)
CD8	%	22.92 ± 3.47 (n=7)	25.13 ± 2.33 (n=8)
CD4/CD8	%	2.27 ± 0.36 (n=6)	1.72 ± 0.12 (n=7)
CD19	%	18.19 ± 1.11 (n=6)	20.29 ± 2.59 (n=7)
CD16	%	15.67 ± 0.76 (n=7)	15.78 ± 1.12 (n=9)
BTR-PHA10µg/ml	IS	45.40 ± 6.40 (n=6)	75.54 ± 22.07 (n=6)
IgA	G/l	2.54 ± 0.30 (n=15)	2.57 ± 0.24 (n=12)
IgG	G/l	14.04 ± 0.62 (n=13)	13.13 ± 0.47 (n=12)
IgM	G/l	1.37 ± 0.17 (n=14)	1.03 ± 0.11 (n=12)
CIC	V	12.96 ± 1.29 # (n=10)	9.49 ± 0.92 (n=8)
Chlsp	mV	26.50 ± 8.05 (n=7)	13.84 ± 3.12 (n=7)
ChlPh	mV	266.30 ± 85.58 (n=8)	282.86 ± 42.17 (n=7)

\* Significant at p<0.05 (the Student's criterion); # Significant at p<0.05 (Wilcoxon- Mann- hitney's criterion)

by Sochnev et al. (1993) and in HLA-B8,DR3-positive individuals by Monica et al. (1990). The diminished functional activity of lymphocytes may result in ineffective clearance from immunogen, which may contribute to the development of pathological conditions (Modica et al. 1990). The correlation between the HLA-A1B8 haplotype and increased lymphoproliferative activity stimulated by several concentrations of PHA and Con-A have been reported by Makhatadze et al. (1997).

Besides, we revealed the increase in the percentage of lymphocytes. The regulating mechanisms of the number of lymphocytes in the blood are uncertain, although it is likely that hormones, homing factors and cytokines determine the lymphocyte number.

Table 3. Characteristics of the HLA-A1B8 halotype carriers exposed to chemicals

HLA-A1B8 carriers	Males	Females
Age Yrs	39	40
Nonsmokers %	17	86
Exsmokers %	8	7
Smokers %	75	8
Smoking duration Yrs	21	10
Cigarettes/day	17	10
IgE	135.65±41.73	19.72±3.34

MHC appears to be involved in these mechanisms also. Caruso et al. (1997) have shown that HLA-B8,DR3 positive subjects displayed significantly lower values of lymphocytes as compared to HLA-B8,DR3 negative ones. A significant increase of spontaneous apoptosis of lymphocytes observed in HLA-B8,DR3 positive.

We have found a significant increase of IgG and IgA levels in exposed females with the HLA-A1B8 haplotype. The increase of IgM levels in HLA-A1B8 positive Venezuelans was reported by Makhatadze et al. (1997). The results obtained by these authors may suggest that some of the genes responsible for these levels could be localised in or along the length of the HLA-A1B8 haplotype between the A and B loci of the MHC. The decrease of the IgA level in HLA-A1B8 positive healthy females was demonstrated by Sochnev et al. (1993). It may result from genetic control of IgA level

partly by MHC genes and partly by sex chromosomes, and both effect do not depend on each other. We revealed that neutrophil phagocytic activity in females, carrying HLA-A1B8 haplotype, was decreased that may reflect reduced immunoreactivity providing defence against infectious agents. It is in contrast with other studies (Sochnev et al. 1993).

Our data revealed that the exposed males with HLA-A1B8 haplotype have a significantly greater total serum IgE concentrations than females with the same haplotype. These findings confirms the ob-

servations made by Sapigni et al. (1998), whereas it is in contrast with other studies (Holford-Strevens et al., 1984; Bousquet et al. 1982). Sex variation in the IgE responses may be the result of the combination of many mechanisms: genetic, hormonal, and environmental (Omenaas et al. 1994). In our samples men had a greater prevalence of active smoking and the frequency of smoking. Many authors found that cigarette smoking was associated with an elevated total serum IgE levels (Omenaas et al. 1994). The relationship between IgE and the dose of smoking was also recently shown especially in males, by a longitudinal study (Sherril et al. 1994). Therefore, other mechanisms should be responsible for the gender-related difference.

### Conclusions

The environmental chemical exposure may cause disorders of immunoregulating in females HLA-A1B8 carriers resulting in the suppression of cellular immunity. The reduction of phagocytic activity of neutrophils and an increase in immunoglobulin levels may reflect disorders in native and humoral immunity. The presence of HLA-A1B8 haplotype in females exposed to environmental chemical may be at highest risk of developing chemical-related disease.

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**MHC (HLA) ANTIGENS IN GASTRIC CANCER PATIENTS****Jaunalksne I., Engele L., Sochnevs A., Donina S., Zakenfelds G., Januskevics V.**

Jaunalksne I., Engele L., Sochnevs A., Donina S., Zakenfelds G., Januskevics V. 2001. MHC (HLA) antigens in gastric cancer patients. *Acta Biol. Univ. Daugavp.*, 1 (2): 86 - 89

MHC antigens play an important role in antigen recognition mechanisms. One of the possible ways which allow tumor cells to escape from immunocompetent cells are these changes in MHC antigen expression. With the aim to analyse immune answer we determined MHC expression of 89 gastric cancer patients, 32 patients with benign gastric diseases and 59 healthy volunteers using microlymphocytotoxicity test. The dominant MHC antigens in all groups were the same, but gastric cancer patients had decreased MHC A10 expression (2%), but patients with gastric ulcer had increased MHC A28 (40%), patients with gastric polyps had statistically significant increase in MHC B17 (33%). Gastric cancer patients with MHC A3 antigen had the highest CD4, CD8, CD38, HLADR cell level, but patients with MHC A28 had the lowest immunocompetent cell level. Therefore antigens are related to different immune answer.

Key words: MHC, Gastric cancer, gastric cancer risk group.

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**Introduction**

The gastric cancer is the second most important cancer in Latvia. These solid tumors respond poorly to chemotherapy and radiotherapy, thus surgical treatment is the method of first choice. Transformed cells express surface antigens or other structures, components of which (in form of polypeptides connected with HLA molecules on the surface of APC) recognize immunocompetent cells and induce immunological effector mechanisms which can eliminate the transformed cells or impede the progression and spread of tumour.

The MHC (HLA) major histocompatibility complex play a main role in immunological answer involving in the presentation of foreign antigen for their recognition CD8 (CTL) cytotoxic T lymphocytes. Therefore CD8 lymphocytes are those cells which can monitor genetic changes in cells, MHC class I antigens provides an enormous diversity in potential antigenic peptide presentation including recognition of tumor cells by autologous CTL in man and animals (Van den Eynde et al. 1997).

Human tumors of various origin have been shown to lose completely MHC antigens. Those tumors are more aggressive and those patients have poorer prognosis

(Hicklin et al. 1999). For the first it was shown in 1976 in murine lymphoma case and 1977 at first in human malignancy. Total HLA antigen loss is shown 9 - 52% in all tumours. 19% in laryngs, 14% in colorectal 10% in gastric cancers and melanomas. 39 - 88% HLA haplotype loss was shown in rectal, pancreas and melanoma tumor cases. HLA locus A loss was determined in 3 - 19%, HLA B 5 - 19%. Another authors showed 16 - 29% (Garrido et al. 1997). In colorectal cancer patients more frequently was determined HLA A2 locus absence.

70% from tumours had decreased number of HLA antigen expression on cell surface, for example patients with cervix uteri tumours had diminished HLA B44 expression.

There may be also B<sub>2</sub> microglobulin defects, peptide insufficiency in endoplasmic reticulum and TAP defects. In primary melanoma TAP defects were shown in 15%, in metastatic melanoma in 22% (Ruiz-Cabello et al. 1998).

These all above mentioned situations may help tumours to escape from immune answer. For another hand there are observations that exact MHC antigens present tumour antigens which may recognize CTL (Yasoshima et al. 1995, Brauer et al. 2000).

**Materials and methods**

Searching the answer why gastric cancer patients differ by immunological reactivity, differ in effect of therapy, in course of disease we determined: 1) HLA I class antigens, HLA phenotype of gastric cancer patients and patients who suffer from chronic gastric diseases with high cancer development risk (patients with chronic ulcer and polyps of stomach), 2) peripheral blood lymphocyte subpopulation level of gastric cancer patients. We have examined: 59 healthy control persons, 50 gastric cancer risk group patients, 18 patients with gastric polyps, 32 patients with gastric ulcer, 80 patients with gastric cancer. HLA antigens were determined using two lymphocyto-toxicity test. Immunocompetent cell level was determined using laser flow cytofluorimeter Ortho Spectrum III by using monoclonal antibodies.

**Results**

HLA antigen prevalence is shown in table 1. If we compare HLA antigen expression among our control

group and large control group and large control group from the literaturae (Balchenko et al. 1994) the dominant HLA antigens are similar. From the latvian population group one of the prevailing HLA B alleles were B40. It was not observed in our control group. Gastric cancer patients group have the same dominant HLA A and B antigens our control group, only gastric cancer patients had statistically significant decrease in HLA A10 antigen. Patients with gastric ulcus in comparison with control group had statistically significant higher expression of HLA A28 and higher expression of B27, but patients with gastric polyps had statistically significantly increased HLA B17 antigen.

On table 2 are shown prevailing haplotypes in different groups. HLA A1B8 is a prevailing haplotype in all group. Gastric cancer patients had HLA A2B7 haplotype, the same which was in latvian population dominant. We determine # distribution of HLA antigen expression in patients with different vastness of malignant process (table 3). Our results show that the patients with more advanced disease had higher HLA B12 and HLA B40 alleles.

Table 1. Distribution of HLA antigens

HLA antigens	Latvian population n = 525	Control group n = 59	Gastric cancer group n = 89	Chr. gastric ulcus group n = 32	Gastric Polypus group n = 18
a) Frequency of locus A antigens in					
A1	19.05	20.33	28.43	18.75	5.55
A2	56.38	49.15	45.97	28.12	55.55
A3	26.86	22.03	22.98	18.75	27.70
A9	18.48	16.94	19.54	12.50	-
A10	12.95	20.33	2.29*	25.00	27.77
A11	13.33	16.94	2.29	3.12	11.11
A19	5.52	n.d.	-	3.12	-
A28	4.19	10.16	10.34	40.62	11.11
A30	n.d.	1.69	1.14	-	-
b) Frequency of locus B antigens					
B5	11.05	15.25	11.36	9.37	11.11
B7	27.82	32.20	21.59	15.62	22.22
B8	15.43	16.94	12.50	18.75	11.11
B12	19.62	22.03	15.90	6.25	5.55
B13	10.10	8.47	4.54	3.12	16.66
B14	3.43	1.69	2.27	3.12	-
B15	11.81	8.47	4.54	9.37	5.55
B16	2.86	1.69	2.27	-	-
B17	8.57	5.08	7.95	3.12	33.33*
B18	7.23	8.47	5.68	-	5.55
B27	10.10	8.47	4.54	18.75	5.56
B35	14.10	13.55	3.40	-	-
B38	6.19	n.d.	1.13	-	-
B40	16.38	6.77	7.95	6.25	5.55

\*Statistically signif. difference (Fishers meaning).

MHC (HLA) antigens in gastric cancer patients

Table 2. Dominant HLA haplotypes

Place		1 st place	2nd place	3rd place	4th place	5th place
Latvian population	n=525	A2B7	A2B12	A2B40	A3B7	A1B8
Control group	n=59	A2B12	A11B7	A1B8	A9B35	A1B7
Gastric cancer patients group	n=89	A2B7	A2B12	A1B8	A3B7	A1B17
Patients with gastric ulcer	n=32	A1B8	A28B27	A3B7	A9B27	A3B8
Patients with gastric polyps	n=18	A2B17	A3B5	A2B7	A2B7	A1B8

Interesting (Pic. 1) that the patients with HLA A3 antigens had higher level of CD4, CD8, CD38, HLADR immunocompetent cells, but patients with HLA a28 had the lower immunocompetent cell level. It was not possible to determine subpopulations to the HLA B locus antigens because of grate spread.

Discussion

Ogosni K. (1997) and his colleagues showed that gastric cancer patients with HLA A2 have a lower probability for metastazing in lymph nodes, but patients with HLA B52 have an increased risk for lymph node involvement. Our results show that patients count with HLA B12, B40 increase with vastness of malignant process. Yaroshima (1995) reported that the patients with HLA A31 could present tumour antigen to CTL. Moureen C. (1999) proposed than CTL are killed if the tumour antigens are in a high level, that would

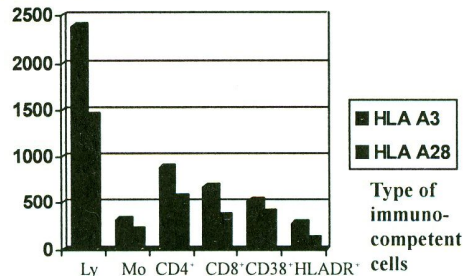
Table 3. HLA antigen in various stages of malignant process

HLA antigens	HLA A1	HLA A2	HLA A3	HLA A9	HLA A28
I + II stage %	58%	49%	68%	%#%	%&%
III + IV stage %	42%	51%	32%	47%	43%

HLA B antigens	HLA B5	HLA B7	HLA B8	HLA B12	HLA B40	HLA B17
I + II stage %	62%	69%	58%	36%	43%	57%
III + IV stage %	38%	31%	42%	64%	58%	43%

Absolute immuno-competent cell level



Pic. 1. Gastric cancer patients average Immunocompetent cell absolute count with HLA A3 and HLA A28 antigen expression

happened if the patients have a large tumour. We can say that the patients with HLA A3 have highest immunocompetent cell level, may be these patients have better prognosis. From our results patients with gastric cancer had decreased HLA A10, may it is a protective antigen. In another way patients with Gastric ulcer much more higher level of HLA A28, HLA B27, but patients with polyps had high expression of B17, may be these HLA as indicators of disease.

Hicklin D.I. (1999) reported: "Selective HLA class I allele loss is not unique to malignant cells, similar phenotypes have been found although with low frequency in peripheral blood Lymphocytes." Therefore the tumour cells can escape from immune answer.

Conclusions

The patients with chronic of stomach are characterized by elevated frequency of A28, B27 antigen.



The patients with gastric polyps are characterized by statistically significant increase of B17 antigens.

The gastric cancer patients are characterized by decreased frequency of HLA A10.

Gastric cancer patients with HLA A3 have a higher immunocompetent cell level, but patients with HLA A28 – lower immunocompetent cell level.

The questions, whether patients differ in HLA and their immune state differ also in clinical outcome and modus of treatment.

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**GENETIC BASIS OF BIODIVERSITY AND METHODS OF ITS DETERMINATION****Natālija Škute**

Škute N. 2001. Genetic basis of biodiversity and methods of its determination. *Acta Biol. Univ. Daugavp.*, 1 (2): 90 - 93.

Genetic levels of biodiversity can be divided in to components too the genetic levels diversity exist at tree levels. Genetic variation at this level it was consider. It was shown, the methods of molecular hybridisation, of determination of primary structure of gene, immunologic methods and methods of genetic markers, can be used for determination of genetic diversity.

Key words: biodiversity, genetic diversity, heterozygosyts, polymorphisms, molecular hybridisation

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Biodiversity is not just diversity of species. A comprehensive approach to biodiversity conservation must address multiple levels of organization and many different spatial and temporal scales. Most definitions of biodiversity recognize its hierarchical structure, with the genetic, population-species, community-ecosystem, and landscape levels considered most often. Each of these levels can be further divided into compositional, structural and functional components. Composition includes the genetic constitution of populations, the identity and relative abundance of species in a natural community, and the kinds of habitats and communities distributed across the landscape. Structure includes the sequence of pools and riffles in a stream, downed logs and snags in a forest, and the vertical layering and horizontal patchiness of vegetation. Function includes the climatic, geologic, hydrologic, ecological and evolutionary processes that generate biodiversity and keep it forever changing.

Genetic level can be divided into compositional, structural and functional components too. Compositional components of genetic level include the allelic diversity, presence or absence of rare alleles. Structural components of genetic level include the heterozygosity and phenotypic polymorphism. Functional components of genetic level includes the symptoms of inbreeding depression or genetic drift (reduced survivorship or fertility, abnormal sperm, reduced resistance to disease, morphological abnormalities or asymmetries), the inbreeding or outbreeding rate, the rate of genetic interchange between populations (measured by rate of dispersal and subsequent reproduction of migrants) (Reed F. Noss 1994).

A species' pool of genetic diversity exists at three fundamental levels: genetic variation within individuals (heterozygosity), genetic differences among individuals within a population and genetic differences among populations.

**Individual level**

Heritable genetic variation is the basis for evolutionary change and is essential for natural selection. With the exception of identical twins and clones, every individual of a species is genetically unique. Some of these phenotypic or physically expressed variations are due to variation in the genotype, or the genetic constitution and some are due to environmental modification.

Quantitative genetics describes this relationship between an organism's phenotype, its genotype and environmental influences as  $V_p = V_g + V_e + V_{ge}$ , where  $V_p$  is overall phenotypic variation among individuals,  $V_g$  is variation due to genotype,  $V_e$  is variation due to environmental influences, and  $V_{ge}$  is variation due to genetic-environmental interaction.

The genetic variation is important at the level of the individual because it forms part of the basis for its phenotype. At any particular gene locus there are two alleles or copies of the gene inherited from two parents. Each locus can be either monomorphic (both copies of the allele are always the same, there is no variation at that locus) or polymorphic (there are multiple types of alleles at that locus). For any particular

individual and polymorphic locus may be either homozygosity (two copies of the same allele) or heterozygosity, or the proportion of these gene loci in an individual that contains alternative forms of alleles, which is one measure of individual genetic diversity.

Heterozygosity may correlate with higher individual fitness. The ultimate origin of such genetic variation is mutations, but within individual variation each generation is produced by recombination during sexual reproduction.

Genetic variation at the individual level can reflect the action of selection, or can indicate low levels of diversity relevant to conservation. The individual is the level, on which genetic problems such as inbreeding occur. Genetic variation is always measured in individuals, but then summed up across all populations and compared at the higher level.

#### Population level

Population –level of genetic variation consist of the types of alleles present and frequencies of their appearance across all members of a population considered the whole (the gene pool). Genetic variation at the population level described by types and frequencies of alleles presents and in the particular combination of alleles. Gene frequencies within a population generally change over time, due to selection, random processes such as genetic drift or immigration from or immigration to other populations, called gene flow. Species rarely exist as single, randomly interbreeding or panmictic populations.

#### Genetic diversity among population

On the contrary, genetic differences typically exist among populations; these geographic differences are an important component of overall genetic diversity. Genetic diversity in this case consists of within population diversity (mean individual heterozygosity level within population) and among-population divergence (mean genetic differences among geographical locations). A genetic model of this diversity is  $H_t = H_p + D_{pt}$ , where  $H_t$  is total genetic variation in the species,  $H_p$  is average diversity within populations or among individuals,  $D_{pt}$  is average divergence among population across the total species range (Nei M., 1975). There are different approaches to the study genetic diversity among individuals.

#### Methods of determination of genetic diversity

It is the presence of identical or similar consecution of nucleotides of sufficient length in genomes of different individuals that must be the evidence of kinship such individuals. The more such homologies between their DNA are found then closer must be their kinship. The method of determining genetic homology is called molecular hybridisation.

#### Molecular hybridisation

This method is based on thermal denaturation of double-spiralled DNA molecules and their further reconstruction during the cooling. It appears that reconstruction of separate DNA spirals is not a species-specific process and can occur between separate DNA chains of different species. And the more homologue are DNA chains – the more hybrid spirals will be formed. Half of such hybrid chain will belong to one species and the other half to the second species. By means of this method, taxonomic positions of rodents, hominids and other groups were proved. It was also shown not long ago that cetaceans are close relatives of ungulates. This evidence was later confirmed by other techniques. Method of molecular hybridisation brought a lot to taxonomy, however now is not in use. It appeared that organization of genome of eucaryotes is so complicated that it is too difficult to interpret the data of DNA molecular hybridisation for this group of organisms. This method was successfully implemented for the study of small and simply organized genome of prokaryotes. However, even in prokaryotes' genetic taxonomy applicability of this method is limited to genus level. Besides, while determining homologies of DNA we do not know about the genes they belong to.

#### The determination of primary structure of individual genes

The best method of researching particular gene (or other DNA part) is determination of its complete primary structure. Particular gene is extracted from DNA of the researched individual. Then a complete sequence of nucleotides is determined for this gene for further comparison with similar genes of other individuals. In every case only one gene becomes the object of comparison, its function must be a known issue and homology is determined with single nucleotide accuracy. For such purpose, the RNA gene of smaller ribosomal subunit is often used. Other genes, however, are also

used, not only those of caryotic DNA, but also of mitochondrial DNA and chloroplastal DNA. It can also be performed for ribosomal proteins, genes of some DNA polymerases, cytoskeletal proteins, ATF-synthetases, glycolitic ferments, histones etc. Those genes are so popular because of their conservative character.

For analysis of vegetation species not only ribosomal RNA genes are used, but also their parts, located between those two genes (rbcL gene). It has turned out that subdivision of angiosperm species to monocotyledonous and dicotyledonous groups contradicts to the data of molecular research (Красилов В.А., Буглаева Е.В., Маркевич В.С., Маслова Н.П. 1997). Study of more conservative parts of chloroplastic DNA (gene cpIIIS) showed that species taxonomy determined by classical phenotypic methods differs from the one determined by means of molecular genetics research and therefore needs revision (Antonov, A.S., Troitsky, A.V. 1995).

Considerable success has been achieved in establishing systematic of prokaryotic genes while comparing 16r RNA genes. On the basis of this comparison of r16s genes relationship among the known groups of prokaryote were determined. This method was also used in identification of the unknown microorganisms. The filogenetic tree of the prokaryots and eukariots was created thanks to this method. The obtained data have revised our understanding of biodiversity. It was found out that the major part of diversity of organisms are made up by microscopic life forms, such as procariots and eucariotes (Aravind L., Tatuzov R., at all, 1998, Заварзин Г.А. 1974). This phylogenetic tree might describe only the 16s RNA genes evolution or the structure, which they determine and is not entire organisms. The investigation of primary structures of the entire genome may give answer to this question.

#### **Immunological research**

Immunological research has mainly concentrated on the pathology of infection. With the discovery of specific tissue antigens, it has found application in identifying differences among various species of animals as well as within these species. Further development of this applied use of immunological research made it possible to discover intra-species differences in various groups of animals: rodents, birds, guinea-pigs, fishes, drozofilles.

#### **Determination of DNA polymorphism**

New technological development have expanded the range of DNA polymorphism assays for genetic mapping, marker assisted plant breeding, genome fingerprinting and for investigating genetic biodiversity. These technologies include restriction fragment length polymorphisms (RFLP), random-amplified polymorphism DNA markers (RAPD), amplified fragment length polymorphisms (AFLP) and simple sequence repeat polymorphisms or micro satellites (SSR). These methods detect polymorphism by assaying subsets of the total amount of DNA sequence variation in a genome (Mazur B.I., Tingey S.V. 1995). By markers we understand the genes, the function of which results in particular characteristic of organisms.

The application of DNA-based diagnostic markers is determined, to a large extent, by the technology that is used to reveal DNA polymorphisms. The available assays fall into two broad categories: restriction enzyme-based assays, and DNA amplification-based assays. In the first category there are restriction fragment length polymorphism (RFLP) assays, which detect DNA polymorphisms through restriction endonuclease digestions followed by visualization via DNA blot hybridizations RFLP markers are attractive because they are co-dominant (i.e. each polymorphic allele at a locus is detected in the assay). RFLP markers are also useful for detecting locus-specific polymorphisms across species boundaries (Pawel W., Morgante M., Andre C. et al. 1998, Morell M.K., Peakall R., Appels R. et al. 1995).

Molecular markers have found widespread application in the characterization of systematic, phylogenetic and evolutionary relationships among diverse species. This approach has also been used to describe genetic relationships and genetic diversity within populations of a particular species.

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## THE INVESTIGATION OF MORPHOLOGICAL AND ECOLOGICAL PECULIARITIES OF *SORBUS* GENERA SPECIES AND SORTS IN THE BOTANICAL GARDEN OF VILNIUS UNIVERSITY

Evaldas Navys

Navys E. 2001. The investigation of morphological and ecological peculiarities of *Sorbus* genera species and sorts in the Botanical Garden of Vilnius University. *Acta Biol. Univ. Daugavp.*, 1 (2): 94 - 98.

The article deals with investigation of the next biological, morphological and ecological peculiarities of *Sorbus* genera species and sorts connection between the sugar content and taste of fruits, structure of crown and resistance to winter damages.

Key words: Mountain Ash, species, sort, group of sorts, fruit stalks, frost damages.

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### Introduction

There are known about 85 species and over 100 subspecies, varieties, forms and sorts of the Mountain ash and Rowan (*Sorbus* L.). The collection of the Botanical garden of Vilnius University contain 68 taxa. Not all the species and loner ranking botanical taxonomic units and cultivars, however can grow in Lithuanian climate and only part of them are worth to be grown in the orchards and forests. All they are important as taking part in metabolism and improving soil and forest sanitary state.

The author of this article studies the biology, morphology and ecology harvest size and quality under Lithuanian's conditions to selection of species and sorts fit to grow in Lithuania.

When accumulating the genefund of *Sorbus* genus species and sorts in Botanical garden of Vilnius university it is important to research the scientific and utilitarian value of these plants. Studies of mountain ash *Sorbus* L. genus taxa as well as species *Sorbus aucuparia* L. in Lithuania are among the sparest among those on local arboreal plants. The article contains data of ten-year studies carried out in order to determinate fitness of *Sorbus* L., order species and sorts to be grown in Lithuanian orchard and forests.

### Materials and methods

The main objective in the study of the Vilnius University Botanical garden were to define biological, morphological and ecological properties of *Sorbus aucuparia* L., alien species and sweet fruit cultivars grown in dendrological collections and town plantations.

We have studied content of sugar, acids and vitamin C in the fruits. Ordered by us the analyses of sugar and acids were done by AB Anyksciu Vynas laboratory. Since the laboratory analyses were done once, we have generalised them by comparing with the foreign data published (calculated averages) (Cinovskis 1986; Pürs 1999).

After detailed studies of morphological peculiarity of leaves we (Navys 2001) proposed the table for definition of *Sorbus* species. The table is compiled according to the morphology of leaves for species in the Botanical garden on Vilnius University. Literary sources have also been used (Prokudin 1987; Snarskis, Galinis 1974; Natkevičaitė - Ivanauskienė 1971; Rolof & Bärtels 1996; McAllister 1986).

In the collection of the VU Botanical Garden we have studied morphological features of the crown structure. Applying methods worked out by prof., dr. habil. J. Galvydis (1999), we have defined types of fruit stalks and their distribution in the crown for 2 species and 12 varieties.

In the Botanical Garden of Vilnius University and

Table 1. Connection between the sugar content in mountain ash garden sorts and its taste (positive degree - 10)

Sort	Sugar (in % of green weight)	Degree of taste	Coefficient of connection
'Alaya Krupnaya'	6,88	7	1,02
'Burka'	7,43	7	0,94
'Granatnaya'	7,03	8	1,14
'Nevezhenskaya'	10,5	9	0,86
'Nevezhenskaya Oranzhevaya'	12,0	10	0,83
'Titan'	7,6	7	0,92

Latvian National Botanical Garden we have assessed influence of various temperature factors on 14 *Sorbus* species and 19 varieties during the resting phase from the end of the arboreal plant vegetation to the start of swelling of buds - the consequences of such impact are called in the present paper the winter damages. For this purpose we have applied a 10-point scale for assessment of deciduous trees and shrubs (Navys 1999, 2000).

## Results and discussion

Sugar content in the fruits is a variable parameter depending not only on biological features of a variety but also on ecology, in particular on insolation degree, geographical zone and climatic changes during the vegetation.

Table 2. Distribution of fruit stalk numbers for mountain ash

Species, group of sorts, sort	Number of fruit stalks							
	twig-like		Peaked		Small		Total	
	No.	%	No.	%	No.	%	No.	%
<i>S. aucuparia</i> L. (typical species)	120	93,75	5	3,91	3	2,34	128	100
'Edulis' group sorts:								
'Konzentra'	59	93,66	2	3,17	2	3,17	63	100
'Moravskaya Krupnoplodnaya'	173	95,58	4	2,21	4	2,21	181	100
'Rosina'	231	97,06	4	1,68	3	1,26	238	100
'Rossica' group sorts:								
'Krasnaya Krupnaya'	146	92,99	3	1,91	8	5,10	157	100
'Neveshenskaya'	184	95,83	4	2,09	4	2,08	192	100
'Neveshenskaya Oranzhevaya'	189	94,97	8	4,02	2	1,01	199	100
'Rossica'	107	96,40	4	3,60	-	-	111	100
'Rossica group sorts:								
'Alaya Krupnaya'	47	37,90	5	4,03	72	58,07	124	100
'Burka'	-	-	-	-	57	100	57	100
'Granatnaya'	3	0,99	10	3,30	290	95,71	303	100
'Likernaye'	1	1	2	2	97	97	100	100
'Titan'	5	4,95	15	14,85	81	80,2	101	100
<i>S. hybrida</i> L. (typical species)	3	4,06	3	4,05	68	91,89	74	100

Comparison of the data obtained to *S. aucuparia* sugar content (5,8%) showed that sorts studied were really the sweetest - sugar contents in their fruits were considerably (1,2-2,1 times) higher.

Taste characteristics are not based by any laboratory data and they are absolutely subjective and dependent on opinions of 50 persons, who took part in tasting the fruits. Of 15 varieties covered by tasting in the Botanical Garden of Vilnius University on August 29, 2000 the sorts of 'Edulis' and 'Rossica' groups were the best (from 8 to 10 points), followed by slightly less tasty 'Rossica Major' group varieties.

We have defined morphological features of leaves for 11 mountain ash species and compiled a peculiar table for definition of these species leaves.

Margins of compound leaflets for the varieties of 'Rossica' group are deeply or sharply lacerated, hence according to this feature, they can be easily distinguished from 'Edulis' group varieties with even-margin leaflets from the base to 1/4 or 1/3 of their length.

We tried to evaluate important morphologic features - we have classified the fruit stalks into tree above types and calculations each-type fruit stalk number in the tree. We have calculated 7-row branches (including the main) for the mountain ash and its sorts. Fruit stalks formed on the secondary branches of the crown can be small, peaked or

Table 3. Evaluation of *Sorbus* genus species resistance to winter damages

	Degree of resistance	Species	Quantity	
			Number	Per cent
1.	No frost damages	<i>S. americana</i> Marsh., <i>S. aucuparia</i> L., <i>S. hybrida</i> L., <i>S. intermedia</i> (Ehrh.) Pers. <i>S. sibirica</i> Hedl., <i>S. × splendida</i> Hedl.	6	42,86
2.	Up to 70 % of flower buds damaged.	<i>S. aria</i> (L.) Crantz., <i>S. sambucifolia</i> (Cham. et Schltld.) Roem	2	14,29
3.	Up to 70 % of vegetative buds and 30 % one year shoot tops damaged.	<i>S. latifolia</i> (Lam.) Pers., <i>S. rufo-ferruginea</i> C.K. Schneid.	2	14,29
4.	30-100 % of one – year shoots or up to 30 % of older shoots damaged entirely or partly.	<i>S. × hostii</i> (Jacq. f.) K. Koch.	1	7,14
5.	Up to 70 % two – year and older branches (or shrub steams) damaged.	<i>S. torminalis</i> (L.) Crantz.	1	7,14
6.	Trunks cracked (up to 3 cm width) above the snow cover.	<i>S. caucasica</i> Zinserl.	1	7,14
7.	Trunks cracked (up to 3 cm width) at the root collar or any height the bark is come off and damaged by warmth – caused rot inside the crack wide than 3 cm.	-	-	-
8.	The above – ground part damaged (over 70 %), but the plant can regenerate from the bast.	-	-	-
9.	The part above the ground dead, but roots remain alive, and the plant can form root -shoots.	-	-	-
10.	The above – ground part and roots destroyed.	<i>S. domestica</i> L.	1 14	7,14 100

twig-like, small fruit stalks are 1-5 cm long and have from 2 to 10 dense leaves and one terminal flower bud. Peaked fruit stalks with terminal flower bud and lateral vegetative buds are 5-15 cm long. The twig-like fruit stalk with terminal flower buds is longer than 15 cm.

We have observed that fruit stalks types differ with mountain ash species and sorts. Branch morphology for spontaneous and created sorts within the *S. aucuparia* species is similar to that of the type species: twig type fruit stalks prevail in the crown, whereas ‘Rossica Major’ group sorts possess few fruit stalks of such a type, while small fruit stalks dominate.

Branching of sorts from the ‘Edulis’ and ‘Rossica’ groups resembles that of *S. aucuparia*. Morphology of branches for ‘Rossica Major’ group is close to that of *S. hybrida*. During blossom and fruit developing the mountain ash with twig like fruitstalks wild species, ‘Edulis’ and ‘Rossica’ group sorts have greatly magnificent look (flower and fruit racemes outside).

Resistance to winter damages is the most important factor determining success in plant’s introduction (McAllister 1986). We proposed the scale for evaluation of frost damages to the deciduous trees and shrubs (Navys 1999, 2000).

We have got the following frosting data for evaluated 14 *Sorbus* species: 6 species (42,86%) were found to absolutely frost resistant, 5 ones (35,72 %) affected slightly and rapidly regenerated, whereas serious damages and long regeneration was typical of 2 species (14,28 %), to absolutely frost destroyed 1 species (7,14 %).

We have got the following frosting data for 19 *Sorbus* sorts: 57,8 % were found to be absolutely frost resistant 26,3 % affected slightly and rapidly regenerated, whereas serious damages and long regeneration was 15,8 % sorts. All the ‘Rossica’ group sorts studied are frost-resistant, and some sorts of other groups are just slightly less frost hardy, i.e. there are no such trees with their above-ground part and roots damaged by frost (70% and more).

Root system of mountain ash is well developed, wide, but rather shallow. Up to 80 % of roots lie not deeper than 40 cm with the largest density at 20 cm depth. Small roots are found at 3-5 cm depth from the surface.

## Conclusions

1. There are direct connections between the sugar content in mountain ash garden sorts and its taste. The



Navys E:

Table 4. Evaluation of *Sorbus* sorts resistance to winter damages

Degree of resistance	'Edulis' group sorts	'Rossica' group sorts	'Rossica Major' group sorts
1. No frost damages		'Nevezhenskaya', 'Nevezhenskaya Kubovaya', 'Nevezhenskaya Krasnya' 'Nevezhenskaya Oranzhevaya' 'Nevezhenskaya Sachamaya' 'Nevezhenskaya Zholtaya'	'Alaya Krupnaya', 'Burka', 'Businka', 'Granatnaya', 'Titan'
2. Up to 70 % of flower buds damaged.	'Krasnaya Krupnoplodnaya'	-	'Desertnaya'
3. Up to 70 % of vegetative buds and 30 % one year shoot tops damaged.	'Konzentra', 'Rosina'	-	'Likemaya'
4. 30-100 % of one – year shoots or up to 30 % of older shoots damaged entirely or partly.	-	-	'Krasavica'
5. Up to 70 % two – year and older branches (or shrub steams) damaged.	-	-	-
6. Trunks cracked (up to 3 cm width) above the snow cover.	-	-	'Rubinovaya'
7. Trunks cracked (up to 3 cm width) at the root collar or any height the bark is come off and damaged by warmth – caused rot inside the crack wide than 3 cm.	-	'Moravica'	-
8. The above – ground part damaged (over 70 %), but the plant can regenerate from the bast.	-	-	-
9. The part above the ground dead, but roots remain alive, and the plant can form root - shoots.	-	-	-
10. The above – ground part and roots destroyed.	-	-	-

sorts of 'Edulis' and 'Rossica' groups were really the sweetest and the best tasty.

2. Branch morphology for spontaneous and created sorts within the *S. aucuparia* species is similar to that of the type species: twig type fruit stalks prevail in the crown, whereas 'Rossica Major' group varieties possess few fruit stalks of such a type, while small fruit stalks dominate.

3. *S. aucuparia* and introduced sweet-fruit species can be defined from the leaves, and sorts groups can be distinguished, however for definition of the sorts within

groups the leaf features are not sufficient.

4. We have got the following frosting data for evaluated 14 *Sorbus* species: 42,86 % were found to absolutely frost resistant, 35,72 % affected slightly and rapidly, 21,42% whereas serious damages and absolutely frost destroyed.

5. We have got the following frosting data for 19 *Sorbus* sorts: 57,8 % were found to be absolutely frost resistant 26,3 % affected slightly and rapidly regenerated, whereas serious damages and long regeneration was 15,8 % sorts.

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## PROBLEMS AND PROSPECTS OF DENDROLOGY IN LITHUANIA IN THE BEGINNING OF THE THIRD MILLENIUM

**Audrius Skridaila**

Skridaila, A. 2001. Problems and prospects of dendrology in Lithuania in the beginning of the third millenium. *Acta Biol. Univ. Daugavp.*, 1 (2): 99 - 102.

Nowadays there are in Lithuania more than 3200 taxa of woody plants. Most of them (about 1700 taxa) were introduced in last ten years. With the growing of woody plant diversity in Lithuania come to light a lot of old problems of dendrology. This article attempt to show different contemporary problems of dendrology (in a wider sense) in Lithuania. It presents the heritage from soviet times, obstacles for development of dendrology in Lithuania nowadays and prospects of this science in future.

Key words: Lithuania; dendrology; organising; biological-ecological, taxonomic-nomenclature problems, prospects

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### Introduction

There are more than 3200 taxa of woody plants in Lithuania at present. In 1987 in our country were about 1500 taxa of woody plants only (Januškevičius, Budriūnas, 1987). This significant grow of diversity of woody plants in Lithuania were dependent on especially rich increasing of cultivars of ornamental trees and shrubs in last ten years (Skridaila, 2000). Those new cultivars made a lot of private gardens and collections of state institutions – botanical gardens and arboreta rich more. However come to light a lot of old problems of dendrology (in a wider sense) in Lithuania. Conditional it is possible to divide these problems up to tree groups: of organising character, biological-ecological and taxonomic-nomenclature.

### Problems of organising character

One of the most actually problem of this character – not even distribution of sources (diversity of woody plants) in our country (Fig. 1). There are the most rich collection in Lithuania at present in Vilnius university Botanical Garden – more than 2400 taxa of woody plants (Skridaila, 2001). The second collection by numbers of taxa of woody plants in Kaunas Vytautas Magnus university (VMU) Botanical garden contains 1100 taxa (Januškevičius, 1999). Then are collections

of Dubrava arboretum and park Skinderiškis – there are growing woody plants of 900 taxa about. Like those state institutions, 900 taxa of trees and shrubs are growing in private garden by Mr. S. Juknevičius, in garden by Mr. L. Januškevičius (800 taxa). But there are not one big collection of woody plants in North East Lithuania and in region near by Baltic See (in this region, there are the mildest climate conditions of Lithuania). It is not normal from the point of view researches in problems of introduction new plants in Lithuania.

Other collections of trees and shrubs in our country are less. There are growing in 150 collections about woody plants of 100-400 taxa (Baronienė, Januškevičius, 2001). From them it is worth to mark: private garden by Mr. A. Čiapas in Naujoji Akmenė (358 taxa), memorial holding “Obelynė (near by Kaunas) by professor. T. Ivanauskas (300 taxa), private garden in Mažeikiai by Mr. And Mrs. J. Butas (210 taxa) etc. The most collections like them are concentrated in one region of Lithuania– Žemaitija, in other regions there are a few collections only. In thousands holdings, gardens other green plantations common for Lithuania are growing less numbers of woody plants – 10-30 taxa (Baronienė, 1999). It means that there are possible to cultivate much more ornamental woody plants in those gardens.

Poor net of nurseries in soviet time that is reason of differences of distribution of woody plants in Lithua-

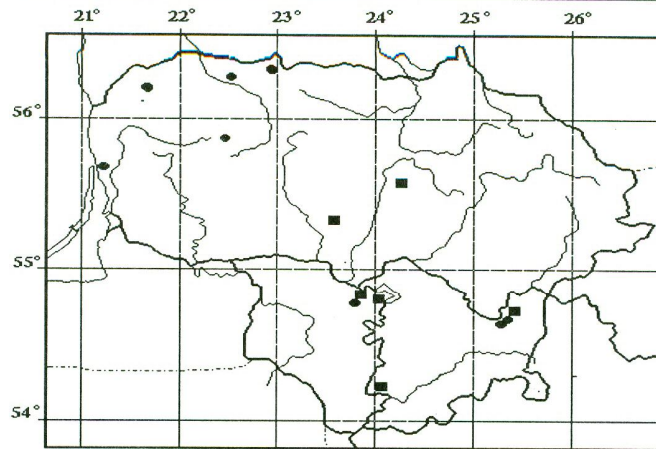


Fig. 1. Distribution of main woody plant collection in Lithuania (■ - collections of 800 – 2400 taxa; ● - collections of 200 – 400 taxa)

nia. In soviet times ornamental woody plants in our country produced state forest nurseries only. The main aim of its were producing seedlings for replanting forests. Growing of ornamental woody plants in these nurseries depends from enthusiasm every chairman. A few best nurseries only, for example in Raudondvaris (near Kaunas) Rokiškis, Plungė and a few more, in that time produced woody plants up to 100 taxa. There were not in soviet time private nurseries in Lithuania. Official it was impossible to introduce new plants into Lithuania from foreign countries, but amateurs introduced some woody plants illegal. That is reason why a few of the most devoted for gardening amateurs only have quite interesting collections. Not one of nursery or gardeners was working in soviet time on selection of ornamental woody plants – nowadays we have not one original Lithuanian cultivar of ornamental woody plants. In the same time our neighbours Polish, Byelorussian, Latvian, Russian created a lot of its.

After borders of Lithuania were open a lot of new woody plants were coming into our country from West. Nowadays in Lithuania are growing easy as well old as new collections of trees and shrubs. It is good for working in selection of new ornamental woody plants, for gardening, but in the same time comes into Lithuania not resistance (for climate conditions) trees and shrubs. Firms introduced a lot of plants, cultivars of *Acer palmatum* Thunb. ex Murr., *Chamaecyparis lawsoniana* (A. Murr.) Parsl. and etc., which are not tested in our country. These plants during hard or contrasting winters in Lithuania are damaged and killed

by frost. Any institutions control this process in Lithuania not. It is question of competence of specialists working for firms of plants introducing in our country still.

The biggest botanical gardens of Lithuania (in Vilnius and Kaunas) produced ornamental woody plants too. Every year those institutions distribute woody plants up to 500 names. Plants from botanical gardens are tested for climate conditions, diseases and pests. It is a pity quantity and diversity of those plants is not enough for market of Lithuania. In future (5-10years later) it is possible new botanical gardens of Klaipėda and Šiauliai universities will carry forward this work, but today they are on organising stage only. There are in Lithuania a few private nurseries but they can not to produce enough of ornamental woody plants for our country too.

### Biological-ecological problems

Problems of this character closely connect with alien, wild woody plants introduced from other geographical regions into Lithuania in XVIII-XIX century. Common they present typical species or varieties from nature of other countries. Today some of them make ecological problems in Lithuania. Some alien trees and shrubs, good adapted for climate and other ecological conditions of our country, are quite aggressive and intervene into natural biotops in different regions of

Lithuania. For example: *Acer negundo* L., (in cities and in some forests), *Acer pseudoplatanus* L. (in forest in districts near See), *Malus toringo* (Sieb.) Sieb. ex de Vriese (in National park of Trakai), *Populus* □ *canescens* (Ait.) Sm., *Robinia pseudoacacia* L. (in manor parks and neighbouring areas), *Spiraea chamaedryfolia* L. (groups of local character in parks) etc. In good ecological conditions quite aggressive behave species *Pinus strobus* L. (if trees are not injured by fungi disease). In forest near Valkininkai (there are area of 0,6 ha of trees *Pinus strobus* L. in age 95 years) seedlings of this species intervene into young culture of *Pinus sylvestris* L. in neighbouring area. Those spontaneous young plants of *Pinus strobus* L., second generation in Lithuania, grows better and over seedlings of our indigenous *Pinus sylvestris* L.

However we have not observed facts of large intervention of alien woody plants into natural biotops of our country, this process have more local character. Usually problem comes in areas injured by anthropogenic factors. Researches in this problem in Lithuania carry out Institute of botany (Gud□inskas, 1997-2000). These researches have more statement character but it is indispensable more comprehensive work. Especially as botanical gardens and arboreta of Lithuania introduced new alien plants further.

Some other biological characteristics of introduced alien woody plants are undesirable too. For example: some trees of genus *Populus* L. during fructification disseminate a lot of downs, trees are huge, and sometimes become danger for people. Until 80-th of XX century in Lithuania were a lot of poplars were planted in streets of cities, in middle Lithuania near roads. Today the most of poplars are removing from green plantations and in future this problem may be in Lithuania not pressing.

### Taxonomic-nomenclature problems

Botanical science of Lithuania and dendrology nearly a half of last century were influenced by scientists of Russia. Limited relationships with other Europe countries that is reason why standard of dendrology science in Lithuania in soviet times was not high, influence of this heritage we have actually still.

1. In soviet period amateurs introduced into Lithuania a lot of woody plant cultivars of not clear origin. These plants more or less were spread in our country and today it is a problem for Lithuanian dendrologists to identify them.

2. Actually we have not in Lithuania standard collections of woody plants, which can serve as assistant means for plants identification. This problem especially urgent identification of new introduced cultivars. Botanical gardens and arboreta of Lithuania still have not rich libraries, there are only a few newest scientific publications of the World, herbariums become old (in urgency mean).

3. Names of plants (in Latin) still are not unified and not settled. The reason is the most botanists of Lithuania treat some genus following example of Russian scientist. The problem to give new name for new cultivars coming from West – nevertheless prompt dendrologists of Lithuania accept the nomenclature following new gardening publications of West Europe countries and USA, especially in respect of genus *Prunus* L., *Acer* L., *Cytisus* L. etc.

4. Until today have not all plants have standard Lithuanian names. The attempt of Lithuanian botanists to solve a problem, they published two dictionaries (Jankevičienė, 1998, Gud□inskas, 1999), not received general recognition by Lithuanian dendrologists.

5. Lithuanian dendrologists have not decide still about using of some terms: “decorative form” or “cultivar” etc. It is possible to see in publications by different authors (Janučkevičius, 1994, 1999; Baronienė, 1999; Navys, 2001 etc.).

### Conclusions

The dendrologists of Lithuania endeavour to solve aforementioned problems. They are working on: collecting new plants with good documentation, creation data base of woody plants of Lithuania, near finish repeated inventory of old manor parks trees. In base of collections of botanical gardens Vilnius and Kaunas universities and Dubrava arboretum carried out (1998-2002) researches in state scientific programme “Genofund”, in 2002-2004 provide inventory of rare and valuable woody plants in cities and small towns of Lithuania etc. In short time will start work on writing new “Dendroflora of Lithuania”. Ten years in our country exist Lithuanian dendrology society which unit amateurs as well professional from state institutions, private nurseries etc. too. The yearbook of society “Dendrologia Lithuaniae” (in publishing is 6 number) present the main problems and achievements of dendrology in Lithuania. In 2001 Parliament of Lithuania promulgate “The law of national plant genetic sources”. The law was created by assistance of scientists working in state “Genofund” programme. It will

take help for plant conservation and investigation in future, including the most valuable woody plants. The last time we feel growing interest in gardening and dendrology in our country. It makes optimistic about development of dendrology in Lithuania

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SOME RARE AND UNCLEAR WILLOW (*SALIX* L.) SPECIES IN LATVIA

Pēteris Evarts-Bunders

Evarts-Bunders P. 2001. Some rare and unclear willow (*Salix* L.) species in Latvia. *Acta Biol. Univ. Daugavp.*, 1 (2): 103 - 105.

Investigations of rare and unclear willow species in Latvia were initiated in 1993. During these investigations the distribution of such rare species as *Salix repens* and *S. myrtilloides* was explained and the spread of several unclear species was precised. The explorations pay great attention to the species which have their border of spreading areal in Latvia. The areal spreading border in Latvia of those species as *S. lapponum* and *S. phylicifolia* which distribution is not even within the whole territory was explained. The research does not comprise all rare and taxonomically unclear species, but only four – *S. lapponum*, *S. repens*, *S. myrtilloides* and *S. phylicifolia*

Key words : willow, *Salix*, areal, Latvia, dendrology

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## Introduction

Willow genus is the largest arboreal group in Latvia. It comprise 19 wild growing or naturalized species and more than 50 hybrids among them. Even though scientists have been interested in willow genus for a long period and many scientific papers on it have been developed in Latvia and in Baltics, it should be admitted that there is a lack of detailed information on the spread of many willow species. It refers to these species whose areal border crosses Latvia – *S. phylicifolia*, *S. repens*, and those species whose spreading is connected to the particular habitat. There are several species ( e.g. *S. lapponum*) which, due to the lack of suitable habitats, are not evenly spread in whole Latvia that is why they are considered to be ordinar species. The existing of one of species described in research paper (*S. repens*) not been proved before in Latvia. That is why it was not included into the list of the Latvian rare and endangered species. Investigations of the distribution of the *S. repens* showed presence of this species in coastal habitats.

## Materials and methods

Investigations on the spreading of willow genus species in Latvia were initiated in 1993. Researches were made during scientific expedition in different locations of Latvia, especially in the regions, which lack herbarium materials and it is not possible to judge equita-

bly about the distribution of the particular species. The collected herbarium materials is preserved in the herbarium of Dendroflora department of the National botanic garden (HBN). At the same time the analysis of all available literature was done, and herbarium material was examined in the largest herbariums (LATV, RIG, HBN, LDM, DPU, TVR).

## Results and discussion

*S. repens* should be considered as one of the most taxonomically unclear willow genus species in Latvia. The works of previous authors (Starcs 1925; Rasiņš 1959) and herbarium materials give rather contradictory data about spreading of species and determining essential morphological features. It was combined with *S. rosmarinifolia* and determined only as one of variety. This species have been divided for the first time in Latvia by A. Rasiņš (Rasiņš 1959). Analysing herbarium collected by A. Rasiņš it was found out, that these materials do not contain a samples of *S. repens* from Latvia. The nearest collection from Kuršu spit, where concentrated the primary habitats of *S. repens* in Lithuania (Smaliukas 1997). While investigation suitable for the growing of species in Liepāja and Ventspils district in the period from 1996 to 1999 it was managed to find the species in western part of the Tosmare lake, at Šķēde, Ziemeupe and in the dune part of Ventspils city (Fig. 1). At the same time several undetermined herbarium samples were found at RIG (University of Latvia, Faculty of Biology). Analysing

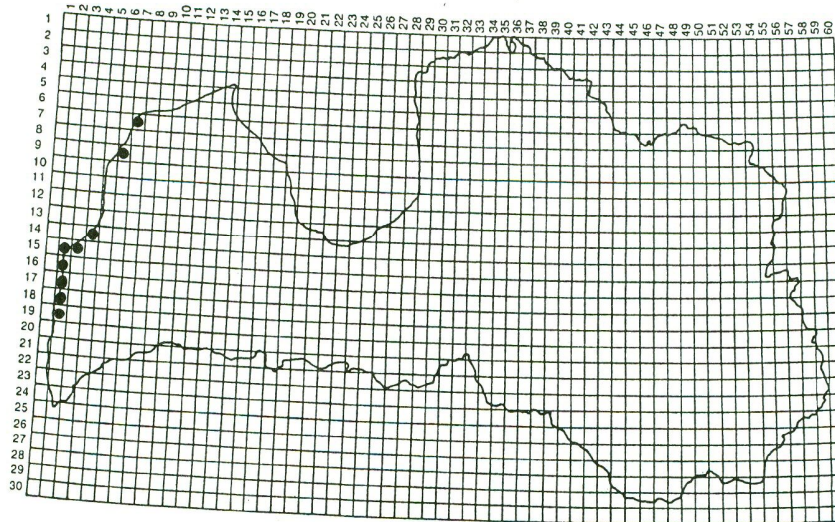


Fig. 1. Distribution of *Salix repens* in Latvia

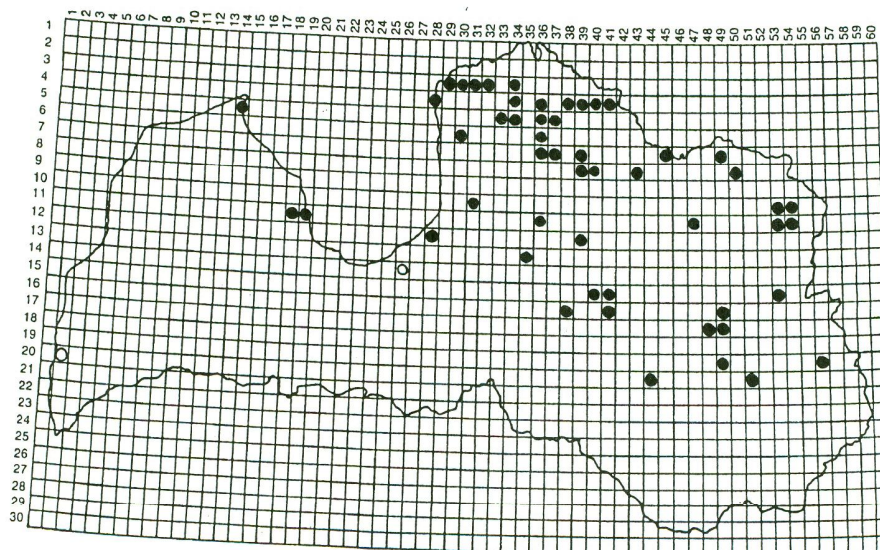


Fig. 2. Distribution of *Salix phylicifolia* in Latvia  
(● - herbarium materials and author's collection data, ○ - literature data)



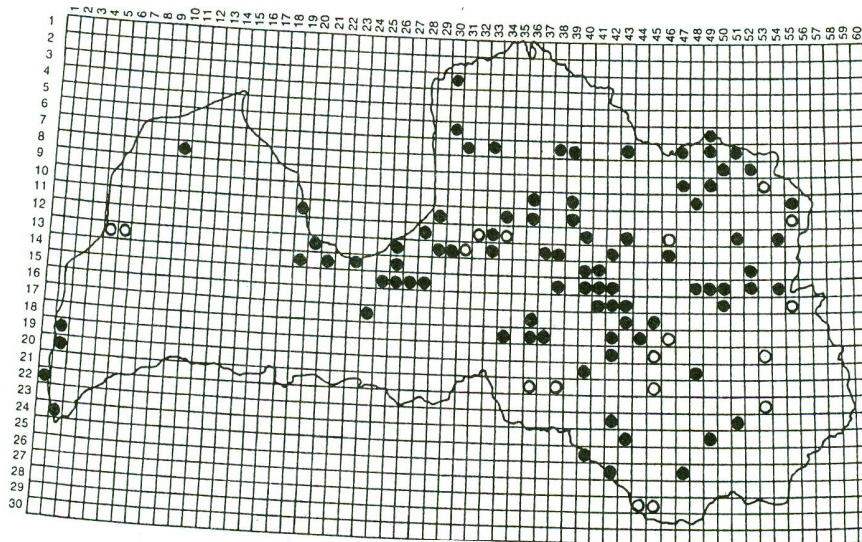


Fig. 3. Distribution of *Salix myrtilloides* in Latvia  
(● - herbarium materials and author's collection data, ○ - literature data)

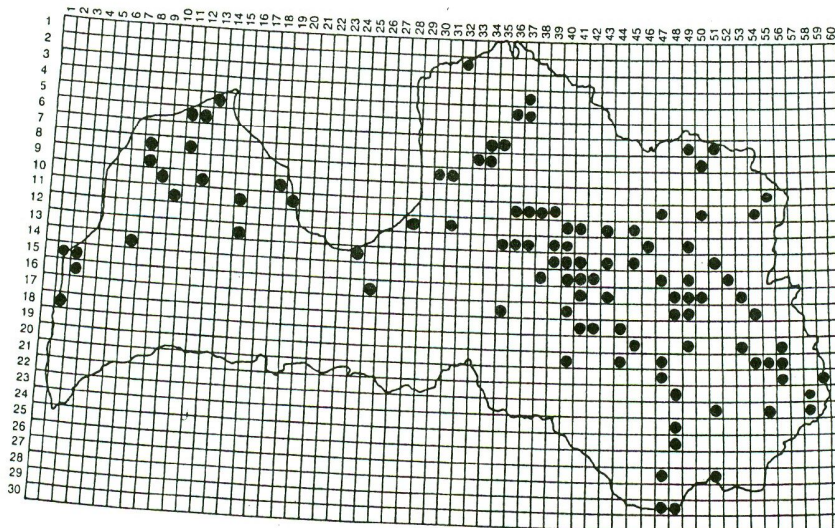


Fig. 4. Distribution of *Salix lapponum* in Latvia

the most essential morphological features in the determination it was defined, that the specimens growing in Latvia are 0,3 – 0,5 m high creeping shrubs with more or less lanceolate leaves – length : 2 – 8 cm, width : appr. 2. cm. One third of upper part of some leaves are denticulated. The most specific feature determining this species is bare capsules. Capsules of similar *S. rosmarinifolia* are pilous. The species areal comprises the north and middle part of western Europe, the eastern border of species areal is located alongside the western coast of Latvia.

The middle part of Latvia is crossed by southern border of *S. phyllicifolia* areal. In Estonia (Krall, Viljasoo 1965) and at the northern part of Vidzeme and Latgale the species are more or less common, but in Lithuania and at the southern part of Latvia this species is not found. The southern extrimity of the continuous areal of the species is located at the southern part of the Lubāna lake in Madona and Rēzekne district (Fig. 2). Further to the south only fragmental habitats of the species are possible. The species widely hybridizate with allied *S. myrsinifolia* - very common *Salix* species in Latvia. This transition forms are found up to the river Daugava or even further. These hybrids caused unclearness and misunderstandings in spreading of *S. phyllicifolia* not only in Latvia, but in all territory of distribution (Meikle 1984). In 1999 the species was found for the first in the northern Kurzeme – Talsu district at Uši. This areal border bespeak about wider distribution of this species in boreal period, when climate was colder. When climate became warmer, the competitiveness of this species decreased and now it is found only in marshy meadows and other marshy habitats, though within the central part of the areal the species grows in completely different habitats, for example, in the northern Estonia in sandy sea dunes.

Latvia is crossed also by the western border of *S. myrtilloides* areal. The border of continuous areal spreads to the line Rīga–Jelgava. In Kurzeme the species is found only at some disjuncted habitats. In the eastern part of Latvia the species is found evenly rare on the whole territory at suitable habitats (Fig. 3). The written materials indicate the largest amount of habitats in central and northern Vidzeme, less in Latgale, possibly it is due to deficient floristic examination. In some districts (Krāslava, Daugavpils) the latest data on this species were found in the work by E. Lehmann (Lehmann 1895). The research resulted also finding the species in the given territories, though only in some habitats (Evarts-Bunders 2001). The species mostly is found at mesotrophic marshes, at the side of Sphagnum – type marshes and in wet and marshy meadows. In this case the existance of many habitats does not

testify the occurrence of species, but that the species is relatively more examined than other willow genus species. The species included into the list of rare and protecting species for a long time, that is why more attention was paid direct to *S. myrtilloides*.

Typical sample of the species which, on the contrary to general opinion, has no even spreading in Latvia is *S. lapponum*. In this case uneven spreading of the species is connected not with the fact, that areal crosses southern part of Latvia, but the lack of suitable habitats in the particular territory (Fig. 4). The species grows in high and mezotrophic marshes, marshy meadows, at the banks of marshy lakes. Although the *S. lapponum* considered to be frequent, the distribution is not even. In agrarian territories – Zemgale plain the species is found only in some habitats. Such situation is spread in several other places not only in Zemgale, but in Latgale and Kurzeme too.

The research does not deal with all *Salix* species, whose distribution is not clear. Additional investigations will be necessary for the such species as *S. acutifolia*, *S. alba*, *S. burjatica*, *S. daphnoides*, *S. fragilis* and *S. starkeana*.

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## AGROBOTANICAL FEATURES AND PRODUCTIVITY OF WILD AND CULTIVATED POPULATIONS OF *ORIGANUM VULGARE*

Ieva Žukauska

Žukauska I. 2001. Agrobotanical features and productivity of wild and cultivated populations of *Origanum vulgare*. *Acta Biol. Univ. Daugavp.*, 1 (2): 107 - 109

The objective of our study was to test different wild types of *Origanum vulgare* genotypes for further experiments and conversation. The experiment was carried out in 25 natural growing sites located in the 7 different districts in Latvia during 1996 -2000. Phenological phases and growth habit were observed. Genotypes observed according to the specific International Plant Genetic Resources Institute (IPGRI) descriptors, data evaluated in points from 1 to 9. Characteristics wild plants were vegetatively propagated and replanted to the MAP collections at the Department of Horticulture LUA. The preliminary experiments suggest that there are possibilities for producing plant material from field cultivation as well. Species originally belonging to the Latvian flora show a good adaptability to more intensive growing conditions in field. Productivity in the plantations is higher than in nature, but more detailed researches needed to develop suitable growing methods.

Key words: *Origanum vulgare*, aromatic medicinal plants, genetic resources, conversation.

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### Introduction

The use of herbal preparations over the millennia in the history of mankind, growing awareness of the role of medicinal plants in drug development and the modern "back to nature" trend, have increase global attention on medicinal and aromatic plants (MAP) research (Baričevič, Zupancic 1999). Herbal medicines represent an important economic factor in the European Union Sales figures in Europe are estimate at US 7 billion per year (Lange 1998). The species have been classified according to their main use in nine groups.

There are *Origanum vulgare* classified as medicinal, spice and ornamental plants (Hammer, Spahillari 1999).

*Origanum vulgare* is a wild plant at the Latvian flora. It is a traditional medicinal and culinary species plant, an excellent plant for attracting bees and attractive landscape ornamental, *Origanum vulgare* is one of these species which is the basis for the development of suitable agrosystems. This is one of the species, which could be as botanical composition plant grown in multiple - component mixtures with the aim to

reconstruct overcropped lands and rise natural plants reproduction (Zukauska 2001). *Origanum vulgare* flowering tops and leaves are used in the food, drink and pharmaceutical industries. *Origanum vulgare* wide usage is one of the reasons why the population of this plant severely depleted. (*Origanum vulgare* are commonly found, but in natural populations plants density and yields are low). Suitable use of *Origanum vulgare* in Latvia can be achieved by promoting the cultivation. In Europe MAP are cultivated on some 70 000 ha (Laghetti et. all. 1993).

The aim of our researches is to find and test suitable types of *Origanum vulgare* wildgrowing plants from different parts of Latvia in order to study agrobotanical properties suitable for cultivation, further selection and conservation.

### Materials and methods

The experiment was carried out in 25 natural growing sites located in the 7 different districts in Latvia during 1996 - 2000. Phenological phases and growth habit were observed. The list of MAP (Medicinal Aromatic

Plants) descriptors was used for plants and sites characteristic. The "Multi - crop passport descriptors" developed by IPGR and FAO 1997, have been used as a basis for description. Quantitative characters that are continuously variable are recorded on 1 - 9 scale: 1 very low, 2 very low to low, 3 low, 4 low to intermediate, 5 intermediate, 6 intermediate to high, 7 high, 8 high to very high, 9 high is the expression areas and distributions are present in table 1. Characteristics wild plants were vegetatively propagated and replanted to the collection. The experimental plots were situated at the experimental collection field of the Department of Horticulture LUA.

The plant materials were harvested, fresh and dry yields were calculated kg m<sup>-2</sup>. The plants were measured and harvested during full flowering period. Each plant was

hand harvest. The seeds from non-cut plants were collected and their biological value was studied after four months. The germination test carried out by the top-paper method using 4 x 50 seeds. In spring seed samples of various sites were sown into pots and kept in plastic house for 30 -35 days. From each course 50 seedlings were transplanted into experimental plots with density 6 plants per m.

The seed samples are often mixed and it is quite difficult to obtain uniform plants populations for specific requirements. *Origanum vulgare* were propagated by stem cutting in a greenhouse. In summer 50 plants of each type were planted in the experimental plots. The plant density was 0,3 x0,4 m. The soil of the plots was clayey P<sub>2</sub>O<sub>3</sub> 225 mg<sup>-1</sup>, K<sub>2</sub>O 150 mg<sup>-1</sup>, N 110 mg<sup>-1</sup> of pH 6,5, soilorgm 3,5 %.

Table 1. Location and plant characterisation of *Origanum vulgare* L. collected from nature in Latvia in 1996 - 2000

Numb.	Location	Year	Habitat	Soilorgm	Soil pH	Plant Characterisation		
						plant high, m	beginning flowering	flowers colour
I	Jēkabpils	1996	5	1	5	0,70	late	Pink
II	Limbaži	1997	6	2	9	0,55	early	Light red
III	Tukums	1997	5	1	7	0,93	very early	Light pink
IV	Madona	1996	6	2	7	0,47	early	Red or violet
V	Talsi	1997	5	1	5	0,65	late	Dark pink
VI	Jelgava	1996	9	2	7	0,50	late	Dark red
VI	Rīga	1997	6	2	7	0,72	intermediate	Pink
VIII	Tukums	1998	10	1	5	0,60	intermediate-late	Light pink
IX	Rīga	1997	5	1	3	0,84	early	Mixed colours
X	Tukums	2000		1	5	0,32	intermediate	Dark violet

Table 2. Productivity of wild *Origanum vulgare* L. collected from nature in Latvia 1996 - 2000

Number	Location	Growing site	Year	Collection area total m <sup>2</sup>	Fresh yield g m <sup>-2</sup>
I	Jēkabpils	dry meadow	1996	350	250
II	Limbaži	middle humidity meadow	1997	175	678
III	Tukums	dry meadow	1997	280	823
IV	Madona	middle humidity meadow	1996	185	915
VI	Rīga	middle humidity meadow	1997	87	329
V	Talsi	dry meadow	1997	420	376
VI	Jelgava	field border	1996	115	95
VIII	Tukums	dry hill	1998	268	415
IX	Rīga	dry meadow	1997	93	82
X	Tukums	field border	2000	62	117

*Agrobotanical features and productivity of wild and cultivated populations of Origanum vulgare*

Table 3. Productivity of cultivated *Origanum vulgare* L. in experimental field in 1997 - 2000

Number	Location	Year	Fresh yield gm <sup>-2</sup>			
			1997	1998	1999	2000
I	Jēkabpils	1996	214	675	1240	2355
II	Limbaži	1997	-	417	506	1080
III	Tukums	1997	-	317	995	2115
IV	Madona	1996	115	1050	2135	2240
V	Talsi	1997	-	217	917	1113
VI	Jelgava	1996	85	815	990	1005
VI	Rīga	1997	-	113	414	516
VIII	Tukums	1998	-	-	125	1228
IX	Rīga	1997	-	414	715	2414
X	Tukums	2000	-	-	-	-

Table 4. Percentage germination of the seeds of *Origanum vulgare* L.

Numb.	Germination %				
	1996	1997	1998	1999	2000
I	43*	78	85	65	73
II	-	57*	75	57	81
III	-	69*	87	64	79
IV	63*	65	72	70	82
V	-	49*	79	63	86
VI	56*	77	78	52	66
VI	-	67*	81	79	67
VIII	-	-	43*	62	58
IX	-	34*	42	52	62
X	-	-	-	-	39*

\* - collected from nature

## Results

*Origanum vulgare* wild types differ in:

- plant high 0,47 - 0,93 m;
- flowers colour - pink, light pink, dark pink, light red, dark red, violet, mixed colours;
- leaf colour tones - yellow green - light green - dark green;
- Beginning of flowering - very early (beginning of the June) to late (middle of the July).

The average fresh herb yield was. The lowest yield - were collected in dry meadow, poor in humus, soil pH

4,1 - 4,5. The highest yield in sunny middle humidity meadow, middle poor to rich in humus, soil pH 6,1 - 6,5. The productivity depending of the growing site.

The productivity of the experimental plots was 4-20 times higher than that of the wild populations (table 2). The fresh yield of *Origanum vulgare* in the first growing year was 9 low, but in the second and third year was significantly higher. The germination of natural seeds varied between 34 - 78% and cultivated plants.

Based on our results of the last four years the *Origanum vulgare* showed it's overwintering was safe the growth was normal and it procured good quality seeds.

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## BIODIVERSITY OF FLORA AND THE POSSIBLE UTILIZATION OF MEADOWS IN TĒRVETE NATURE PARK

Dzigunda Kļaviņa, Aleksandrs Adamovičs, Inga Straupe

Kļaviņa Dz., Adamovičs A., Straupe I. 2001. Biodiversity of flora and the possible utilization of meadows in Tērvete Nature park. *Acta Biol. Univ. Daugavp.*, 1 (2): 110 - 112

The phytocenological investigation of natural meadows has been carried out in Tērvete Nature park in 88.7 ha area. Types of soil, plots of massif, relief and phytocenological character of meadows have been determined. Silārbiešu and Auziņu meadows are typical dry meadows. The hay harvest is the lowest (1.9 t ha<sup>-1</sup>), but it has the highest botanical value of plants. Iļļu, Pļavnieku and Sprīdīšu meadows are medium moisture meadows. The hay harvest is 2.1 to 2.4 t ha<sup>-1</sup>. The meadows are rich in plants, but in Iļļu meadows a very rare plant - *Genticana pneumonanthe* (Linnaeus, 1753) has been found.

Key words: Tērvete Nature park, meadows, botanical composition

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### Introduction

Meadow is a unique natural complex and an object of nature protection. Meadow ecosystems are based on the occurrence of perennial grass communities, which are connected with a whole complex of microorganisms and animal species. Meadows are habitats most abundant in plant species with 40 to 50 flowering plants covering the area of 1 m<sup>2</sup>, and the survival of which is ensured by the human activities – cutting and pasturing. Meadows that are permanently receiving no fertiliser and are not improved are most significant for biological diversity. A fourth out of more than 1650 vascular plants have been found in meadows. About a hundred plant species such as orchid, shingle gladiolus, Siberian iris, gentians and other have been enrolled in Latvian Red Book. During the last decade in Latvia, the area under nature meadows is decreasing due to overgrowing. The establishment of new meadows is a lasting process which requires particular measures of management. The distribution of plant groups is affected by soil moisture conditions. Therefore cutting and grazing treatments are the most simple measures of nature protection in meadows.

Nature parks should become a model in the management of particular territory allowing the survival of distinguished formations, organizing recreation in natu-

ral environment and providing normal conditions for the development of agriculture and forestry. Tērvete Nature park is abundant in protected plant species. Therefore the function of its unique vegetation and the survival of flora genofund in the future must be as important as the recreation function of the park.

### Material and Methods

Tērvete Nature park (total area 1350 hectares, including 88.7 hectares of nature meadows) is the territory protected by the State, and the utilization and admissible activities by man are regulated by particular State law.

Approbated required methods are used for the analysis of meadow vegetation. (Fatare 1992; Pakalne, Znotiņa 1992)

For determination of the botanical composition of a sward, record plots 1m<sup>2</sup> in size were established in each meadow massif in 8 to 12 replications. The total mass of plants, the abundance of plants by Hensen - Druide scale were determined in each record plot.

The hay yield (t ha<sup>-1</sup>) was determined by naturally dried average plant samples. Architectonics and

projective covering of plants (using Ramenskis net) for economically significant species, phenological phase (at the moment of recording), as well as nutritive value of plants were determined. On experimental data basis, phytocenological characteristics with ecological and biological estimation criteria and recommendations for further use were drawn up for each meadow massif.

## Results

Our research showed great botanical diversity of meadows in Tērvete Nature Park (Table 1).

Silalībiešu meadows (total area 21.1 ha, hay yield 1.9 t ha<sup>-1</sup>) are typical dry meadows with plant communities consisting of 35% grasses, 20% legumes, 40% broad-leaved plants, and 5% horsetails. In these meadows there are found six indicator plant species which characterize uncultivated meadows: *Dianthus deltoides* (Linnaeus, 1753), *Filipendula hexapetala* (Linnaeus, 1781), *Trifolium montanum* (Linnaeus, 1753), *Polygala vulgaris* (Linnaeus, 1753), *Galium verum* (Linnaeus, 1753), *Plantago media* (Linnaeus, 1753).

Auziņu meadow (total area 4.6 ha, hay yield 1.9 t ha<sup>-1</sup>) is as well a typical dry meadow with plant communities consisting of 40% grasses, 10% legumes, 50% broad-leaved plants, including 5% cow parsley (*Anthriscus sylvestris* Linnaeus, 1814).

There are found the following indicator plant species: *Dianthus deltoides* (Linnaeus, 1753), *Polygala vulgaris* (Linnaeus, 1753), *Plantago media* (Linnaeus, 1753), *Pimpinella saxifraga* (Linnaeus, 1753). A lot

of medical plants have been found there.

Silalībiešu and Auziņu meadows have been utilized as objects for preserving biological diversity of Tērvete Nature park. The biological composition of a sward suggests that meadows have developed from forest, and are free from fertilization and liming. Silalībiešu and Auziņu meadows haven't been cut for several years. For that reason, these meadows are in need of extensive farming – they should be cut once in two years, preferably in July to favour the spreading of flowering plant seeds, or they should be grazed. For further survival, bushes and trees are recommended to be cut out. These meadows must be preserved as integral part of Tērvete Nature park with their esthetic meaning – “flower meadows” due to low productivity of a meadow.

Iļļu meadows (total area 3.7 ha, hay yield 2.1 t ha<sup>-1</sup>) are medium moisture meadows, where plant communities are formed by 70% grasses, 1% legumes and 29% broad-leaved plants. The botanical composition of a sward suggests that these are improved meadows with sown grasses. For that reason, there are not found indicator plant species except *Plantago media* (Linnaeus, 1753). There are not found any legumes in the meadow. There is observed regular grassland management – cutting and improvement with mineral fertilizers. In the future, cutting treatment is preferable, and for the improvement of grassland agronomic value the sowing of legumes would be recommended.

Sprīdiņu meadows (total area 7.7 ha, hay yield 2.4 t ha<sup>-1</sup>) are here and there improved with legumes. The botanical compositions of grasses suggest that meadows are of natural origin. In the grassland, there are identified indicator plants characteristic to unimproved meadows: *Filipendula hexapetala* (Linnaeus, 1781), *Galium verum* (Linnaeus, 1753),

Table 1. Botanical composition of meadows in Tērvete Nature park, %

Herbaceous plants	Meadow massifs					
	Silalībieši	Auziņas	Iļļi	Pļavnieki	Sprīdiši	Floodlands of the Tērvete river
Grasses	35	40	70	50	50	50
Legumes	20	10	1	25	10	-
Broad-leaved plants	40	50	29	25	40	30
Horsetails	5	-	-	-	-	-
Sedges	-	-	-	-	-	20

*Plantago media* (Linnaeus, 1753), *Geranium palustre* (Linnaeus, 1756), *Pimpinella saxifraga* (Linnaeus, 1753). The botanical composition of a sward is as follows: 50% grasses, 10% legumes and 40% broad-leaved plants. Here and there, the grassland has overgrown with deciduous trees. Sprīdīšu meadows are medium moisture and moderately fertile grasslands. These meadows occur in the territory of castle ruin. This territory is relating to the history of civilization. For that reason, in grassland management should be considered the historical traditions: flowering meadows up to solstice, meadows for haymaking, etc., preserving landscape and esthetic value. These grasslands are extensively utilized.

Plavnieku meadows (total area 39 ha, hay yield 2.2 t ha<sup>-1</sup>) are medium moist and partly improved, and of high value. The indicator plants which are characteristic to unimproved grasslands occur in the remote corners of the meadows. There are *Dianthus deltooides* (Linnaeus, 1753), *Trifolium montanum* (Linnaeus, 1753), *Polygala vulgaris* (Linnaeus, 1753), *Galium verum* (Linnaeus, 1753). The botanical composition of a sward is the following: 50% grasses, 25% legumes including 20% alfalfa, and 25% broad-leaved plants. The central flat part of the relief has been partly improved by alfalfa. Plavnieku grassland has been regularly managed – pasture grazing is the recommended utilization of meadows because of difficulties in cutting due to rough relief.

Alluvial meadows of the Tērvete river (total area 2.6 ha, hay yield 2.9 t ha<sup>-1</sup>) are fertile flooding grassland, that periodically overflow and seasonally enrich with organic and inorganic substances which are brought by high water. There occur particular microrelief with characteristic hygrophilous vegetation. Alluvial meadows are the habitat for a lot of Bern convention plant species and animals. Only *Plantago media* (Linnaeus, 1753), an indicator plant for unproved meadows, was identified there. Plant clusters are composed of 50% grasses 30% broad-leaved plants including 15% *Anthriscus sylvestris* (Linnaeus, 1814), 5% *Heracleum sibiricum* (Linnaeus, 1753), 20% Sedges including 15% *Scirpus sylvaticus* (Linnaeus, 1753). Which is a common plant for the habitat of this kind. Alluvial meadows of the Tērvete river are utilized for cutting the grass during dry summers.

Owing to the occurrence of sedges, the cutting of grass should be done in late May and early June when the nutritive value of sedges is higher. Under rainy conditions, the cutting of grass is possible in July. In this case, the grass could be used as bedding material due to its low nutritive value. For the preservation of

biological diversity of plants, the height of cutting grass should be at least 10 cm from the ground level in the alluvial meadows, as this cutting height protect many animal species from being injured.

Separate strips of land 1 – 2 m<sup>2</sup> in size should be left uncut. These strips of land should be changed yearly.

## Conclusions

Tērvete Nature park is geographically peculiar object relating to the history of civilization, characterized by great biological diversity, with definite abundance of medical and rare plants. Irregular, extensive utilization of these grasslands is necessary for the preservation of biodiversity.

The function of Nature protection, recreation and biological diversity preservation must be in conformity with the corresponding methods of grassland management.

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## SOIL TOXICITY AND MICROORGANISMS QUALITATIVE AND QUANTITATIVE COMPOSITION CHANGES IN THE SUGAR-BEETS SOWING

Laila Dubova, Dzidra Zariņa

Dubova L., Zariņa Dz. 2001. Soil toxicity and microorganisms qualitative and quantitative composition changes in the sugar-beets sowing. *Acta Biol. Univ. Daugavp.*, 1 (2): 113 - 116.

The aim of this study was to study herbicides pollution effects on soil microflora activity and find out the potential of Toxkit microbiotests as alternative cost-effective methods for the detection and control of herbicides and their decomposition components in agricultural soils, which might be contaminated by such compounds. We detected the total number of bacteria, micromycetas, the first and the second step nitrificators. Intensive agriculture practise the treatment of different pesticides several times during the vegetation period. The chemical compounds can be highly concentrated in the upper soil layers or leaching from the subsoil to groundwater. This is particularly true if treatment is repeated several times during the vegetation period. Pesticides are broken down or degraded at different rates by soil microorganisms, chemical reactions, and sunlight, some pesticides form intermediate substances during the breakdown process may become more toxic than the original compounds. Effects on soil microorganisms strongly depend on the pesticide dosage, but the influence may be varied by its availability in soil. The ecotoxicological risk can be greater for those non-targeted soil and water organisms, which belong to the same systematic group as the target organisms. Herbicides contamination can essentially influence fertility of soil. Such influence is a result of changes in soil biological activity and diversity.

Key words: microorganisms, herbicides, soil toxicity, microbiotes.

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### Introduction

Microorganisms are one of the most sensitive links in food-chain after herbicide application. Therefore, microorganisms can be used as sensitive bioindicators to control herbicides pollution level in agricultural soil and water. It is possible use microorganisms as bioindicators in field conditions as well as in laboratory experiments. Nevertheless, some biological methods including microbiological methods are still underestimated, because not all are standardised methods and are not comparative (Žēčkōāšā 1983; Žšóćēīā 1991; Blaise 1991). In contrast to chemical analyses, which mainly focus on the quantitative and qualitative determinative of defined contaminants in enviromental samples, biological test systems can detect the combined effects of different pollutants, the mobility and the bioavailability of contaminants (Schuber 1985; Hund 1997; Hammel et al. 1998). The fate of herbicides in soil are controlled by chemical, biological and physical forces, but activity and variety

of microorganisms depends on soil's chemical and physical forces. Therofere we compare changes of soil toxicity with changes in variety of microorganisms (Scuber 1985; Foissner 1999).

### Materials and methods

Soil sampling. The samples were taken from the Agrecultural Experimental Station at the Inc. "Lielmezotne", Latvia. The samples were taken from the sod-calcareous leached loam soil of a sugar – beet fields. Cores were taken randomly at depths of 0 – 10 cm and 10 – 20 cm from each variant. The variants of experiment and time of samples collection is presented Table 1.

Microbiological analysis. We have performed quantification of the total number of microorganisms, fungi and nitrificators in soil. The plate cuont method for estimating the total number of microorganisms and

Table 1. Variants of the soil samples and estimation of soil toxicity

Variants	Time of collection	Used herbicides** (l/ha <sup>-1</sup> )	Mikrobiotests					
			Algtoxkit		Protoxkit		Rotoxkit	
			EC %	Tox. Class	EC%	Tox. Class	EC%	Tox. Class
P1	1999 May	Befor herbicides	0*	1	1,5	1	0	1
P2	1999 May	Control (without herbicides)	0*	1	5,5	1	0	1
P3A	1999 October	Control (without herbicides)	0*	1	18,5	1	0	1
P3B	1999 October	Control (without herbicides)	0*	1	54,5	2	0	1
P4A	1999 October	P.T.4	0*	1	6,5	1	0,01	1
P4B	1999 October	R.2 + N.0,2	0*	1	62,5	3	0	1
P5A	1999 October	B.P. OF 1,25	0*	1	68,4	3	0	1
P5B	1999 October	B.P. OF 1,25	0*	1	70,5	3	0,3	1
P10A	2000 July	Control (without herbicides)	0*	1	61,9	3	1,6	1
P10B	2000 July	Control (without herbicides)	0*	1	50,3	2	1,6	1
P8A	2000 July	MCPA 2	0*	1	86,7	3	0	1
P8B	2000 July	MCPA 2	0*	1	37,2	2	0	1
P9A	2000 July	B.P. OF 3	0*	1	66,8	3	4,9	1
P9B	2000 July	B.P. OF 3	0*	1	69,8	3	6,4	1

A – Depth of the ample collecting 0 – 10 cm  
 B – Depth of the sample collecting 10 – 20 cm  
 \* - Algae growth was not inhibited

\*\* - Used Herbicides: P.T – Piramyn Turbo  
 R. – Regio  
 N. – Nortron  
 B.P.OF – Betanal Progress OF

fungi per gram of dry soil was used. For estimating other microorganisms we used the most-probable-number method and the results estimated by McCrady tables. The medium for quantification of the total number of bacteria: Bacto nutrient agar (Difco, USA) and for fungi: Zapec medium. The medium composition for the first and the second step of nitrifiers

contained per litre: I step – 1g (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, 1g K<sub>2</sub>HPO<sub>4</sub>, 2g NaCl, MgSO<sub>4</sub>·7H<sub>2</sub>O, 0,01g FeSO<sub>4</sub>·7H<sub>2</sub>O, 0,3g MgCO<sub>3</sub>; II step 1g NaNO<sub>2</sub>, 1g Na<sub>2</sub>CO<sub>3</sub>, 0,5g K<sub>2</sub>HPO<sub>4</sub>, 0,5g NaCl, 0,3g MgSO<sub>4</sub>·7H<sub>2</sub>O, 0,3g Microbiotests. We used TOXKIT microbiotests, which have been developed, at the Laboratory for Biologica Research in Aquatic Pollution at the University of Gent in Belgium. Five microbiotests were applied to all soils extract:

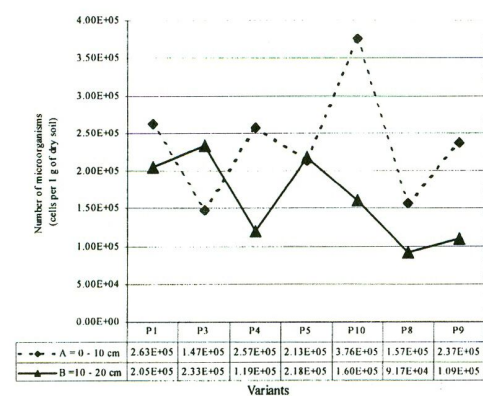


Fig. 1. The total number of microorganisms in soil

1. The Algtoxkit F<sup>TM</sup> with micro-algae *Selenastrum capricornutum* (renamed *Raphidocelis subcapitata*), with determination of the algal growth inhibition after 72h exposure, following the protocol (Persoone 1998a).
2. The Protoxkit F<sup>TM</sup> with protozoa *Tetrahymena thermophila*, with determination of the ciliaten protozoan growth inhibition after 24h exposure, following the protocol (Pauli 1993).
3. The Rotoxkit F<sup>TM</sup> which measure the lethal effect of toxicants on rotifers freshly hatched from cysts (rotifers *Brachionus calyciflorus*), after 24h exposure (Snell et al.1991; Persoone 1998b).

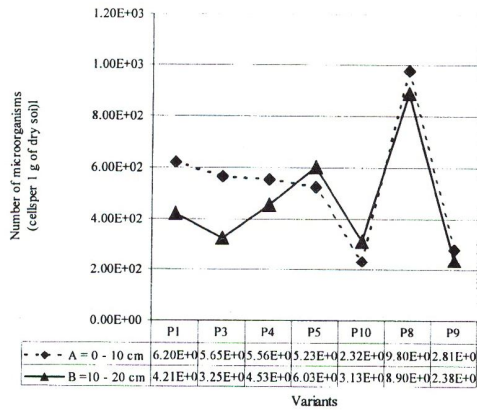


Fig. 2. The number of micromycetes in soil

The results of microbiotesting we represent, according to the regulations of method's authors, in % effects observed in the non-diluted sample (FITA, 2000).

We have used the following hazard classification system for determination of the degree of toxic contamination in soil:

Class I: no acute hazard = none of the tests shows a toxic effect.

Class II: slight acute hazard = a statistically significant EP is reached in at least one test, but the effect level is below 50%.

Class III: acute hazard = the EP50 is reached or exceeded in at least one test, but the effect level is below 100%.

Class IV: high acute hazard = the EP100 is reached in at least one test.

Class V: very high acute hazard = the EP100 is reached in all the tests.

### Results and discussion

Results of soil toxicity are presented in Table 1. Soil toxicity changes in dependence of herbicides form and application sequence during two vegetation periods.

Microbiological analysis showed that application of herbicides changes the activity of microorganisms as well. The changes of microbiological activity was various. The total number of microorganisms (Fig. 1.) and the number of micromycetes (Fig. 2.) in the year 1999 after herbicide application did not change so significantly than in the year 2000. Considerable changes of the number of micromycetes and bacteria compared with control variants P8 in the year 2000, when sugar-beet field was sowed with grain-crops and was used herbicide-MCPA. The results show that microorganism activity and variety depends not only on the natural environmental factors but also on used agrochemicals and pesticides. These variations of soil biological processes also can influences the agroecosystems.

Nitrificators were most of all influenced by herbicides. Number of I and II step nitrificators decreased in variation with herbicide's application (Fig. 5 and 6). Comparison of the total numbers of microorganisms (Fig. 1 and 2) and micromycetes showed that nitrification was the most sensitive among several other different environmental factors and can serve as good indicator of soil biological activity disturbances.

The results of microbiological analyses were confirmed by Algaltokit's algae growth. Our experiments showed that herbicides and their metabolites provided stimulating growth conditions in soil for algae and in some variants also for microorganisms. The results of microbiotests and soil microbial community changes could serve as first indicators of soil functioning trends and soil quality assessment.

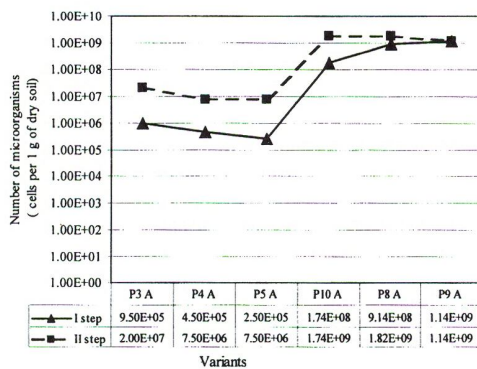


Fig. 3. Number of I and II step nitrificators in soil (sampling in depth 0 - 10 cm)

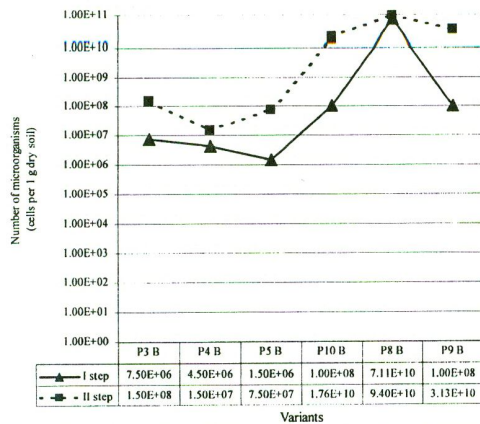


Fig. 4. Number of I and II step nitrifiers in soil (sampling in depth 10 - 20 cm)

### Conclusions

Activity and variety of microorganisms depends on herbicides form and application sequence during two vegetation periods. Nitrifiers and denitrifiers are more sensitive indicators in comparison with the total number of microorganisms and fungi. Application of biotests battery detects test organism sensitivity differences at various trophic levels. The selected test batteries are indispensable for the pesticide pollution assessment.

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**TROPHIC STATE OF THIRTEEN LAKES OF DAUGAVPILS REGION****Sandra Poikane, Vita Licite, Lelde Enģele**

Poikane S., Licite V., Enģele L. 2001. Trophic state of thirteen lakes of Daugavpils region. *Acta Biol. Univ. Daugavp.*, 1 (2): 117- 126.

Thirteen lakes in the Daugavpils region of Latvia were investigated in relation to their current trophic status and historical trends of eutrophication. The lakes varied in morphometrical and hydrochemical parameters, and included both small shallow and large deep lakes, as well as softwater lakes of suffusion origin: Lake Čortoks and Lake Melnezers. Measured parameters included dissolved oxygen and temperature, pH, conductivity, colour, Secchi depth, total phosphorus, and chlorophyll-a. The species composition of phytoplankton, zooplankton, and macrophytes was used for trophic classification of the lakes. Among the lakes examined, some are unique due to their water purity and low trophy (deep, slightly eutrophic lakes): Lake Briģenes, Lake Sventes, Lake Riču, Lake Čortoks and Lake Jazinkas. Four of the lakes were moderately eutrophic, 2 were strongly eutrophic, and 2 were dyseutrophic. Eutrophication has strongly progressed in these lakes during the last decades, indicated by a significant decrease of Secchi depth and hypolimnetic oxygen concentrations, and by changes in the species composition of phytoplankton, zooplankton and invertebrates.

Keywords: Lake; trophic state; eutrophication

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**Introduction**

Standing water are of paramount importance in nature conservation because of their numerous interactions with the surrounding ecosystems, their essential role in the life of many biota, and role as indicators of the general condition of nature. Considerable work has been conducted on the typology (Cimdiņš 1995 a, b) and monitoring (Latvijas... 1997, LVA 2000) of rivers of Latvija. In contrast, standing waters have received a little attention so far. However, these biotopes have been affected at least as much, if not more, by various anthropogenic factors. In order to implement management of standing water in nature development projects, a lake typology which provides information on natural diversity, ecological, and potential for further development would be useful. Such a typology should be based on an integrated ecosystem approach which includes water chemical parameters, composition of biological communities, and land-use.

Development of a lake typology in Latvia was began by Bruno Berzins (Bērziņš 1949), and continued by Kumsāre (Кумса́ре 1995) and Spuris (Спу́рис 1967). The most recent typology was developed in 1976 (Вадзис и др. 1976), but this system is not adequate

for the current conditions. Various international (Carlson 1977, OECD 1982) and national classification (Carlson 1977, OECD 1982) and national classification (Māemets 1974, Ott & Kõiv 1999) schemes are used in Latvia. Due to the natural features of Latvian lakes (shallowness high content of organic matter), the typologies developed in other countries have only limited value in Latvia (Poikane, Līcīte 1999).

Gaugavpils region is one the richest region of Latvia in lakes. There are 194 lakes in Daugavpils District and 273 lakes in Kraslava District, which together cover 2.5% of the total surface area. There is lack of data on the hydrochemical and hydrobiological features of most of these lakes.

The present study is part of a project to investigate the chemistry, phytoplankton, zooplankton and macrophytes of 50 lakes of Latvia.

The aims of project were

- to evaluate the current trophic status of the lakes, based on their chemistry and biology;
- to identify the most important factors for an ecological classification of lakes of Latvia, based on their morphology, chemistry and biology, which

- would be useful for the purposes of water quality monitoring and lake management;
- to determine the main lake types and to describe their chemical and biological features;
- to evaluate trends of lake development where historical data is available.

### Material and methods

Lakes were sampled during winter and summer stratification periods following standard procedures (ISO 5667).

The variables analyzed for the national lake survey were:

- dissolved oxygen and temperature profile (ISO 5814:1990), pH (DIN 38404 Teil 5 (1984)), conductivity (ISO 7888: (1985), colour (ISO 7887: 1985), Secchi depth, total phosphorus (ISO 6878 – 1:1986), chlorophyll-a (ISO 10260:1992), phytoplankton (APHA 10200), zooplankton (APHA 10200), and macrophytes (APHA 10700) during summer stratification;
- dissolved oxygen and temperature profile, total phosphorus, total ammonium (ISO 7150 – 1:1984) and nitrites (ISO 6777:1984) during winter.

Trophic state was evaluated using hypolimnetic oxygen content and Carlson's trophic state index (Carlson 1977), and based on species composition and abundance of phytoplankton, zooplankton and macrophytes.

### Results and discussion

Slightly eutrophic lakes formed a distinct group: Lake Brigenes, Lake Čortoks, Lake Jazinkas, Lake Riču, and Lake Sventes.

Common morphological, hydrochemical features of these lakes were:

- deep or medium deep (mean depth 8-10 m)
- large surface area (100-1000 ha)
- small specific water catchment basin (<10)
- low water renewal coefficient (<25%/year)
- relatively low sensitivity to anthropogenic eutrophication.
- Low concentrations of humic substances, corresponding to ultraoligohumic – oligohumic

- lakes (colour 10 – 30 mg Pt/l);
- low  $P_{tot}$  concentrations both during summer and winter (10 – 30  $\mu\text{g/l}$ ), unlike eutrophic lakes where temporary nutrient decrease is possible in the epilimnion due to sedimentation (Lake Čortoks was an exception due to intense accumulation of nutrients Oxygen in the deepest layers caused by incomplete mixing and unique form in profile of this lake);
- low chl a concentrations (from 2 – 4  $\mu\text{g/l}$ );
- high Secchi depth (more than 3 m).

Based on epilimnetic trophic parameters (summer  $P_{tot}$ , chl a and Secchi depth), some lakes of the slightly eutrophic group can be considered to be mesotrophic (Lake Brigenes, Lake Sventes) or even oligotrophic (Lake Čortoks). Oxygen concentrations and composition of biota in the hypolimnion are parameters which indicate eutrophic conditions.

The phytoplankton of slightly eutrophic lakes was characterized by:

- low biomass (0.2 – 0.5 mg/l)
- dominance of dinophyta or diatomea, small colonial cyanophyta (*Merismopedia tenuissima*, *Mikrosystems renboldii*, *Aphanothece clathrata*, *Snowella kacustris*), and chrysophyta *Dinobryon* sp.
- an abundance of eutrophic indicators (filamentous blue-green algae *Oscillatoria agardhii*, *Oscillatoria* sp., *Lyngbya limnetica*, *Aphanizomenon flos-aqua*, *Anabaena* sp., and green algae *Crucigenia tetrapedia*, and *Tetraedron minimum*) in all of these lakes, despite a low biomass.
- The zooplankton of slightly eutrophic lakes had
- A low density (less than 300 thous/m<sup>3</sup>), with Rotatoria contributing 20 – 50 % of the total number of individuals (average 30%)
- Dominating species *Keratella cochlearis* (in Lake Brigenes and Sventes), *Pompholyx sulcata* (in Lake Jazinkas), *Polyarthra* sp. (in Lake Riču), and *K. quadrata* (77% of the total number of zooplankton individuals Lake Čortoks)
- subdominants *Asplancha major* and *Polyarthra major*; Cladocera species (11 – 22 % of the total number), *Diaphanosoma brachyrum* (the dominant pelagic species in all lakes), *Bosmina obtusirostris*, *Daphnia cucullata*, *D. cristata*, and *Chydorus sphaericus* in some of the large lakes (Sventes, Riču), and *Leptodora kindtii*, *Polyphemus pediculus*, and *Bythotrephes* sp. Nauplii dominated the Copepoda group.
- both Cyclopoida and Calanoida in all of lakes,

### Trophic state of thirteen lakes of Daugavpils region

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| <p>with a predominance of Cyclopoida over Calanoida</p> <ul style="list-style-type: none"> <li>- a low trophy, indicated by the presence of oligotrophic species such as <i>Bosmina obtusirostris</i>, <i>Polyphemus pediculus</i>, and <i>Bythotrephes</i> sp. in the pelagic zooplankton, and by the lack of eutrophic species.</li> </ul> <p>Macrophyte vegetation of slightly eutrophic lakes was characterized by:</p> <ul style="list-style-type: none"> <li>- a relatively low number of species and low abundance</li> <li>- a sparse helophyte belt composed of <i>Phragmites australis</i>, <i>Equisetum fluviatile</i>, and <i>Carex</i> sp.</li> <li>- a fragmentary belt of floating – leaved macrophytes (<i>Potamogeton natans</i>, <i>Nuphar lutea</i>, <i>Polygonum amphibium</i>)</li> <li>- a well – developed submerged macrophyte zone (Charophyta, <i>Potamogeton lucens</i>, <i>P. perfoliatus</i>).</li> </ul> <p>A moderately eutrophic lake group was identified, which include lake Dārza, Lake Dervānišķu, Lake Melnezers and Lake Sasaļu. Melnezers was included in this group, but it differed in having extremely soft water (conductivity 25 µS/cm), a very small area considerable depth.</p> <p>Characteristic features of the moderately eutrophic lakes were:</p> <ul style="list-style-type: none"> <li>- mesohumic by humic content (60 –70 Pt mg/l);</li> <li>- medium high concentrations of nutrients (<math>P_{tot}</math> 25 – 35 µg/l during summer)</li> <li>- medium high chl a concentrations 8 - µg/l;</li> <li>- medium high Secchi depth 1.7 – 2.0 m.</li> </ul> <p>Two groups of moderately eutrophic lakes were distinguished based on qualitative and quantitative features of phytoplankton composition:</p> <ul style="list-style-type: none"> <li>- hard-water lakes – medium high biomass (1.2 mg/l) with considerable dominance of filamentous Cyanophyta (&gt;50% of the total biomass) and subdominance of eutrophic diatoms (<i>Asterionella formosa</i>, <i>Aulacoseira</i> sp., <i>Fragilaria crotonensis</i>, <i>Synedra acus</i>) and dinophyta (<i>Ceratium hirundinella</i>, <i>Peridinium willei</i>);</li> <li>- soft-water lakes (Lake Melnezers) with a low biomass (0.3 mg/l) and very poor and specific species composition dominated by green algae <i>Botryococcus braunii</i> (Chlorophyta, Chlorococcales) and <i>Staurodesmus incus</i> (Chlorophyta, Desmidiaceales), which are indicators</li> </ul> | <p>of oligotrophy, and subdominated by blue-green algae <i>Limnothrix redekei</i> and <i>Mikrosystems reinboldii</i> as well as the dinoflagellate <i>Peridinium willei</i>;</p> <p>Characteristics of the zooplankton of moderately eutrophic lakes were as follows:</p> <ul style="list-style-type: none"> <li>- The Cladocera group contributed 7 – 25% of the total number of individuals, with lakes dominated by different species – <i>Chydorus sphaericus</i> and <i>Bosmina coregoni thersites</i> in Lake Darza, <i>Bosmina obtusirostris</i> and <i>Daphnia cucullata</i> in Lake Dervānišķu, <i>Daphnia cucullata</i> and <i>Bosmina longispina</i> in Lake Sasaļu, and <i>Ceriodaphnia quadrangulara</i> and <i>Bosmina longirostris</i> in Lake Melnezers. Subdominants included <i>Daphnia cucullata</i> and <i>Diaphanosoma brachyurum</i>. Comparing to slightly eutrophic lakes, oligotrophic species were rare and eutrophic species dominating;</li> <li>- Rotatoria, which contributed 25 – 57 % of the total number of individuals, were dominated by <i>Keratella cochlearis</i> or <i>Pompholyx sulcata</i>, with the subdominants <i>Asplancha priodonta</i>, <i>Trichocera cylindrica</i>, <i>Euchlanis dilatata</i>, and <i>Conochilus</i> sp.;</li> <li>- Nauplii dominated the Copepoda group, with Calanoida as subdominants.</li> </ul> <p>The macrophyte vegetation of moderately eutrophic lakes was formed by:</p> <ul style="list-style-type: none"> <li>- a helophyte belt, dominated by <i>Phragmites australis</i>, <i>Scirpus leustris</i>, <i>Equisetum fluviatile</i>, and typical eutrophic species as (<i>Acorus calamus</i>);</li> <li>- a floating-leaved macrophytes belt dominated by <i>Nuphar lutea</i>, <i>Potamogeton natans</i>, <i>Polygonum amphibium</i>, and <i>Nymphae candida</i>;</li> <li>- a submerged macrophyte zone dominated by <i>Potamogeton lucens</i>, and subdominated by <i>Potamogeton perfoliatus</i>, <i>Fontinalis antipyretica</i>, <i>Ceratophyllum demersum</i>, and <i>Elodea canadensis</i>.</li> </ul> <p>The strongly eutrophic lake group includes Lake Abiteļu and Lake Šenheidas. These lakes have been subjected to a major anthropogenic impact and significant eutrophication. Common features were high trophic parameters:</p> <ul style="list-style-type: none"> <li>- high <math>P_{tot}</math> concentrations (62 µg/l in Lake Šenheidas and 77 µg/l in Lake Abiteļu);</li> <li>- high chl a concentrations (42 µg/l in Lake Šenheidas and 55 µg/l in Lake Abiteļu);</li> <li>- a low Secchi depth – 0,6 m;</li> </ul> |
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- oxygen depletion under ice.

Phytoplankton of strongly eutrophic lakes were characterized by:

- a high biomass (Abiteļu 12 mg/l and in Lake Šenheidas 8,8 mg/l);
- a dominance of filamentous blue-green algae (*Aphanizomenon flos-aquae*) or eutrophic diatomea (*Synedra acus*). The latter case is not typical can be explained by a rather low water temperature (17,5°C) and complete mixing, which together create unfavourable conditions for massive blue-green algae blooming.

Features of the zooplankton of strongly eutrophic lakes were:

- a high density (685 thous/m<sup>3</sup> in Lake Šenheidas and 1467 thous/m<sup>3</sup> in Lake Abiteļu)
- a high contribution of Rotatoria (53 – 62 % of individuals), dominated by indicators of eutrophy – *Pompholyx sulcata*, *Filinia longiseta*, *Asplancha priodonta*, *Keratella quadrata*, *Polyarthra euryptera*, and *Trichocerca capucina*;
- in the Cladocera group, the dominants were *Chydorus sphaericus* and *Daphnia cucullata*, and the number of species was very small, with mainly indicators of a eutrophic condition;
- nauplii dominated the Copepoda group (>50% of individuals), Cyclopoida were subdominant, and Calanoida were infrequent.

Macrophytes of strongly eutrophic lakes had:

- a dense but uniform vegetation (species diversity low),
- an abundance of emerged (*Phragmites australis*, *Scirpus leustris*) and floating-leaved plants (*Nuphar lutea*, *Potamogeton natans*, and *Nyphae candida*) covering ~ 15% of the lake surface area;
- a sparse submerged plants belt.

The dyseutrophic lakes had features of both eutrophy (high content of nutrients) and dystrophy (high content of humic substances). These lakes were rather different from each other. Lake Bruņū is polyhumic lake by content of humic substances (colour 135 mg Pt/l), but the biota do not show dystrophic features, possibly due to high to nutrient concentrations which counteract the impact of humic substances. Both abiotic and biotic features in Lake Bruņū indicated high trophic: - hypolimnetic oxygen depletion during summer and anoxic conditions under ice;

- Hypolimnetic oxygen depletion during summer and anoxic conditions under ice;

- high P<sub>tot</sub> (66 µg/l in summer and 81 µg/l in winter), high chl a concentration (55 µg/l) and low Secchi depth (1,1 m);
- accumulation of ammonium (N/NH<sub>4</sub> – 1,47 mg/l) and nitrites in high concentrations under ice;
- high phytoplankton biomass and eutrophic Cyanophyta species;
- high zooplankton density and presence of eutrophic Rotatoria and Cladocera species, as well as strong Cyclopoida predominance over Calanoida;
- dense macrophyte vegetation, with a well-developed emerged and floating-leaved plant belt covering 15-20% of the lake area.

In comparison, in Lake Sila the humic content was lower (80 mg Pt/l), along with lower nutrient levels (54 µg/l in summer and 32 µg/l in winter) and dystrophic features in zooplankton and the macrophyte community. Biota of Lake Sila was characterized by:

- dominance of *Oscillatoria* sp. (The dominance of *Oscillatoria* in this lake may be explained by a low light level rather than by high nutrient availability (Scheffer 1998));
- dominance of eutrophic species of the Cladocera group (*Bosmina coregoni thersites* – 21% of individuals), with some oligotrophic species, and Cyclopoida dominance over Calanoida, an indicator of dyseutrophy;
- poor vegetation with *Phragmites australis* and *Scirpus leustris* in the emergent plant belt, *Nuphar lutea*, *Potamogeton natans* and *Amphibium polygonum* in the floating-leaved plants belt and *Potamogeton lucens*, *Fontinalis antipyretica*, *Myriophyllum* sp. and *Elodea canadensis* in the submerged plants zone.

Permanent anthropogenic activity during the last decades has strongly influenced the natural development of the lakes investigated in our study, and rapid changes in the trophic state have occurred. In Lake Svantes the Secchi depth had decreased from 8.75 m in 1960 (Kumsāre 1960) to 4.9 m, and the oxygen concentration in the bottom layer during summer stagnation from 4 mg/l in 1955 and 3 mg/l in 1986 to 1.5 mg/l (20 m) in 1998. In Lake Jazinkas and Lake Čortoks, the Secchi depth decreased from 4.6 m (Bērziņš 1949) and 12 m (Leinerte, pers.comm.) to 2.7 m 7.15 m in 1998, respectively.

More detailed historical records are available for Lake Svantes. In 1952, Lake Svantes was mesotrophic, indicated by:

- a high Secchi depth (maximum 8.8) (Пер,



- Школьникова 1955);
- the composition of phytoplankton (dominance of chrysophyta *Dinobryon sociale*, and to a lesser extent, of *Dinobryon divergens*, small contribution of Cyanophyta, large contribution of diatoms, including *Tabellaria fenestra* var. *asterionelloides*, an indicator of a low trophic condition, and presence of the green algae *Sphaerocystis Schroeteri*) (Кумсапе 1955);
  - a very low density (15 thous./m<sup>3</sup>) of zooplankton (small contribution of Rotatoria, presence of *Eurytemora lacustris*, oligotrophic indicators among the dominating species – *Bosmina obtusirostris*, *Daphnia cristata*) (Селкере 1955);
  - the composition of benthic invertebrates fauna formed by species characteristic of mesotrophic lakes – *Mikrospectra* ex gr. *praecox*, *Prodiamesa* ex gr. *balthiphylla*, *Sergentia* ex gr. *longiventris*, *Protanypus* sp. (Спурис).

In 1992, the lake showed strong features of eutrophication:

- Secchi depth 3.5 m,
- water-blooming by the blue-green algae *Oscillatoria lacustris*;
- increased abundance of the eutrophic indicator *Chydorus sphaericus* in pelagic zooplankton;
- benthic invertebrates dominated by typical species of eutrophic lakes – *Chironomus plumosus*, *Potamothenis hammoniensis* and *Limnodrilus hoffmeisteri*.

An increase of Secchi depth and change of phyto- and zooplankton communities showed considerable improvement of the trophic condition in 1998 to slightly eutrophic (Table 6).

## Conclusions

Of the investigated thirteen lakes, four were evaluated as slightly eutrophic, four as moderately eutrophic, two as strongly eutrophic, and two as dyseutrophic. Some of the lakes were observed to be particularly valuable in regard to water purity and low trophy, and included the deep, slightly eutrophic lakes Briģenes, Sventes, Riču, and Čortoks and to a lesser extent, Lake Jazinkas.

Investigation of historical records showed that eutrophication has strongly proceeded in the lakes during the last few decades: there was a significant decrease of Secchi depth and hypolimnetic oxygen concentrations, as well as changes in species composition

of phytoplankton, zooplankton and benthic invertebrates.

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## Appendixes

Table 1. Morphometric and hydrological characteristics of lakes studied

Lake	Surface area (f), ha	Catchment area (F)km <sup>2</sup>	Index of catchment area (F/f)	Mean depth, m (m <sup>3</sup> 1000/m)	Volume/shore line (%/year)	Water renewal
Abitelu	97,5	9,7	9,9	2,3	0,51	85
Brigēnes	136,5	7,5	5,5	10	1,4	10,5
Brunu	36,8	16,6	45	3	0,34	286
Cortoks	1,9	1,3	68,4	8		
Darza	51,1	5,5	10,7	4,8	0,4	54
Dervanisku	68,7	7,5	10,9	5,5	0,7	38
Jazinkas	260	17,3	6,7	8,1	1,1	22
Melnezers	2			22*		
Ricu	1286	145	11,2	9,7	4,2	25
Sasalu	27,4	1,97	7,2	4,5	0,4	111
Sventes	735	18,5	2,5	7,8	2,6	6
Senheids	59,8	10	17	2,4	0,41	137
Sila	31,6	47	18	41	0,73	90

\* maximal depth

Trophic state of thirteen lakes of Daugavpils region

Table 2. Main abiotic characteristics of lakes studied

Lake	O <sub>2</sub> conditions in summer, mg/l	O <sub>2</sub> conditions in winter, mg/l	hH	Conductivity, μS/cm	Colour, mg Pt/l	Chlorophyll a, μg/l	P <sub>tot</sub> summer, μg/l	P <sub>tot</sub> winter, μg/l	Secchi depth M
Abitelu	mixing*	anoxic (2 m)***	8,9	284	60	55	77	38	0,55
Brigenes	anoxic**	6,5 (20 m)	8,4	314	15	3,3	66	12	3,2
Brunu	anoxic	anoxic (3 m)	8,4	421	135	55	9	81	1,15
Cortoks	anoxic	anoxic (12 m)	5	16	5	2	27	35	7,1
Darza	anoxic	anoxic (3 m)	8,3	350	55	7,9	20	20	2,05
Dervanisku	anoxic	anoxic (7 m)	8,2	436	70	9,2	26	30	1,75
Jazinkas	mixing	4,7 (20 m)	8,5	269	15	4,3	27	23	2,3
Melnezers	anoxic	anoxic (0,5 m)	7,3	25	55	10	35	360	1,9
Ricu	mixing	-****	8,2	243	30	2,7	17	-	3,9
Sasalu	anoxic	anoxic (3 m)	8,4	384	40	9,5	32	92	1,75
Sventes	1,5 (20m)	3,0 (20 m)	8,7	221	10	2	13	19	4,9
Senheids	mixing	anoxic (1 m)	8,5	393	60	42	62	67	0,6
Sila	Mixing	Anoxic(4 m)	8,1	282	80	24,5	56	32	0,85

\*Lake don't stratify during summer, \*\* complete hypolimnetic oxygen depletion, \*\*\*in brackets – depth where oxygen concentrations less than 2 mg/l, \*\*\*\* - not measured

Table 3. Macrophyte vegetation of lakes studied (relative abundance)

Species	Abiteļu	Brigēnes	Brunu	Čertoks	Dārza	Dervaniškū	Jazinkas	Melnezers	Riču	Sasalu	Sila	Sventes	Senheids
<i>Acorus calamus</i>		2		3				3		3	2	1	
<i>Alisma plantago-aquatica</i> L.			4										
<i>Batrachium circinatum</i> (Sibth.) Spach		3		2			2					1	
<i>Batrachium</i> sp.													4
<i>Calla palustris</i> L.								6					
<i>Carex rostrata</i> Stokes				2							2		
<i>Carex</i> sp.						4	2	4		4		4	
<i>Ceratophyllum demersum</i> L.		2			2		2			2		2	
Charophyta		6	4		4		6		2			4	
<i>Cicuta virosa</i> L.													4
<i>Drapanocladus</i> sp.													2
<i>Eleocharis</i> sp.	2	1		1							2	2	
<i>Elodea canadensis</i> Michx.	2	2						2			1	4	
<i>Equisetum fluviatile</i> L.	2	3		2	2					3	2	4	
<i>Fontinalis antipyretica</i> Hedw.		2	3	4	2			2	4		2		
<i>Glyceria maxima</i> (Hartn.) Holmb.													2
<i>Hydrocharis morsus-ranae</i> L.								4					
<i>Lemna trisulca</i> L.		1											
<i>Menyanthes trifoliata</i> L.						6							
<i>Myriophyllum</i> sp.		1		1								4	
<i>Myriophyllum spicatum</i> L.				4							2		
<i>Myriophyllum verticillatum</i> L.			4										
<i>Naumburgia thyrsiflora</i> (L.) Rehb.				2			2						2
<i>Nuphar lutea</i> (L.) Smith		7		2	7		2	6	2	7	4		
<i>Nuphar pumila</i> (Timm) DC.		1	7		1								
<i>Nymphaea candida</i> C. Presl	2				2					3			7
<i>Nymphaea</i> sp.			2	1		1							
<i>Phragmites australis</i> (Cav.) Trin. Ex Steud.	6	7	2	7	4		6		6	7	6	6	
<i>Polygonum amphibium</i> L.		3	7	3	2		4				2		2
<i>Potamogeton compressus</i> L.											1		
<i>Potamogeton crispus</i> L.				7	7		6		4	7	4	4	7
<i>Potamogeton lucens</i> L.	4	6		3	2		3	6	2	1	4	4	
<i>Potamogeton natans</i> L.		2			2								
<i>Potamogeton pectinatus</i> L.													
<i>Potamogeton perfoliatus</i> L.	2	2		3			4			3		2	
<i>Potamogeton praelongus</i> Wulfen		2	4										7
<i>Ranunculus reptans</i> L.													4
<i>Sagittaria sagittifolia</i> L.	2	4					2			2		1	
<i>Scirpus lacustris</i> L.	2			6	6		2		6	3			
<i>Scirpus tabernaemontani</i> C.C. Gmel.			2										
<i>Sium latifolium</i> L.													4
<i>Sparganium</i> sp.						2	2	2					
<i>Sphagnum</i> sp.													
<i>Stratiotes aloides</i> L.		3											
<i>Typha angustifolia</i> L.		1											
<i>Typha latifolia</i> L.		2	4										
<i>Typha</i> sp.								2					
<i>Utricularia</i> sp.		4											
<i>Utricularia vulgaris</i> L.								4	4				

1 – very rare; 2 – rare; 3 – in places frequently; 4 – frequently; 5 – in places very frequently; 6 – very frequently; 7 - dominance

Table 4. Phytoplankton of lakes studied ( % from the total biomass)

Species	Abiteļu	Enģenes	Brunu	Čertoks	Dārza	Dervaniš-ku	Jazinkas	Meln ezers	Sasaļu	Sila	Sven-tes	Šenheids
<b>Bacillariophyta</b>												
<i>Asterionella formosa</i> Hassal						15,3			1,0			
<i>Aulacoseira</i> sp.	32,9	8,4	0,9		8,4							5,7
<i>Aulacoseira granulata</i> var. <i>angustissima</i> (O. Muller) Sim.		0,8			0,8	3,8						
<i>Aulacoseira islandica</i> (O. Muller) Simonsen									1,4	24,8		
<i>Cyclotella</i> sp.		3,0			3,0	1,8		0,5			1,5	0,8
<i>Flagilaria crotonensis</i> Kitton						1,1		2,0			88,0	
<i>Flagilaria ulna</i> (Nitsch) Lange-Bertalot	0,4					9,0	1,8	8,1	2,1			54,0
<i>Nitzschia acicularis</i> W. Smith			1,3					0,9				
<i>Nitzschia</i> sp.	0,2		0,1								0,1	6,0
<b>Chlorophyta</b>												
<i>Botryococcus braunii</i> Kutzing								31,1				
<i>Chlamidomonas</i> sp.			1,3					0,5				1,4
<i>Coelastrum microporum</i> Nageli	0,6		0,2									
<i>Crucigenia tetrapedia</i> W & G. S. West		0,2			0,2	0,2						
<i>Dictiosphaerium pulchellum</i> Wood								0,9				
<i>Monoraphidium griffithii</i> (Beikley) Komarkova-Lagener.									0,1			
<i>Oocystis</i> sp.		0,1			0,1	0,1						0,1
<i>Pediastrum boryanum</i> (Turpini) Meneghini	2,4											
<i>Pediastrum duplex</i> Meyen			0,3									
<i>Pediastrum tetras</i> (Ehrenberg) Ralfs	0,6		0,1									
<i>Scenedesmus</i> sp.	0,7	0,3	0,1		0,3	0,5			0,1			0,3
<i>Staurastrum</i> sp.						2,6						
<i>Staurodesmus incus</i> (Brebisson) Teiling								29,8				
<i>Tetraedron minimum</i> (A. Braun) Hansgirg		0,3	0,1		0,3	0,1	0,3				0,1	0,4
<b>Chrysophyta</b>												
<i>Dinobryon</i> sp.							4,5				0,2	
<b>Cryptophyta</b>												
<i>Cryptomonas</i> sp.	0,7	5,4	1,1	0,4	5,4	15,5			10,1	0,8	1,8	7,0
<b>Cyanophyta</b>												
<i>Anabaena lemmermannii</i> P. Richter												
<i>Anabaena</i> sp.	19,4		0,6				2,5		8,7			
<i>Aphanizomenon flos-aquae</i> f. <i>gracile</i> Lemmermann	23,7	30,7	0,5		30,7	9,7	2,7			3,6		3,7
<i>Aphanothece clathrata</i> W. et. G. S. West							0,8					
<i>Chroococcus</i> sp.							0,4					
<i>Lyngbya limnetica</i> Lemmermann	1,8	3,0			3,0		3,3		1,5			
<i>M. wesenbergii</i> (Komarek) Starmach	6,7											
<i>Merismopedia tenuissima</i> Lemmermann		0,3			0,4	0,8	0,3				1,8	
<i>Microcystis aeruginosa</i> Kutzing	0,5											0,7
<i>Microcystis reinboldii</i> (Richter) Fortii	1,0	4,1	0,1		4,1		3,8	1,1	0,1		0,1	0,9
<i>Oscillatoria agardhii</i> Gomont	1,0	2,8	6,1		2,8	35,6	7,1	7,7	0,6		3,0	
<i>Oscillatoria redekei</i> van Goor						1,8		7,7	31,6			
<i>Oscillatoria</i> sp.	5,6	1,9	0,5		1,9		50,4		8,0	68,7		19,5
<i>Snowella lacustris</i> (Chodat) Komarek et. Hindak	1,7		0,1									
<i>Synechococcus elongatus</i> Nageli				26,0								
<b>Dinophyta</b>												
<i>Ceratium hirundinella</i> (O. F. Muller) Schrank		23,6			23,5		16,2		11,0			
<i>Peridinium</i> sp.			2,8	65,0			6,2	16,7	12,6		3,1	
<b>Euglenophyta</b>												
<i>Euglena</i> sp.			0,5						98,8			
<i>Euglenates</i>			83,1									
<i>Phacus pleuronectes</i> (O. F. Muller) Dujardin			0,4			1,9						
<i>Phacus pyrum</i> (Ehrenberg) Stein									1,2			
<i>Trachelomonas</i> sp.		15,0	0,1		15,0							
Total biomass, mg/l	12,4	0,4	7,4	0,2	0,8	1,5	0,5	0,3	1,9	5,7	0,3	8,8

Trophic state of thirteen lakes of Daugavpils region

Table 5. Zooplankton of studied ( % from the total number)

Species	Abi- tēju	Brīg- enes	Bru- ņu	Čer- toks	Dār- za	Der- vaniš- ķu	Jazin- kas	Meln- ezers	Riču	Sasa- ju	Sila	Sven- tes	Šen- heids
<i>Rotatoria</i>	62,5	24,9	57,6	82,8	26,5	56,7	26,5	29	50,4	24,8	23,2	17,8	52,9
<i>Anuraeopsis fissa</i>	0,1										0,9		0,3
<i>Gosse</i>													
<i>Asplanchna priodonta</i>	0,2	1,2	8,5		3,1	0,8	1,7	0,4	1,7	0,2	2,6	1,3	1,4
<i>Gosse</i>													
<i>Brachionus angularis</i>	0,6		6,3							0,4			
<i>Gosse</i>													
<i>Brachionus calyciflorus</i>													0,1
<i>Pallas</i>													
<i>Brachionus diversioomis</i>	4,3			0,3			0,3						
<i>Daday</i>													
<i>Conochillus unicomis</i>										0,2			
<i>Rousselet</i>													
<i>Filinia longiseta</i>	2,5				1,1	1,1	0,3						16,0,4
<i>Ehrenberg</i>													6
<i>Kellicotna longispina</i>	0,5	1	4		1,5	1,3	2,5		0,8	0,4		0,4	0,1
<i>Kellicott</i>													
<i>Keratella cochlearis</i>	5,9	8,6	13,6	0,3	7,3	17,5	6,3	15,1	13,6	22	2,6	10,3	0,9
<i>Gosse</i>													
<i>Keratella cochlearis</i>	0,8		0,3			2,3	0,1				0,3		0,6
<i>Gosse</i>													
<i>Keratella quadrata</i>	5,7			76,7	5,7	2		0,4				0,2	0,4
<i>Müller</i>													
<i>Keratella quadrata</i>		0,2	3,5				0,8						
<i>Müller</i>													
<i>Lecane (Monostyla) sp.</i>				0,3								0,2	
<i>Polyarthra euryptera</i>	0,2					1,5	0,3	3,6	7,6	0,2		0,2	1,1
<i>Wierzejski</i>													
<i>Polyarthra major</i>		0,8	0,1				0,2	1,1	1,7			3	
<i>Burckhardt</i>													
<i>Polyarthra sp.</i>	0,6	4,6	3	3,4	1	3,1	2,8		22,5		8,9	2,1	24
<i>Pompholyx sulcata</i>	37,9	7,4	18,1	0,3	5,9	26,4	9,2	0,4	1,3				2,9
<i>Hudson</i>													
<i>Trichocerca capucina</i>	0,1	0,8		0,8		0,1	0,4	0,4		0,4	0,9		3,5
<i>Wierz. et Zach.</i>													
<i>Trichocerca cylindrica</i>	0,1				1	0,3		8,7		1			
<i>Imhof</i>													
<i>Trichocerca sp.</i>	3,1		0,4				0,7		1,3		7,4		0,9
<b>Cladocera</b>	21,4	11,5	9,6	3,7	24,6	13	22,3	12,7	11,4	6,7	29,2	11,8	12,4
<i>Bosmina coregoni</i>		0,6											
<i>coregoni Baird</i>													
<i>Bosmina coregoni</i>										0,4			
<i>gobbera Schoedier</i>													
<i>Bosmina coregoni</i>	1		1		7,3						20,9		
<i>thersites Poppe</i>													
<i>Bosmina longirostris</i>			3,1		0,2	0,5	0,6	0,4		1,4	1,7		
<i>O.F.Müller</i>													
<i>Bosmina longispina</i>									0,4				0,6
<i>O.F.Müller</i>													
<i>Bosmina obtusirostris</i>		2,2			0,2	5,5	6,3		1,3			0,4	
<i>Sars</i>													
<i>Eythotrepes sp.</i>													
<i>Ceriodaphnia pulchella</i>	0,1		0,1		0,6	0,1	0,6			0,2	0,3		0,2
<i>Sars</i>													0,4
<i>Ceriodaphnia</i>	0,1		0,3		0,4	0,1	0,1	8,3					0,7
<i>quadrangula O.F.Müller</i>													0,1
<i>Chydorus sphaericus</i>	17,9	1,2	1,4		9,2	0,9	7,5			0,4	2,3	0,4	10,1
<i>O.F.Müller</i>													
<i>Daphnia cristata Sars</i>		0,8	0,6			1,3	1,9		0,4	0,2			
<i>Daphnia cucullata Sars</i>	2	4,2	2,7		3,2	3,1	1,5		1,7	3,1	3,4	1,3	1,5
<i>Daphnia longispina</i>								3,2			0,6		
<i>O.F.Müller</i>													
<i>Diaphanosoma</i>	0,3	2,4	0,4	3,7	3,6	1,6	3,8	0,8	6,4	1		8,4	
<i>brachyurum Liévin</i>													
<i>Leptodora kindtii Focke</i>									0,8				
<i>Polyphemus pediculus</i>									0,4			0,2	
<i>Sida crystallina</i>		0,2											
<i>O.F.Müller</i>													
<b>Copepoda</b>	16,1	63,6	32,7	13,5	48,9	30,4	51,3	58,3	38,1	68,5	47,6	70,4	34,8
<i>Nauplii</i>	10,4	32,2	24,4	4,5	34,7	14,9	27,9	4,6	24,6	5,2	23,8	46,2	18,4
<i>Cyclopoida I-II</i>	1,5	7	2,4		4,8	4,8	3,2	2,4	2,1	4,9	0,9	2,4	2,5
<i>Cyclopoida III-IV</i>	1	9,9	1		1,9	3,3	4	2	1,7	1,6	4	4,3	2,7
<i>Cyclopoida V-VI</i>	2,1	11,1	4,2	0,3	3,1	6,5	9,1	5,2	8,1	7,3	18,6	5,6	9,4
<i>Calanoida I-II</i>	0,2	1	0,4	0,8	0,6	0,3	2,2	1,6	0,4	1		5,6	0,6
<i>Calanoida III-IV</i>	0,4	1	0,1	1,1	1	0,3	1,5	0,8		1		1,9	0,4
<i>Calanoida V-VI</i>	0,6	1,2	0,1	6,7	2,9	0,2	3,3	0,4	1,3	1	0,3	4,3	0,8
<b>Total (1000 x Eks./l)</b>	<b>1467</b>	<b>201</b>	<b>561</b>	<b>25</b>	<b>398</b>	<b>668</b>	<b>273</b>	<b>207</b>	<b>45</b>	<b>221</b>	<b>272</b>	<b>101</b>	<b>685</b>

Table 6. Changes of Lake Sventes trophic state from 1952 – 1998

	1952	1992	1998
Secchi depth, m	8,8	3,5	4,9
Phytoplankton, dominating species	<i>Dinobryon sociale</i> , <i>D. divergens</i> , <i>Fragilaria crotonensis</i> , <i>Tabellaria fenestrata</i> <i>Anabaena circinalis</i> , <i>Sphaerocystis Schroeteri</i>	<i>Oscillatoria lacustris</i> , <i>Fragilaria crotonensis</i> , <i>Tabellaria fenestrata</i> , <i>Asterionella formosa</i> , <i>Anabaena spiroides</i>	<i>Fragilaria crotonensis</i> , <i>Cyclotella sp.</i> , <i>Oscillatoria agardhii</i> , <i>Merismopedia tenuissima</i> , <i>Tabellaria fenestrata</i> .
Zooplankton, dominating species	<i>Eurytemora lacustris</i> , <i>Bosmina obtusirostris</i>	<i>Chydorus sphaericus</i> , <i>Polyarthra major</i> , <i>Euchlanis dilatata</i> , <i>Kellicotia longispina</i>	<i>Diaphanosoma brachyurum</i> , <i>Polyarthra major</i> , <i>Daphnia cucullata</i> , <i>Bosmina obtusirostris</i> , <i>Chydorus sphaericus</i>
Benthic invertebrates, dominant species	<i>Mikrospectra sp.</i> , <i>Procladius sp.</i> , <i>Sergentia sp.</i> , <i>Protanypus sp.</i>	<i>Chironomus plumosus</i> <i>Ephemera vulgata</i> <i>Limnodrilus hoffmeisteri</i> <i>Potamothenis hammoniensis</i>	
Trophic state	Mesotrophic	Eutrophic	Slightly eutrophic

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