

NEW SPECIES OF CHAROPHYTA *NITELLA TRANSLUCENS* (PERS.) C. AGARDH 1807 IN LATVIA

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Charophyta *Nitella translucens* (Pers.) C. Agardh 1807 was recently recorded in Latvia for the first time. Two sterile individuals were found in Dobeles district, in the Slagūne water pond behind a dam on the Balžņa River. The individuals grew in slowly running water on mud sediment at a depth of 1.2 m.

Key words: Charophyta, Latvia, macrophytes, *Nitella translucens*

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Introduction

Nitella translucens is a fairly common species found throughout Britain and Ireland. Its distribution extends to western and southern Europe from Sweden to Portugal, and eastward to Austria and Italy (Moore 1986). Nevertheless, *Nitella translucens* is rare in the Baltic Sea region. The species has not been found in Finland (Langangen et al. 2002). It has become extinct in Lithuania (Į Lietuvos Raudoną Knygą įrašytų saugomų gyvūnų, augalų ir grybų rūšių sąrašas 2000) and is included in the Red List of Swedish Species (Gärdenfors 2005). *Nitella translucens* is threatened due to eutrophication and dehumidification (Krause 1997).

Nitella translucens grows in ditches, ponds, lakes, running water and pits in marshes and fens (Moore 1986). The habitat of the species is variable, ranging from clear well lit waters with mud

sediments (Moore 1986) to brown waters rich in humic substances in the western part of Europe (Krause 1997).

It is a monoecious species and gametangia develop late in summer or in autumn (Čiėėšėėđ, Źšėėšėėđ 1986).

Materials and Methods

The studied Slagūne water pond is situated in the central part of Latvia in Dobeles District. The pond formed behind a dam on the Balžņa River. The habitat can be described by slowly running water and hypertrophic conditions, mostly overgrown with aquatic vegetation and swampy shores. Macrophyte vegetation was dominated by *Phragmites australis*, *Potamogeton lucens*, *P. natans*, *Scirpus lacustris*, *Chara contaria* and *C. globularis*.

The habitat was investigated on 16 July, 2003 by boat. Submerged macrophytes were collected using a grapnel. Water pH was determined using a Greisinger GPH 014 pH meter, water colour, nitrite, nitrate, and phosphate concentrations with a Hach DR/2000 spectrophotometer (Hach 1992), and calcium and hydrogencarbonate concentrations by titration (APHA, AWWA, WPCF 1989). All water samples were collected in the central part of the pond at a 0.5-m depth. Water transparency was estimated by Secchi depth.

The *Nitella translucens* collection was determined by Irmgard Blindow, and is stored in the Natural History Museum of Latvia.

Results and discussion

Two individuals of *Nitella translucens* were found in the central part of the Slagūne water

pond (Fig.1), the first record of this charophyte species in Latvia. Individuals grew in a slowly running water free of other macrophytes, on mud sediment at a 1.2-meter depth. The shallowest areas of the pond were overgrown with macrophytes. This might not pose a threat as *Nitella translucens* has been described to compete well with other submerged aquatics (Moore 1986), but in Latvia it was found only in the open water area. Other charophytes *C. contraria* and *C. globularis* were distributed in the shallower water at a 0.2 – 1.1-m depth. Plankton was dense in the Slagūne pond. Secchi depth was 1.7 m, water colour 67 Pt/Co. It is possible that light penetration widely fluctuates seasonally due plankton abundance.

Concentrations of HCO_3^- , Ca^{2+} , N-NO_3^- , N-NO_2^- , and P-PO_4^{3-} were 109.8 mg/l, 21.12 mg/l, 1.3 mg/l, 0.006 mg/l, and 0.0076 mg/l, respectively. Water pH was 8.4, which is quite higher than previous

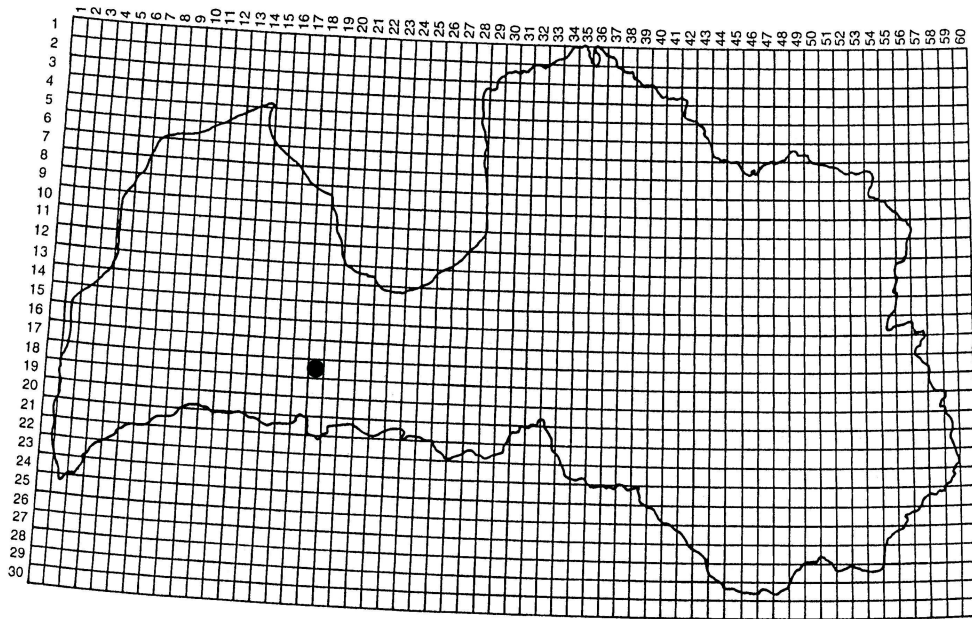


Fig. 1. Distribution of *Nitella translucens* in Latvia

descriptions in its distribution range. Moore (1986) observed *Nitella translucens* in waters with a pH around neutral and Krause (1997) in waters with pH between 5.8 and 7.1. There are other similar habitats to Slagūne pond in Latvia where *Nitella translucens* might be found. Both individuals of *Nitella translucens* were sterile. Gametangia should develop later in summer or in autumn.

Conclusions

Two sterile individuals of *Nitella translucens* (Pers.) C. Agardh 1807, a new species of Charophyta in Latvia, were found in the Slagūne pond, with slowly running water and mud sediment. The description of the Slagūne pond indicated that it is a typical habitat for *Nitella translucens*, excepting a high pH of 8.4, which is higher than that described previously in its distribution range. Further investigation is required to determine the status of species in Latvia. Insufficient information is available on the species distribution and ecology.

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DISTRIBUTION OF EPIPHYTIC BRYOPHYTES IN FIVE LATVIAN NATURAL FOREST STANDS OF SLOPES, SCREES AND RAVINES

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Mežaka A., Znotiņa V., Piterāns A. 2005. Distribution of epiphytic bryophytes in five Latvian natural forest stands of slopes, screes and ravines. *Acta Biol. Univ. Daugavp.*, 5 (2): 101 - 108.

Distribution of epiphytic bryophytes was studied in five specially protected nature territories – Geological and Geomorphological Nature monuments - “Kalamecu un Markūzu gravas”, “Korkuļu saugultne un pazemes upe”, Nature Reserves – “Pilskalnes Siguldiņa”, “Dziļezers un Riebezers” and “Viežu pilskalns” in Gauja National park during 2004 and 2005. Overall, 51 bryophyte species was found including three specially protected species in Latvia (*Dicranum viride*, *Neckera pennata* and *Pterigynandrum filiforme*). Bryophyte flora varied between studied areas and slopes. The number of bryophyte species and indicator species was influenced significantly by tree species, studied area, height on the tree stem and slope exposure. Number of bryophyte species was influenced significantly by slope exposure. Number of bryophyte species and indicator species was higher on northern – eastern, and western slope exposures and on northern and western, but at least on southern and eastern directions of exposures on tree trunks.

Key words: bryophytes, epiphytes, broad – leaved forests, distribution.

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Introduction

Epiphytic bryophyte richness is characteristic in long – term unmanaged forests without human impact (Kuusinen 1996, Znotiņa 2003). Rich epiphytic bryophyte flora can be observed in natural broad – leaved forests (Priedītis 2000, Bambe & Lārmanis 2001, Ek et al. 2001).

Broad – leaved forests occurred fragmentary in Latvia (Dumpe, 1999). River valleys or slopes is one of the habitat, where broad – leaved forests distributed nowadays in Latvia. Nowadays, one of the most diverse natural broad – leaved forests can be found in river valleys or slopes

(Priedītis 1999). Here, the persistence of rare plant and animal species is ensured by the particular microclimate conditions (Znotiņa, 2003).

Rare bryophyte species were used as indicators of natural forests, so called Woodland key habitats (WKH), which are being inventoried in Latvia since year 1997 in Latvia (Ek et al. 2001). However the ecology of these species is less studied.

Air humidity is one of the most important factors effecting the distribution and development of bryophytes (Barkman 1958, Bambe 2002). Therefore, dense epiphytic bryophyte cover can be

observed on deep valleys of rivers as well as at brook edges (Barkman 1958). Especially, hydrophytic epiphyte vegetation is rich in these forests (Barkman 1958).

Forest stand age is an important factor effecting the distribution of bryophytes, especially for rare bryophyte species (Kuusinen 1996, Aude & Poulsen 2000). Also height above the ground, exposition and inclination are important factors. Microclimate varies with them and so does epiphytic vegetation (Barkman 1958).

Epiphytic species composition differs on tree stem relative humidity, light intensity and bark properties are gradually changing (Smith 1982). Epiphytes are distributed more on basal part of tree stem, less – on upper part of tree stem (John & Dale 1995, Franks & Bergstrom 2000). Facultative epiphytes (growing on different type of substratum) generally grow on basal part of tree stem (Smith 1982, Thomas et al. 2001).

By the change of the slope exposure and the distance from the top of slope, also the locality of some bryophyte species on the tree stem changed, indicating the limiting factors for the occurrence of these species (Mitchell et al. 2004).

Thomas et al. (2001) found that all moss species and obligatory epiphytic bryophytes were distributed more on southern and western exposures, but facultatively epiphytic liverworts occurred only at the tree base and mainly on northern and western exposures.

Relative humidity is higher on northern than on the southern side of tree trunk in woods. Temperature fluctuations are largest on the southern side, smallest on the northern side. Maximum temperature on the southern side is just slightly higher than those on other sides, except north. The wettest side is northern – western, receiving much rain and little sunlight. Trees are most frequently wet on the western side, in territories were dominated western winds. The driest is southern – eastern side, what is exposed to the cold dry east winds in winter, when epiphytes are frozen and danger of desiccation is particularly great (Barkman 1958).

The aim of the present study was to find out significance of studied factors influencing the distribution of epiphytic bryophytes in the studied forest stands.



Figure 1. Studied areas. 1 – “Dziļzers un Riebezers”, 2 – “Kalamecu un Markžu gravas”, 3 – “Viešu pilskalns”, 4 – “Korku sausgultne un pazemes upe”, 5 – “Pilskalnes Siguldiņa”.

Methods

Epiphytic bryophytes in natural forests of slopes, screes and ravines were studied in five specially protected nature territories – Geological and Geomorphological Nature monuments – “Kalamecu un Markūzu gravas”, “Korkuļu saugultne un pazemes upē”, Nature Reserves – “Pilskalnes Siguldiņā”, “Dzīlezers un Riebezers” and “Vieču pilskalns” in Gauja National park (Figure 1).

In years 2004 and 2005 – 60 trees were studied in each area. Broad-leaved trees were dominated in all studied territories. Bryophyte species occurrence was estimated separately in two heights (0 – 0,5 m below the ground level and 0,5 – 1,5 m) for each direction of exposure (N, S, E, W) on tree stems. Slope exposures were estimated in each area. The nomenclature for bryophytes follows A. Āboliņa (2001).

Data processing was carried out using SPSS for Windows, Release 11.5. 2002, Multinomial logistic regression.

Results

Overall, 300 trees of 10 species were studied and 51 bryophyte species were found, of them 9, were indicators of natural forests, called woodland key habitats (WKH). Three species (*Dicranum viride*, *Neckera complanta* and *Pterigynandrum filiforme*) are specially protected in Latvia (Latvijas Republikas Ministru Kabinets, 2000).

Bryophyte species composition varied between the studied areas, and also depended on the slope exposure and the tree species. The highest bryophyte species diversity (35 species) was found in “Kalamecu un Markūzu gravas”, but higher number of indicator species (including WKH indicator species and specially protected species (Latvijas Republikas Ministru Kabinets 2000) (8) was observed in “Vieču pilskalns” (Table 1).

The highest number of bryophyte species and bryophyte indicator species was found on *Fraxinus excelsior*, *Acer platanoides* and *Ulmus glabra*, but *Betula pendula* and *Picea abies* hosted lower diversity of bryophyte species (Figure 2). Three bryophyte species only, *Brachythecium rutabulum*, *Hypnum cupressiforme* and *Radula complanata* were distributed on all studied tree species.

The total number of bryophyte species was significantly influenced by tree species, studied area and slope exposure. The number of indicator species was influenced significantly by tree species and area only (Table 2).

Overall, similar bryophyte species composition was observed in all studied slope exposures. However, some species were observed on one slope exposure only. For example, *Dicranum viride*, *Neckera complanata* and *Orthotrichum pallescens* were distributed on northern - eastern slopes, but *Anomodon attenuatus*, *A. longifolius*, *Pterigynandrum filiforme* and *Orthotrichum speciosum* were recorded on southern – eastern slope. The higher bryophyte diversity was observed on northern – eastern (41 species) and on western (39 species) slope exposures, but lower on southern – western (32 species) and southern - eastern (33 species) slope exposures.

Mostly, bryophyte species were found on the lower part of tree trunk and on the northern and western exposures of trees (Table 3). Typical examples of this group were *Eurhynchium hians* and indicator species *Homalia trichomanoides*. Higher on the tree trunk were found *Frullania dilatata*, *Leucodon sciuroides*, *Orthotrichum affine*, *Pseudoleskeella nervosa* and also indicator species *Neckera pennata*. Several bryophyte species, like, *Dicranum montanum* and *Ptilidium pulcherrimum* were similar distributed on lower and higher part on tree trunks.

Table 1. Occurrence of bryophyte species in different areas and slopes (* - indicator species).

Bryophyte species	Studied areas					Slope exposures			
	Dzīļezers un Riebezers	Korkuļu sausgultne un pazemes upe	Pīlskalnes Siguldiņa	Kalamēcu un Markūzu gravas	Viežu pīlskalns	North - East	West	South - West	South - East
<i>Amblystegium serpens</i>	1	1	1	1	1	1	1	1	1
<i>Amblystegium serpens</i> var.	1	1	1	1	1	1	1	1	1
<i>Anomodon attenuatus</i> *	-	-	-	-	1	-	-	-	1
<i>Anomodon longifolius</i> *	-	-	-	-	1	-	-	-	1
<i>Anomodon viticulosus</i> *	-	1	1	1	-	1	1	1	-
<i>Brachythecium oedipodium</i>	-	-	-	-	1	-	-	-	1
<i>Brachythecium populeum</i>	1	1	1	1	1	1	1	1	1
<i>Brachythecium rutabulum</i>	1	1	1	1	1	1	1	1	1
<i>Brachythecium salebrosum</i>	-	-	1	1	-	1	1	1	-
<i>Brachythecium velutinum</i>	-	-	-	1	-	1	1	-	-
<i>Campylium</i> sp.	-	-	-	1	-	1	1	-	-
<i>Cirriphyllum piliferum</i>	1	-	-	-	-	-	1	-	-
<i>Dicranum montanum</i>	1	-	-	1	1	1	1	-	1
<i>Dicranum scoparium</i>	-	-	-	1	1	1	1	-	1
<i>Dicranum viride</i> *	-	-	-	1	-	1	-	-	-
<i>Eurhynchium hians</i>	1	1	1	1	1	1	1	1	1
<i>Eurhynchium praelongum</i>	-	-	-	1	-	-	-	-	-
<i>Eurhynchium pulchellum</i>	1	1	-	-	-	1	1	1	-
<i>Eurhynchium striatum</i>	1	-	1	-	1	1	1	1	1
<i>Frullania dilatata</i>	1	-	-	-	1	-	1	-	1
<i>Homalia trichomanoides</i> *	1	1	1	1	1	1	1	1	1
<i>Homalothecium sericeum</i>	1	1	1	1	1	1	1	1	1
<i>Hypnum cupressiforme</i>	1	1	1	1	1	1	1	1	1
<i>Isoetecium alopecuroides</i> *	1	-	1	1	1	1	1	1	1
<i>Leskea polycarpa</i>	-	-	1	-	-	1	-	1	-
<i>Leucodon sciurioides</i>	1	1	1	1	1	1	1	1	1
<i>Lophocolea heterophylla</i>	-	-	1	1	-	1	1	1	-
<i>Metzgeria furcata</i> *	1	-	-	-	1	-	1	-	1
<i>Mnium stellare</i>	-	-	-	1	1	1	1	-	1
<i>Neckera complanata</i> *	-	-	-	1	-	1	-	-	-
<i>Neckera pennata</i> *	-	1	1	-	1	1	-	1	1
<i>Orthotrichum affine</i>	1	1	1	1	-	1	1	1	-
<i>Orthotrichum pallens</i>	-	1	-	-	-	1	-	1	-
<i>Orthotrichum speciosum</i>	-	-	-	-	1	-	-	-	1
<i>Plagiochila asplenioides</i>	1	-	-	1	-	1	1	-	-
<i>Plagiochila porelloides</i>	-	-	-	1	1	1	1	-	1
<i>Plagiomnium affine</i>	1	1	1	1	1	1	1	1	1
<i>Plagiomnium cuspidatum</i>	-	1	-	1	-	1	1	1	-
<i>Plagiomnium undulatum</i>	1	-	-	-	1	-	1	-	1
<i>Plagiothecium denticulatum</i>	1	-	-	-	-	1	1	1	-
<i>Plagiothecium laetum</i>	1	-	1	1	1	1	1	1	1
<i>Platygyrium repens</i>	1	1	1	1	1	1	1	1	1
<i>Pleurozium schreberi</i>	-	-	1	1	-	1	1	1	-
<i>Pseudoleskeella nervosa</i>	-	1	1	-	1	1	-	1	1
<i>Pterigynandrum filiforme</i> *	-	-	-	-	1	-	-	-	1
<i>Ptilidium pulcherrimum</i>	-	1	-	1	1	1	1	1	1
<i>Pylaisia polyantha</i>	1	1	1	1	1	1	1	1	1
<i>Radula complanata</i>	1	1	1	1	1	1	1	1	1
<i>Sanionia uncinata</i>	1	1	-	-	1	1	1	1	1
<i>Thuidium delicatulum</i>	-	-	1	1	-	1	1	1	-
<i>Ulota crispa</i> *	1	1	1	1	1	1	1	1	1

Discussion

Forest stand age and also microclimate conditions in the studied areas depend on

historical development (geological and geomorphological, soil conditions), climate and also on the previous territory management. “Viežu pīlskalns” is located in Gauja National park and it

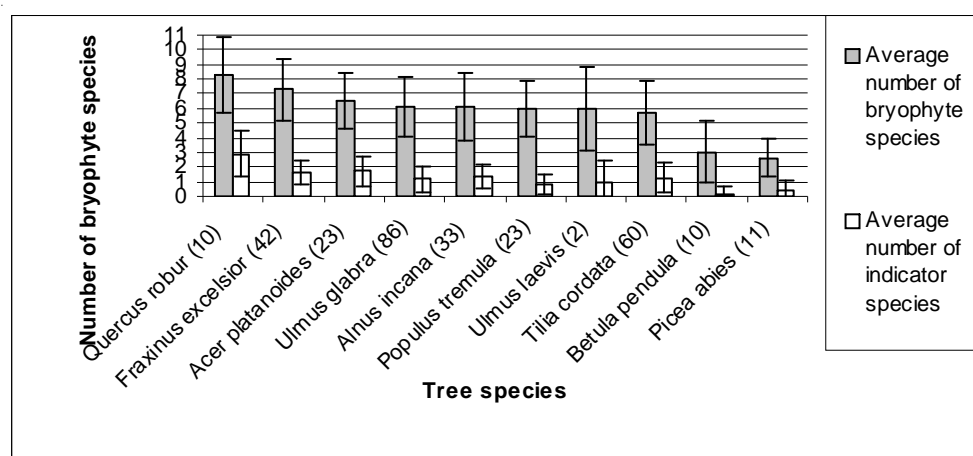


Figure 2. Number of bryophyte species on tree species.

is unaffected by human due to specially protected territory regime in wide scale in this park. This factor, perhaps, ensure the higher presence of bryophyte indicator species in this territory.

Unlike to other studied areas, dolomite outcrops were observed in “Kalamecu un Markūzu gravas”. Dominant tree species in studied plots varied (boreal tree species – *Betula pendula* and *Picea abies* dominated on west slope, but *Populus tremula* and *Ulmus glabra* on north – east slope) in “Kalamacu un Markūzu gravas”. Perhaps these factors together ensure special conditions and were influenced the higher bryophyte species diversity there compared with other areas in the present study.

Betula pendula and *Picea abies* hosted lower number of bryophyte species compared with other tree species (Figure 2). These two species are boreal forest species with different bark properties compare with other studied tree species (Weibull, 2001).

The distribution of indicator species was influenced significantly by tree species and area. Perhaps, there are some other specific factors, which influenced the distribution of indicator species. Therefore, number of bryophyte species was influenced significantly by all studied factors (tree species, studied area and slope exposure) in present study (Table 2). Most of bryophyte species were wide distributed and, probably,

Table 2. Factors effecting bryophyte occurrence ($p < 0.05$). I – number of indicator bryophyte species, A – number of all bryophyte species, - no significant correlations observed.

Factors												
					Areas							
	Kalamecu un Markūzu		Korkulu saugultne		Piiskalnes Siguldiņa		Dzīlezers un Riebezers		Vieðu piiskalns		Overall in areas	
	A	I	A	I	A	I	A	I	A	I	A	I
Tree species	-	-	0.000	0.007	-	-	0.009	-	-	-	0.000	0.009
Studied area (area)	-	-	-	-	-	-	-	-	-	-	0.000	0.008
Slope exposure (slope)	-	-	-	-	-	-	-	-	-	-	0.004	-
Area*slope	-	-	-	-	-	-	-	-	-	-	0.000	-
Area*tree species	-	-	-	-	-	-	-	-	-	-	0.000	-

Table 3. Bryophyte species occurrence in different directions of exposures and height zones on tree stems

Bryophyte species	Direction of exposure				Height above the ground level	
	N	S	E	W	until 0,5 m	0,5 - 1,5 m
<i>Amblystegium serpens</i>	150	140	121	145	365	191
<i>Amblystegium serpens</i> var.	12	11	7	14	31	13
<i>Anomodon attenuatus</i>	31	32	16	39	78	40
<i>Anomodon longifolius</i>	5	3	2	3	8	5
<i>Anomodon viticulosus</i>	22	18	25	22	70	17
<i>Brachythecium populeum</i>	33	45	37	42	100	57
<i>Brachythecium rutabulum</i>	69	52	51	62	210	24
<i>Brachythecium salebrosum</i>	0	0	0	1	1	0
<i>Campylium elodes</i>	1	3	3	2	6	3
<i>Cirriphyllum piliferum</i>	2	1	3	1	7	0
<i>Dicranum montanum</i>	12	12	13	7	19	26
<i>Dicranum scoparium</i>	5	2	0	4	4	7
<i>Dicranum viride</i>	0	0	0	1	0	1
<i>Eurhynchium hians</i>	51	42	54	57	200	4
<i>Eurhynchium praelongum</i>	3	4	3	0	10	0
<i>Eurhynchium pulchellum</i>	4	4	2	5	15	0
<i>Eurhynchium striatum</i>	4	2	6	7	18	1
<i>Frullania dilatata</i>	12	18	13	25	10	58
<i>Homalia trichomanoides</i>	140	103	109	138	420	70
<i>Homalothecium sericeum</i>	4	7	4	2	7	10
<i>Hypnum cupressiforme</i>	222	193	197	208	443	377
<i>Isothecium alopecuroides</i>	16	4	11	11	41	1
<i>Leskea polycarpa</i>	5	5	6	4	11	9
<i>Leucodon sciuroides</i>	36	47	35	31	34	115
<i>Lophocolea heterophylla</i>	5	0	4	5	11	3
<i>Metzgeria furcata</i>	48	40	17	56	86	75
<i>Mnium stellare</i>	4	1	1	3	9	0
<i>Neckera complanata</i>	3	2	3	7	13	2
<i>Neckera pennata</i>	9	2	1	8	6	14
<i>Orthotrichum speciosum</i>	1	1	0	0	0	2
<i>Orthotrichum affine</i>	22	36	18	23	16	83
<i>Orthotrichum pallescens</i>	6	5	9	9	7	22
<i>Plagiochila asplenoides</i>	2	0	2	2	6	0
<i>Plagiochila porelloides</i>	6	2	3	4	12	3
<i>Plagiomnium cuspidatum</i>	2	0	2	2	6	0
<i>Plagiomnium affine</i>	5	10	6	11	30	2
<i>Plagiomnium undulatum</i>	8	1	2	5	16	0
<i>Plagiothecium denticulatum</i>	0	0	1	0	0	1
<i>Plagiothecium laetum</i>	3	4	2	0	8	1
<i>Platygyrium repens</i>	14	11	11	18	14	40
<i>Pleurozium schreberi</i>	2	1	2	1	4	2
<i>Pseudoleskiella nervosa</i>	25	20	16	26	30	57
<i>Pterigynandrum filiforme</i>	5	9	2	8	9	15
<i>Ptilidium pulcherrimum</i>	10	9	6	18	23	20
<i>Pylaisia polyantha</i>	63	56	63	66	103	145
<i>Radula complanata</i>	273	242	231	278	456	568
<i>Sanionia uncinata</i>	6	2	1	2	8	3
<i>Thuidium delicatulum</i>	1	2	2	3	8	0
<i>Ulota crispa</i>	47	34	28	40	23	126

therefore all factors are important for present bryophyte distribution.

Several common bryophyte species – *Hypnum cupressiforme* and *Brachythecium rutabulum* were observed on basal parts of tree in the present study. For these wide distribution in various habitat (soil, grassland, stone) is characteristic (Znotiņa 1968). On the higher part of tree trunk, common bryophyte species was *Radula complanata*. This bryophyte species is widely distributed on trunks of various tree species in Latvia.

Bryophytes were distributed more on lower part of tree stem in the present study. It is agree with John & Dale (1995). The lower zone of the phorophyte trunk ensure the most favourable hydric conditions (Franks & Bergstrom 2000). Epiphytic bryophytes distributed on lower heights on the phorophyte trunk are adapted to low light intensity and have very low resistance to desiccation (Franks & Bergstrom 2000). For example, *Homalia trichomanoides* was distributed mostly on basal part of tree stem,

against to *Frullania dilatata* whose grew mostly on higher part of tree stem in the present study.

Ravine forests and north expositions of slopes and trunks tend to oceanise microclimate, reducing temperature fluctuations. South exposed slopes and south exposures on the trunks tend to continentalise climate, increasing temperature fluctuations (Barkman 1958). In the present study bryophyte diversity were higher on northern – eastern, western slopes and northern, western directions of exposure on tree trunks. Lower number of species was found on southern– western, southern – eastern slopes and southern, eastern directions of exposures on tree trunks. Present study agrees partly with Thomas et al. (2001) research, where bryophyte species were distributed mostly on southern and western exposures due to prevailing southwesterly winds which may increase rain and fog on southern exposure in the region.

Conclusions

Forests of slopes screes and ravines represent rich epiphytic bryophyte flora. The distribution of bryophyte species varied geographically between areas and between slopes of various exposures, indicating various microclimate conditions. Distribution of bryophyte species was influenced significantly by all of studied factors (tree species, slope exposure and territory), but indicator species occurrence was influenced significant by tree species and studied area. The highest bryophyte number was found in sites with the most constant microclimate and relatively high air humidity – in forests on northern – eastern, western slope exposures, on northern or eastern exposures of tree trunks and on basal part of tree trunks.

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LICHENS IN THE UŽAVA NATURE RESERVE

Alfons Piterāns, Brigita Laime, Anna Žeiviniece, Irēna Berga

Piterāns A., Laime B., Žeiviniece A., Berga I. 2005. Lichens in the Užava Nature Reserve. *Acta Biol. Univ. Daugavp.*, 5 (2): 109 - 112.

In the Užava Nature Reserve in the area of grey dunes covered with pebbles 81 lichen species were found, from them 12 are new for Latvia. They are as follows: *Acarospora sinopica*, *Acarospora oligospora*, *Buellia aethalea*, *Diplotomma epipolium*, *Lecanora albescentis*, *Lecanora crenulata*, *Lecidella stigmataea*, *Polysporina simplex*, *Rhizocarpon obscuratum*, *Rhizocarpon umbilicatum*, *Rinodina imersa*, *Rinodina oculata*. All the new species belong to crustose lichens. The other rare species include *Evernia divaricata*, *Fulgensia bracteata* and *Cladonia foliacea*.

Key words: lichen flora, nature reserves, Latvia

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Introduction

The dunes of the coastal part of the Baltic Sea are distinguished by peculiar vegetation. Many rare plant and lichen species grow there. Information about the dune lichens of the Baltic Sea coastal part is available in the publication of A. Piterāns (Piterāns, 1997), where 267 lichen species are mentioned. The study area includes the stretches between Nida and Užava, as well as between Ventspils and Kolka. Rare lichen species were found in this area and include *Cladonia foliacea* that occurs in the vicinity of Pape, Ziemeupe and Saunags. In the surrounding of Akmeņrags and in the Slītere National Park rare lichen species, like *Pycnothelia papillaria* was discovered. In the dune area on trees *Pleurosticta acetabulum* was found. In the Slītere National Park 22 lichen species that are included in the Red Data Book of Latvia were discovered but from the Protected Plant List of the Cabinet of

Ministers 33 species are known (Henīņa, Piterāns 1991, Piterāns 2001, Івчъааааа 1982). Lichen vegetation is greatly influenced by wind that during the storms is often covered by sand. Also the human activities have left an influence on the dune vegetation.

Material and methods

The Užava Nature Reserve is located in the southern part of Ventspils District in the territory of Užava Municipality. The grey dunes at the coast of the Baltic Sea in the territory of the Užava Nature Reserve are formed by rough pebble gravel. Here the influence of the Baltic Sea climate is observed – small temperature fluctuations, higher air humidity. Dunes are less adequate for the growth of vascular plants, although rare plants occur here. The stony dunes are good for the growth of lithophil lichen species that form a

mosaic cover. Lichen studies were carried out in 2002, 2003. and 2004. For the lichen study transects were made starting from the sea coast up to the forest. After every 3 m vascular plants, bryophytes and lichens were determined. The material was collected from the all the study area.

Results

In total 81 lichen species were found in the Užava Nature Reserve that grow mainly on soil and stones. From the discovered lichen species 12 are new for Latvia. In the grey dunes covered with pebble gravel there are smaller and larger stones that overgrow by crustose lichens. On stones the lichens are distributed in a mosaic cover. Here the green-grey thallus change to lighter or darker or orange color thallus. Every stone differs according to the chemical content, therefore different lichen species appear on them. According to the substrata on which all the new lichen species grow they can be divided into 2 groups. One lichen group grows on lime rich pebbles, like *Acarospora oligospora*, *Diplotomma epipolium*, *Lecanora albescens*, *Lecanora crenulata*, *Lecidella stigmataea*, *Rhizocarpon umbilicatum* and *Rinodina imersa*.

The other group occurs on siliceous shingle stones, like *Acarospora sinopica*, *Buellia aethalea*, *Polysporina simplex*, *Rhizocarpon obscuratum* and *Rinodina oculata*.

The discovered lichen species occur in the neighboring countries – Estonia, Sweden Finland where they grow on the sandy coastal part. In Latvia they have found a proper habitat at the coast of the Baltic Sea in the grey dunes covered with pebbles.

In addition, to the new lichen species, also rare species were discovered, such as *Evernia divaricata* that occurs on soil between the pebbles although it is a typical epiphytic species. Similarly, also the rare species, like *Fulgensia bracteata* here has the only known locality in Latvia. Quite rare is also *Diploschistes muscorum* that grows on bryophytes. Also rare lichen species, such as, *Cladonia foliacea* is characteristic for the coastal dunes occurs on soil. Table 1. includes the list of lichen species found in Užava Nature Reserve. The new lichen species for Latvia are marked with asterisk- *.

Table 1. The list of lichens species found in Užava Nature Reserve

Nr.	Lichen species	Bredīķi	Užava
1.	<i>Acarospora fuscata</i> (Schr.) Th. Fr.		+
2.	* <i>Acarospora sinopica</i> (Wahlenb.) Körb.	+	
3.	<i>Acarospora oligospora</i> (Nyl.) Arnold		+
4.	<i>Baeomyces rufus</i> (Huds.) Rebent.		+
5.	* <i>Buellia aethalea</i> (Ach.) Th. Fr.	+	+
6.	<i>Caloplaca citrina</i> (Hoffm.) Th. Fr.	+	
7.	<i>Caloplaca flavovirescens</i> (Wulfen) Dalla Torre & Sarnth.		+
8.	<i>Caloplaca holocarpa</i> (Hoffm.ex Ach.) A. E. Wade	+	+
9.	<i>Caloplaca lactea</i> (A. Massal.) Zahlbr.		+
10.	<i>Candelariella aurella</i> (Hoffm.) Zahlbr.		+
11.	<i>Candelariella vitellina</i> (Hoffm.) Müll. Arg.	+	+
12.	<i>Cetraria aculeata</i> (Schreb.) Fr.	+	+
13.	<i>Cetraria ericetorum</i> Opiz		+
14.	<i>Cetraria muricata</i> (Ach.) Eckfeldt		+
15.	<i>Cladina arbuscula</i> (Wallr.) Hale & W. L. Culb.	+	
16.	<i>Cladina ciliata</i> (Stirt.) Trass	+	+

17.	<i>Cladina mitis</i> (Sandst.) Hustich	+	+
18.	<i>Cladina portentosa</i> (Dufour) Follman	+	+
19.	<i>Cladina rangiferina</i> (L.) Nyl.	+	+
20.	<i>Cladonia cariosa</i> (Ach.) Spreng.	+	
21.	<i>Cladonia chlorophaea</i> (Flörke ex Sommerf.) Spreng.	+	+
22.	<i>Cladonia coccifera</i> (L.) Willd.		+
23.	<i>Cladonia crispata</i> (Ach.) Flot.	+	
24.	<i>Cladonia deformis</i> (L.) Hoffm.		+
25.	<i>Cladonia fimbriata</i> (L.) FR.		+
26.	<i>Cladonia floerkeana</i> (Fr.) Flörke		+
27.	<i>Cladonia foliacea</i> (Huds.) Willd.		+
28.	<i>Cladonia furcata</i> (Huds.) Schrad.	+	+
29.	<i>Cladonia glauca</i> Flörke		+
30.	<i>Cladonia gracilis</i> (L.) Willd.		+
31.	<i>Cladonia phyllophora</i> Hoffm.	+	+
32.	<i>Cladonia polycarpoides</i> Nyl.	+	+
33.	<i>Cladonia pyxidata</i> (L.) Hoffm.	+	+
34.	<i>Cladonia rangiformis</i> Hoffman		+
35.	<i>Cladonia rei</i> Schaer.		+
36.	<i>Cladonia scabriuscula</i> (Delise) Nyl.	+	+
37.	<i>Cladonia subcervicornis</i> (Vain.) Kernst.	+	+
38.	<i>Cladonia subulata</i> (L.) Weber ex F. H. Wigg.		+
39.	<i>Cladonia sulphurina</i> (Michx.) Fr.		+
40.	<i>Cladonia verticillata</i> (Hoffm.) Schaer.		+
41.	<i>Diploschistes muscorum</i> (Scop.) R. Sant. in Hawksw.	+	+
42.	* <i>Diplotomma epipolium</i> (Ach.) Arnold	+	+
43.	<i>Evernia divaricata</i> (L.) Ach.	+	+
44.	<i>Evernia prunastri</i> (L.) Ach.		+
45.	<i>Fulgensia bracteata</i> (Hoffm.) Räsänen	+	+
46.	<i>Hypogymnia farinacea</i> Zopf	+	+
47.	* <i>Lecanora albescens</i> (Hoffm.) Branth et Rostr.		+
48.	<i>Lecanora carpineae</i> (L.) Vain.	+	
49.	* <i>Lecanora crenulata</i> Hook.		+
50.	<i>Lecanora muralis</i> (Schreb.) Rabenh.	+	+
51.	<i>Lecanora rupicola</i> (L.) Zahlbr.		+
52.	* <i>Lecidella stigmatia</i> (Ach.) Hertel et Leuckert	+	
53.	<i>Melanelia exasperatula</i> (Nyl.) Essl.		+
54.	<i>Melanelia olivacea</i> (L.) Essl.		+
55.	<i>Opegrapha rupestris</i> Pers.		+
56.	<i>Peltigera canina</i> (L.) Willd.	+	
57.	<i>Peltigera malacea</i> (Ach.) Funck		+
58.	<i>Peltigera membranacea</i> (Ach.) Nyl.		
59.	<i>Peltigera rufescens</i> (Weiss) Humb.	+	+
60.	<i>Peltigera didactyla</i> (With.) J.R. Laundon	+	+
61.	<i>Physcia tenella</i> (Scop.) DC.	+	
62.	<i>Placynthiella uliginosa</i> (Schrad.) Coppins & P. James		+
63.	<i>Placynthium nigrum</i> (Huds.) Gray		+
64.	* <i>Polysporina simplex</i> (Davies) Vezda	+	

65.	Protoparmelia badia (Hoffm.) Haffellner		+
66.	Pseudevernia furfuracea (L.) Zopf		+
67.	Ramalina sinensis Jatta		+
68.	*Rhizocarpon petraeum (Wulfen) A. Massal.		+
69.	*Rhizocarpon obscuratum (Ach.) A. Massal.	+	+
70.	*Rhizocarpon umbilicatum (Ramond) Flagey		+
71.	*Rinodina immersa (Körb.) Arnold.		+
72.	*Rinodina oculata (Körb.) Sheard		+
73.	Stereocaulon saxatile H. Magn.		+
74.	Tephromela atra (Huds.) Hafellner		+
75.	Thelidium papulare (Fr.) Arnold	+	
76.	Verrucaria maura Wahlenb.	+	+
77.	Verrucaria calciseda DC.		+
78.	Xanthoparmelia somloënsis (Gyeln.) Hale		+
79.	Xanthoria elegans (Link) Th. Fr.		+
80.	Xanthoria parietina (L.) Th. Fr.	+	
81.	Xanthoria polycarpa (Hoffm.) Th. Fr. Ex Rieber	+	

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RE- ESTABLISHMENT OF BIOLOGICAL DIVERSITY AFTER *HERACLEUM* SPP. CONTROL IN RIVERSIDE VAIVE (GAUJA NATIONAL PARK)

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Treikale O., Vanaga I., Priekule I., Gurkina J., Pugacheva J. 2005. Re-establishment of biological diversity after *Heracleum* spp. control in riverside Vaive (Gauja National Park). *Acta Biol. Univ. Daugavp.*, 5 (2): 113 – 125.

In 2003-2004, by the EU funded project, were carried out field experiments with general aiming to develop the strategy to control of *Heracleum* spp. spreading in artificial and in seminatural habitats in Latvia. In the Gauja National Park, where it is not advisable to apply chemical treatment, for current control of *Heracleum* spp. have been tested method of creation of a strong competitive plant community for depression of *Heracleum* spp. by sowing of mesophytic components of grass mixtures which well growth in native bottomland meadows. Changes in natural plant communities were recorded. Significant decrease of the number of susceptible to frequent cutting perennial broadleaved plants was observed. After three cuttings natural biodiversity among annual broadleaved species was also changed: shade-requiring plants were prevailed. Results of assessments clearly demonstrate that native grasses are sufficiently capable of competition to *Heracleum* spp. after frequent cutting. Heavy damage effect of native grass sward (mainly *Elymus repens*, *Poa pratense*) on *Heracleum* spp. was recorded: after three cuttings density of native grasses increased from 48% to 81% of all plants. In the trial sown grasses mixtures (*Dactylis glomerata*+*Festuca rubra*: 50:50%, *F. arundinacea*+*F. rubra*: 35:65%) which were sown by high sowing rate (4000 p. m⁻²) also showed a significant damage effect on density of new-emerged seedlings of *Heracleum* spp. under variable meteorological conditions during the growing season. Frequent cutting of sward negatively influenced the activity of re-growth of invasive species of *Heracleum*. The least re-growth of seedlings of *Heracleum* spp. was recorded after threefold cutting during the hot and dry period of August in 2004. At the same time the components of both grass mixtures well re-grew after each cutting. Analysis of seed bank in riverside Vaive demonstrates the significant differences between *Heracleum* spp. seeds density depending on soil structure and soil layer. In samples from soil layer 0-5 cm the largest amount of viable seeds was found. In samples from floodplain soil significantly lower amount of seeds in comparison with gley-sandy soil were recorded.

Key words: *Heracleum* spp., control, competitor grass mixtures, riverside, biodiversity.

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Introduction

In Latvia three species of *Heracleum* (*H. sibiricum*, *H. mantegazzianum* and *H. sosnowskyi*) are occurred in artificial, semi natural and native habitats.

H. sibiricum as widespread plant in Latvian native flora by G. Fleischer and A. Bunge (1853), I. Klinge (1882) was described. *H. sibiricum* belong to group of plant species with enormous West-European area of distribution (Klinge, 1979), it is frequently found in Nord-Vidzeme geobotanical region (Tabaka et al. 1991), where is recognised National Park Gauja. *H. sibiricum* was recorded by A. Rasiņš (1954) as very polymorphic broadleaved weed distributed in meadows, field sides and roadsides and the species has not significant ecological problem in Latvia. An alien plant *H. mantegazzianum* was introduced to Latvia as garden ornamental in the middle 20th Century (Bērziņš et al. 2003). *H. mantegazzianum* has slow rate of spread in Latvian native flora. About spread of *H. mantegazzianum* in parks and gardens in Latvia was described by A. Pētersone (1957). At present *H. mantegazzianum* is widespread invasive plant in all Europe. More of research has been focused on the elaboration of strategy of current control funded on investigation of its biology and ecology, because the invasive plant has a severe impact on native biodiversity (Tiley and Philp 1994, Pyšek 1995, Ochsmann 1996; Wadsworth et al. 2000, Caffrey 2001, Otte et al. 2004).

H. sosnowskyi was introduced in Latvia for cultivation in agriculture as forage crop in the experimental farms in the 1954 (Rasiņš 1956). The invasive species has now become a widespread problem in Latvia; 12225 ha are infested (Bērziņš et al. 2003). Hazard of the plant intensify of the problem for human health, because the plants, as *H. mantegazzianum*, produces furanocoumarins (Rasiņš et al. 1974, Rasiņš et al. 1984) that induce phytophotodermatitis on contact of sap with human skin (Hipkin 1991).

The Gauja River catchments, located in Nord-Vidzeme, support a rich plant's diversity, in this

area are vegetate over half of the species which are distributed in Latvia (Rasiņš et al. 1979). Vaive River (tributary of Gauja) is one area that is heavy impacted by the invasion of *Heracleum sosnowskyi*. The extremely organic fertile soil with neutral pH in riversides is favourable for rapid growth of these invasive plants. The large plant size, high reproductive output and rapid growth enables it's to dominate in derivate plant communities rapidly that led to the changes of local floral diversity (Rasiņš et al. 1958, Rasiņš et al. 1986, Laiviņš et al. 2003). In nitrophilous floristic communities in riverside Vaive cover of *Heracleum sosnowskyi* reached up to 85% with a resultant loss in number of species up to 7 (Laiviņš, Gavriloova 2003).

In 2003, by the EU funded project, were started field experiments with general aiming to develop the strategy to control alien species of *Heracleum* spreading in artificial and in semi-natural habitats in Latvia. From more field trials with *Heracleum sosnowskyi* during its growing in agriculture as forage crop is known that the species has heightened sensibility to cutting and slight power for regeneration in early period (Rasiņš 1956, Rasiņš et al. 1967, Rasiņš et al. 1975). For reduce of abundance of the invasive plants and management of current control of their further spreading a frequent cutting of the plants above ground during the vegetation season was suggested in riverside's areas, where do not use of chemical treatment in accordance with Law for Plant Protection in Latvia. The strategy based on investigation of method of creation of a strong competitive plant community for depression of re-growth of *Heracleum* spp., damage and remove its seedling's establishment by sowing of mesophytic components of grass mixtures which well growth in native bottomland meadows. The more accession of perennial grasses has shown great diversity in native plant communities and widespread of *Dactylis glomerata*, *Festuca pratensis*, *Festuca rubra*, *Lolium perenne* in wild ecotypes in Latvia (Jansone 2002). Well growing of *Festuca arundinacea*, *Festuca rubra*, *Dactylis glomerata* and *Lolium perenne* under agro ecological conditions of Latvia have been showed

in field trials (Bērziņš at al. 1955, Bērziņš at al. 2002, Antonijs at al. 2002). The data from A. Milbau at al. (2004) suggest that moderately productive, species-rich communities with preferable some planophile species such as *Festuca arundinacea*, *Lolium perenne*, will offer the best defense against invasions with alien species. In riverside Vaive in the Gauja National Park was proposed an experiment to evaluate use of often cutting surface of *Heracleum* spp. for provide effective control of these invasive plants and re-vegetation with perennial grasses *Dactylis glomerata*, *Festuca rubra* and *F. arundinacea*. This paper describes the two-year *Heracleum* spp. control for the Vaive (tributary of Gauja) catchments area and discusses the received results.

Materials and Methods

The experiment was arranged in the Gauja National Park (Priekuli parish) in riverside Vaive with different density of *H. sosnowskyi*.

Characteristic ground flora: trial was arranged on sloping ground overgrown with: *deciduous*: alder, ash, beech, willow, bird-sherry tree, raspberry-yard; *herbaceous*: *Humulus* spp., *Aegopodium podagrarium*, *Taraxacum officinale*, *Tussilago farfara*, *Urtica dioica*, *Cirsium arvense*, *Vicia cracca*, *Trifolium* spp., *Chelidonium majus*, *Galium verum*, *Artemisia vulgaris*, *Mentha* spp., *Glechoma hederacea*, *Linaria vulgaris*, *Viola tricolor*, *Impatiens parviflora*, *Cerastium arvense*, *Lamium amplexicaule*, *Sisymbrium officinale*, *Stellaria media*, *Capsella bursa-pastoris*, *Xanthium strumarium*, *Polygonum convolvulus*, *Myosotis*

spp., *Veronica* spp., *Elytrigia repens*, *Poa* spp., *Carex* spp.

Arrangement of the trial: plot size: 2 m² (2 x 1 m), replicates: 5. Blocks (for 6 plots) was arranged across the slope in accordance with previously observed heterogeneity of site. Replicates were arranged on different soil type, soil reaction, organic matter content and chemical properties (table 1).

After 1st cutting of *Heracleum* spp. (perennial plants) and rake up its grinded residues there were manual sown of the tested grass mixtures in accordance with their components: mixture No. 1: *Dactylis glomerata*, *Festuca rubra* (50:50), mixture No. 2: *Festuca arundinacea*, *Festuca rubra* (35:65) by high sowing rate (4 000 emerged seeds m⁻²). In control plots a grass mixtures were not sown. After sowing all plots were manual harrowed with rake for embedding of seeds to a depth of 1 cm. All plots were covered with crop cover "Capatex" for 1 month for protection from birds.

In all treatments during the vegetation period there were carried threefold cutting of all plants to the ground with hand lawn mower.

The plants density in each plot was recorded by absolute assessment. Individual plants were counted for each species, their height before each cutting was measured. The assessments were carried out on constant places in the center of plot (assessed plot: 0.1 m²).

In 2004 three soil samples were taken from each plot of trial, that was arranged in 2003 on different soil type to examine the seed bank of

Table 1. Description of soil for the trial in riverside Vaive

Replicates	pH	Organic matter content, %	K ₂ O	P ₂ O ₅	Soil structure
I	7.3	1.7	159	89	soddy forest-gley
II	7.6	1.7	90	39	forest gley
III	7.6	1.0	80	40	forest gley
IV	8.0	0.6	42	47	soddy gley-sandy
V	7.8	1.0	63	63	forest gley floodplain

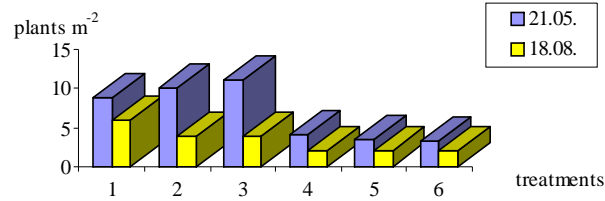


Figure 1. Decrease of density of *Heracleum sosnowskyi* after threefold cutting in 2003.

Heracleum spp. in riverside by slight and heavy invasion with Hogweed. The soil samples were taken on 30 March, 28 July, 11 October, 2004. Each core was taken from 15 x 15 cm places by using a soil hand bore 7cm in diameter to a depth of 15 cm in three replicates for each plot. Each of the replicates was dividend into the following depth section: 0-5, 6-10, 11-15 cm. Cores one month were stored by 8°C. Elutriated seeds were tested for viability by standard method according to International Seed Testing Assotiation Rules (Anon., 1999). Results were expressed as a number of seeds per 1 m².

The weather conditions during the vegetation period of 2003 and 2004 were favourable for growth and development of plants in the trial. The vegetation period in 2003 was delayed on two weeks in comparison with the common term. During the end of April was observed air temperature below-average and a more amount of precipitation compared to common norm. In May

and in the 1st ten-days period of June was marked deficiency of moisture of soil that affected on germination of sown grass in the trial. In June was marked low air temperature in comparison with the common norm.

The vegetation period of 2004 began in the common term: at the first ten-day period of April. During the end of April there were observed high air temperature and insufficient amount of precipitation compared to common norm. During the May-June there were observed lower air temperature in comparison with the common norm, precipitation amount was sufficient for well re-growth of plants after cut. In the trial high air and soil temperature during the 3rd ten-day period of July and the 1st ten-day period of August of 2003 and 2004 were observed.

The data from experiment were subjected to analysis of variance and treatment means were

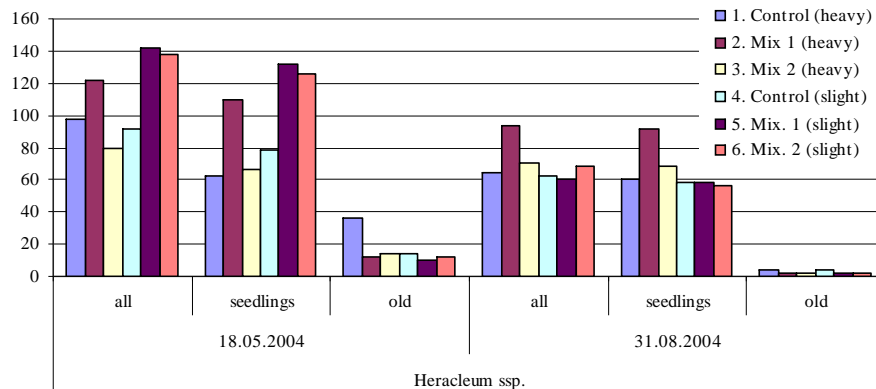


Figure 2. Density of *Heracleum sosnowskyi* after three cuttings in riverside Vaive, 2004

Table 2 *Heracleum* ssp. seeds density in different soil and emergence

Treatments (invasion)	Forest-gley			Floodplain		
	30 March	28 July	11 October	30 March	28 July	11 October
Number of seeds m ⁻²						
1. Control (heavy)	1358,8	1478,3	1913,5	113,2	144,9	144,9
2. Grass mix. 1 (heavy)	1953,3	1521,8	608,7	113,2	202,9	173,9
3. Grass mix 2 (heavy)	1104,0	1130,5	695,7	283,1	202,9	173,9
4. Control (slight)	1868,4	1173,9	1087,0	0,0	57,9	0,0
5. Grass mix 1 (slight)	3991,5	608,7	739,1	226,5	86,9	115,9
6. Grass mix 2 (slight)	1019,2	1478,3	1087,0	169,9	57,9	260,9
Seeds emergence, %						
1. Control (heavy)	81,3	61,5	14,5	100	62,5	0,0
2. Grass mix. 1 (heavy)	82,1	52,9	61,9	100	87,5	16,7
3. Grass mix 2 (heavy)	66,7	45,4	33,3	100	63,0	40,0
4. Control (slight)	92,8	72,2	35,0	-	100	-
5. Grass mix 1 (slight)	72,0	20,8	50,7	100	33,3	100
6. Grass mix 2 (slight)	70,4	38,0	34,1	100	50,0	46,5

separated at the 95% probability level using F-test with program *anova: single factor, correlation and regression* variance analysis.

Results

Studies of changes in density of plants in the experiment in riverside have revealed some tendencies to local succession of species in phytocoenosis by often cutting of small (1-7 p. m⁻²) and heavy (8-17 p. m⁻²) infested with *H. sosnowskyi* places.

The results from the experiment clearly demonstrate that *H. sosnowskyi* had heightened sensibility to often cutting and after 3 cuttings during the first vegetation season can be observed significant decrease of its density per sq. m as in places with heavy invasion (1, 2, 3) as in places with slight invasion (4, 5, 6) (fig. 1). In comparison with the primary number of *Heracleum* ssp. in plots with heavy invasion after 3 cuttings its density per sq. m was decreased to 31.8-63.6%.

In 2004 *Heracleum sosnowskyi* are showed the same heightened sensibility to frequent cutting as in 2003. In comparison with the primary number of *Heracleum sosnowskyi* in plots with heavy invasion density of the species after three cuttings was decreased from 36 to 2, in plots with slight invasion: from 14 to 4 (fig. 2).

After destruction of perennial plants of *H. sosnowskyi* the soil surface beneath the *Heracleum* plants was covered with new-emerged light demanding annual dicots and seedlings of *Heracleum* ssp., which germinated from soil seed-bank or other source of seed propagation. Results of analysis of soil seed bank in 2004 demonstrate the significant differences between *Heracleum* ssp. seeds density depending on soil structure and soil layer. In samples from soil layer 0-5 cm the largest amount of viable seeds was found (table 2). It is important to demonstrate that in samples from floodplain soil significantly lower amount of seeds in comparison with gley-sandy soil were recorded. All seeds from floodplain soil were viable on 30 March. However, in samples from floodplain soil, where were sown grass mixtures, was counted a higher number of seeds of *Heracleum* ssp. in comparison with plots without grass stand.

In control plots after three cuttings during the first vegetative period in 2003 by increasing of density of *H. sosnowskyi* seedlings the total number of *herbaceous* increased up to 25.0-42.5% (table 3). In places by slight invasion of *H. sosnowskyi* after three cuttings *dicotyledons* were superseded by the new-emerged seedlings of *H. sosnowskyi* on the same level as by heavy invasion of the weed. On the figures 3 and 4 are showed the percentage of *H. sosnowskyi* den-

sity regarding to common number of plants per sq. m after 1st cutting (25.06) and after 3 cuttings (18.08) in 2003. There was observed that owing to appearance of new-emerged seedlings of *Heracleum* spp. the density of natural grasses during the first vegetative period was decreased from 52% to 35%, the average number of *dicotyledons* was decreased from 28% to 22% (fig. 3, 4). But in conditions of 2004 three cuttings of all plants gave not significant effect on the number of *Heracleum* ssp. that germinated from seeds in places with heavy invasion of *H. sosnowskyi*. At the same time in these places was observed well re-growth and development of native grass stand (mainly *Elymus repens*, *Poa pratense*) which had damage effect on re-growth of perennial broadleaved plants include *H. sosnowskyi* (fig. 5, 6).

In experimental plots during the two vegetative periods was found tendency to damage of new-emerged seedlings of *Heracleum* ssp. by tested sown grass mixtures and perennial broad-leaved

plants, which were well re-grew after threefold cutting. During the first vegetative period density of sown grass per sq. m was not significantly changed (table 3).

The results from the assessments demonstrate that grass mixture No. 1 (*Dactylis glomerata*, *Festuca rubra* 50:50) by heavy invasion with *H. sosnowskyi* has detrimental effect on new-emerged seedlings of the weed. On figure 7 is showed the density of plants after first cutting in 2003 in plots where grass mixture No. 1 was sown.

There was observed significant damage of seed germination of *H. sosnowskyi* by sown grass mixtures already in first vegetative period. In comparison with control plots there was counted twice less number of *H. sosnowskyi*'s seedlings after third cutting: 19% of all plants (fig. 8). Therefore, there was observed the tendency to suppress of *H. sosnowskyi* on *dicotyledons*: density of the *dicots* was decreased from 92 to 64

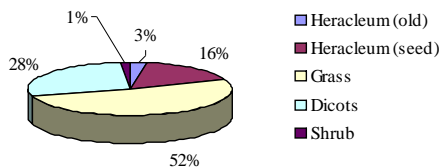


Figure 3. Density of plants in control plots by heavy invasion of *H. sosnowskyi* after 1st cutting (2003)

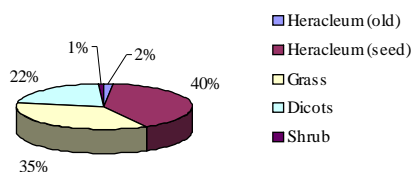


Figure 4. Density of plants in control plots by heavy invasion of *H. sosnowskyi* after 3 cuttings (2003)

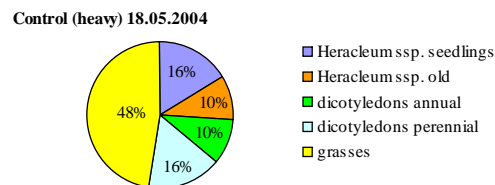


Figure 5. Density of plants in control plots by heavy invasion with *Heracleum* spp.

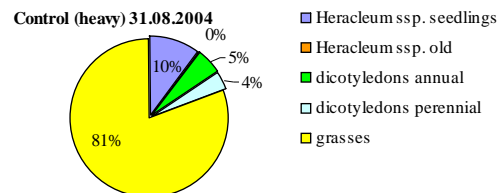


Figure 6. Change in density of plants after 3 cuttings in control plots by heavy invasion with *Heracleum* spp.

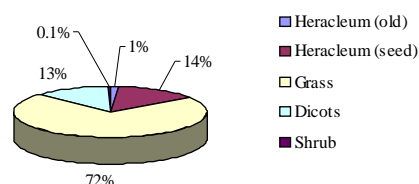


Figure 7. Density of plants after 1st cutting of grass mixture No. 1 by heavy invasion of *H. sosnowskyi*

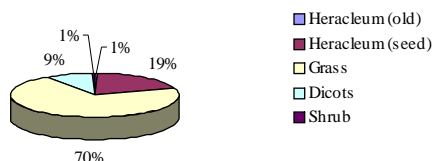


Figure 8. Density of plants after 3 cuttings in plots with grass mixture No. 2 (2003)

plants per sq. m or from 13% to 9% in respect on common number of plants.

Evaluations of sensibility of *Heracleum sosnowskyi* to frequent cutting demonstrate that cutting had negative effect on re-growth of perennial plants *H. sosnowskyi*. Analysis of development of *H. sosnowskyi* in dynamics during the first and second vegetative periods show uneven re-growth of it depending on meteorological conditions and on density of stand of grass in the plots. In all assessments was observed more intensive re-growth of plants after 1st cut-

ting in May-June and low re-growth after following cut (fig. 9).

In 2004 the least re-growth of old plants *H. sosnowskyi* was recorded during the cold period of June-July, after two-fold cutting (fig. 10). There was not observed the damaged effect of three-fold cutting on re-growth of new-emerged *Heracleum* spp. However, after 1st cutting in control plots with heavy invasion of *H. sosnowskyi* was observed more intensive re-growth of new-emerged seedlings during the month owing to creation of favourable conditions of lighting and soil warm up. The least re-growth of seedlings of *Heracleum* spp. was recorded after the third cutting, during the hot and dry period of August (fig. 11).

In experimental plots well re-growth of sown grass mixtures was observed after each cutting (fig. 12).

Results of assessments in riverside Vaive demonstrate that frequent cutting caused the local succession of species in derivate plant communities with *Heracleum sosnowskyi*. Experimental plots were arranged on the places with different *herbaceous* density. In experimental plots were recorded 13 species of perennial and 11 species of annual *dicotyledons*. From annual *dicotyledons* were prevailed: *Stellaria media*, *Impatiens parviflora*; from perennial *dicotyledons* were dominated: *Artemisia vulgaris*, *Urtica dioica*, *Taraxacum officinale*. In places with heavy invasion of *Heracleum sosnowskyi* after threefold cutting common number of broadleaved species was significantly decreased: to 50%.

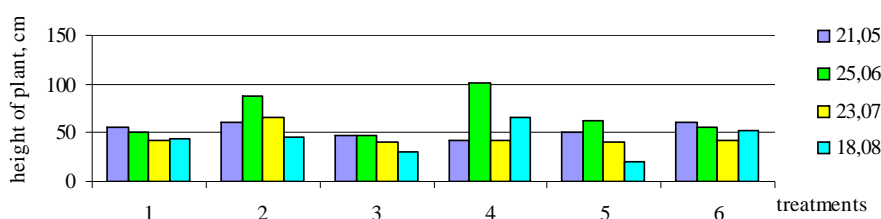
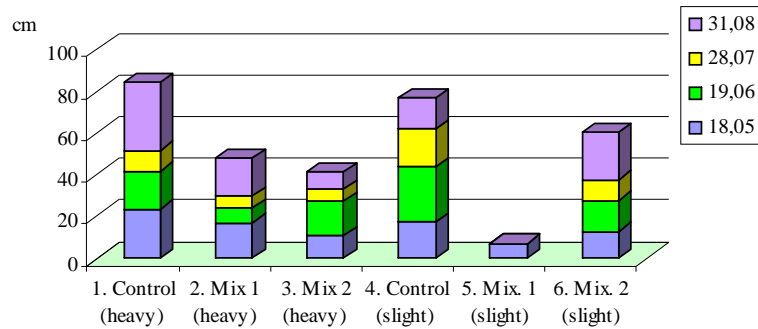
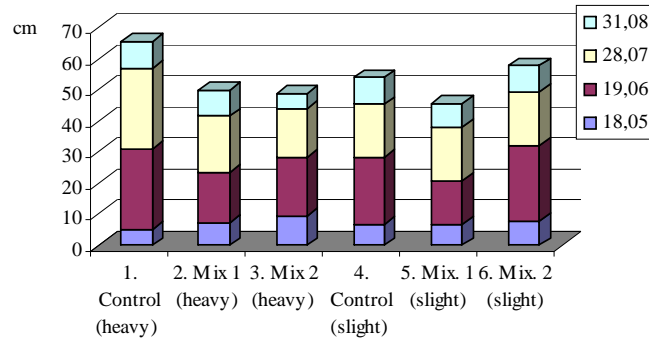


Figure 9. Height of *Heracleum sosnowskyi* (perennial plants) before each cutting (2003)

Table 3. The vegetation in the plots by different density of *Heracleum sosnowskyi* after threefold cutting during the first season (2003)

Treatments	Assess ments	Total plants		<i>H. sosnowskyi</i> (old)		<i>H. sosnowskyi</i> (seedlings)		Grass		Dicotyledons		Shrub	
		p. m ⁻²	% to primary	p. m ⁻²	% to primary	p. m ⁻²	% to primary	p. m ⁻²	% to primary	p. m ⁻²	% to primary	p. m ⁻²	% to primary
1. P. 1 - Control	25.06	312.0	100.0	8.0*	100.0	48.0	100.0	152.0	100.0	84.0	100.0	4.0	100.0
	18.08	390.0	125.0	6.0	75.0	158.0	329.0	138.0	90.8	84.0	100.0	4.0	100.0
2. P. 1 - grass mix 1	25.06	726.0	100.0	10.0*	100.0	102.0	100.0	526.0	100.0	92.0	100.0	2.0	100.0
	18.08	729.0	100.4	4.0	40.0	140.0	137.3	517.0	98.3	64.0	69.6	4.0	200.0
3. P. 1 - grass mix 2	25.06	546.0	100.0	11.0*	100.0	130.0	100.0	386.0	100.0	20.0	100.0	10.0	100.0
	18.08	655.0	120.0	4.0	36.4	218.0	167.7	387.0	100.3	48.0	240.0	2.0	20.0
4. P. 2 - Control	25.06	240.0	100.0	4.2*	100.0	92.0	100.0	52.0	100.0	82.0	100.0	4.0	100.0
	18.08	342.0	142.5	2.0	47.6	138.0	150.0	122.0	234.6	72.0	87.8	6.0	150.0
5. P. 2 – grass mix 1	25.06	910.0	100.0	3.4*	100.0	126.0	100.0	640.0	100.0	92.0	100.0	16.0	100.0
	18.08	963.0	105.8	2.0	58.8	240.0	190.5	653.0	102.0	62.0	67.4	6.0	37.5
6. P. 2 – grass mix 2	25.06	564.0	100.0	3.2*	100.0	134.0	100.0	254.0	100.0	120.0	100.0	12.0	100.0
	18.08	581.0	103.0	2.0	62.5	172.0	128.4	341.0	134.3	60.0	50.0	6.0	50.0
LSD ₀₅	25.06	280.8	-	-	-	131.1	-	272.6	-	64.2	-	19.7	-
	18.08	377.4	-	7.48	-	218.6	-	342.2	-	67.7	-	9.32	-
F _{fact} (F _{crit} = 2.62)	25.06	6.69	-	-	-	0.52	-	5.71	-	2.24	-	0.66	-
	18.08	3.09	-	0.40	-	0.31	-	3.12	-	0.27	-	0.43	-

Figure 10. Re-growth of old *Heracleum sosnowskyi* after cutting in 2004.Figure 11. Re-growth of seedlings of *Heracleum ssp.* after cutting in 2004.

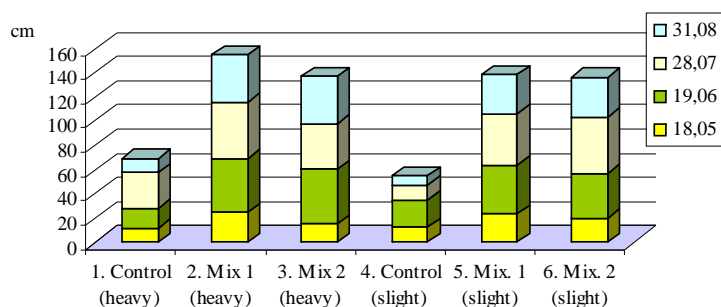


Figure 12. Re-growth of grasses after cutting in 2004.

Table 4. Change in density of broadleaved plants after three cuttings in 2004

URTDI	CHEMA	CIRAR	GALVE	LINVU	TRFPR	ARCLA	GLEHE	ACHMI	ARTVU	DAUCA	all perennial	STEME	IMPPA	LAMAM	XANST	VERONICA	ERICA	MYOAR	VIOIR	all annual
18.05																				
4	4	2	6	0	0	2	0	0	0	0	7	2	24	2	0	8	0	2	0	5
0	2	0	0	2	0	0	0	0	0	0	3	2	18	0	4	2	0	0	0	4
0	2	0	4	0	2	0	6	0	0	0	6	4	26	2	2	6	2	0	12	7
0	0	0	0	0	0	2	0	0	0	0	3	4	16	4	0	2	0	0	0	4
0	0	0	0	0	0	0	0	0	0	0	2	8	8	0	0	0	0	0	0	2
10	0	2	0	0	0	0	0	0	0	0	3	0	14	0	0	10	0	2	0	3
31.08																				
2	2	0	16	0	0	0	0	0	0	0	4	0	10	0	0	6	0	0	0	2
0	0	0	0	0	0	0	0	0	0	0	1	0	18	0	0	8	0	1	0	3
0	0	0	10	0	0	0	0	0	0	0	3	0	20	0	0	6	0	0	0	2
0	2	0	0	0	0	0	0	0	0	0	3	0	4	0	0	2	0	0	0	3
0	4	0	8	0	0	0	0	2	2	4	7	0	18	0	0	2	0	0	0	2
2	0	0	0	0	0	0	0	0	4	0	3	0	8	0	0	6	0	0	0	2

In the trial can be observed that after cutting of perennial plants *Heracleum sosnowskyi* in 2003 in these places were germinated light demanding: *Lamium amplexicaule*, *Sisymbrium officinale*, *Cerastium arvense*, *Veronica arvense*. Besides, after cutting of one of them in the same place were germinated other annual dicots. In next vegetation period three cuttings caused change in biological diversity among annual broadleaved species: *Stellaria media*, *Lamium amplexicaule*, *Xanthium strumaris*, *Erigeron canadensis*, *Viola* ssp. were fully lost, shade-requiring plants: *Impatiens parviflora*, *Veronica* ssp., *Myosotis* ssp. were prevailed (table 4).

After threefold cutting in plots, where were grow raspberry, alder's and bird-sherry tree's sprouts, new-emerged sprouts of these species was not

observed. At the same time the re-growth of *Humulus* spp. was observed in all treatments.

There were observed that susceptible to frequent cutting perennial broadleaved plants: *Arctium lapa*, *Cirsium arvense*, *Taraxacum officinalis*, *Glechoma hederacea* were fully lost. Number of *Urtica dioica*, *Chelidonium majus*, *Aegopodium podagrarium* were significantly decreased. At the last assessment in the trial appear of new-emerged light demanding perennial broadleaved plants: *Galium verum*, *Achillea millefolium*, *Artemisia vulgare*, *Daucus carota* were observed. The results from statistical analysis in the experiment show that there were relationships between the density of perennial plants *Heracleum sosnowskyi* and perennial dicotyledons per sq. m. The small positive correlation was found be-

tween density of shade-resistant *Aegopodium podagrarium* and old *Heracleum sosnowskyi* plants (table 5).

There was found positive correlation between density of new-emerged seedlings of *Heracleum* spp. and annual *dicotyledons*. The mean of new-emerged annual *dicots* was higher in places with high density of new-emerged *Heracleum* spp.

At the same time was found negative correlation between densities of new-emerged *Heracleum* spp. and all *dicotyledons*, perennial *dicots*, sown grass (table 6).

There are no significant relationships between density of seedlings *Heracleum* spp. and competitive plants. However, in results of this analysis was found tendency to damage of new-

Table 5. The relationships between density of *Heracleum sosnowskyi* and competitive plants

Species	<i>Heracleum sosnowskyi</i> (perennial)			
	Multiple R^2	$F_{\text{fact.}}$ ($F_{\text{crit.}} = 2.62$)	t_{Stat}	P-value
annual <i>dicots</i>	0.218	1.40	- 1.18	0.25
perennial <i>dicots</i>	0.323	3.27	+ 1.81	0.08
sown grasses	0.043	0.03	- 0.18	0.86
<i>A. podagrarium</i>	0.201	1.18	+ 1.08	0.29

Table 6. The relationships between density of seedlings *Heracleum* spp. and competitive plants

Species	new-emerged <i>Heracleum</i> spp.			
	Multiple R^2	$F_{\text{fact.}}$ ($F_{\text{crit.}} = 2.62$)	t_{Stat}	P-value
all <i>dicots</i>	0.557	12.62	- 5.55	0.001
perennial <i>dicots</i>	0.271	2.21	- 1.49	0.148
annual <i>dicots</i>	0.563	13.03	+ 3.61	0.001
sown grasses	0.167	0.52	- 0.72	0.482
sown grasses + all <i>dicots</i>	0.244	1.14	- 1.07	0.299
<i>A. podagrarium</i>	0.188	1.04	- 1.02	0.317

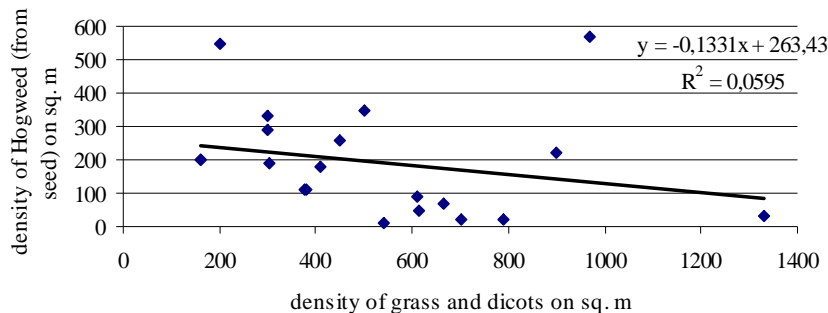


Figure 13. The relationship between density of seedlings *Heracleum* spp. and competitive plants on sq. m in riverside Vaive

emerged *Heracleum* spp. by sown grasses and *dicotyledons* which were re-growth in the experimental plots after threefold cutting (fig. 13).

Discussion

Analysis of development of *Heracleum sosnowskyi* in dynamics during the vegetative season show uneven re-growth of it depending on meteorological conditions and on density of stand of grass in the plots. In the experiment frequent cutting showed negative effect on re-growth of perennial plants *Heracleum sosnowskyi*, but had not influenced on re-growth of new-emerged seedlings of *Heracleum* spp., besides, after three cuttings observed significant decrease of number of annual broadleaved plants. In the field experiment clearly demonstrated that by threefold cutting the changes of density of plants in plots with different species has taken place. There was observed a tendency in change of specific composition of plants after threefold cutting during the vegetation period as in plots by heavy invasion of *Heracleum sosnowskyi* as by slight invasion of it. After frequent cutting during the first vegetation season was germinated light demanding: *Lamium amplexicaule*, *Sisymbrium officinale*, *Cerastium arvense*, *Veronica arvense*, but in the next vegetative period three cuttings caused significant change in biological diversity among annual broadleaved species: light demanding plants were fully lost, shade-requiring plants were prevailed.

For depression of *dicots*, including *Heracleum sosnowskyi*, selected mesophytic components of grass mixtures, which were well growth in natural grass stand on different soil type in riverside, gave good effect. The *dicots* have heightened sensibility to the cutting, owing to that after frequent cutting in riverside depending on soil type and provision with moisture of soil there are prevailed some perennial grass species, mainly *Elymus repens* and *Poa pratense*. Therefore, natural grasses are sufficiently capable of competition to *Heracleum sosnowskyi* after their cutting. In results of statistical analysis was found tendency to damage of new-emerged *Heracleum*

spp. by sown grasses and *dicotyledons* which were re-growth in the experimental plots after threefold cutting. Between grasses mixtures, which were sown by high sown rate in the experiment, were not significant differences in damage effect on *Heracleum sosnowskyi*. Well grass sward in second growing period gave significant damage effect on new-emerged seedlings of *Heracleum* spp. But sowing grass mixtures on floodplain soil should be not recommended, because seeds of *Heracleum* spp., which deposit with flood or other seed propagated way, are retentive in grass stand and well germinate in spring.

Conclusion

This investigation have revealed some tendencies to change of specific compositions in phytocoenosis of riverside Vaive by frequent cutting of small and heavy infested places with *Heracleum sosnowskyi*. Frequent cutting caused the local succession of species in derivate plant communities with *H. sosnowskyi*. In places with heavy invasion of *H. sosnowskyi* after threefold cutting common number of broadleaved species was decreased to 50%.

The results from the assessments are showed that grass mixture No. 1 (*Dactylis glomerata* 50%, *Festuca rubra* 50%) by rate 4000 seeds on sq. m in heavy infested with *H. sosnowskyi* plots had significant damaged effect on seed germination of *H. sosnowskyi* during the 1st vegetation period. Grass mixture No. 2 (*Festuca arundinacea* 35%, *Festuca rubra* 65%) in the first vegetative season gave lower effect on new-emerged seedlings of *Heracleum* spp. in comparison with grass mixture No. 1. During the second vegetation period between grasses mixtures the differences in damage effect on *H. sosnowskyi* were not been. Because both grass mixtures well re-grew after each cutting, in these plots was observed significant damage effect of grass sward on density of perennial plants of *H. sosnowskyi* and their seedlings.

The investigations are showed that frequent cutting had negative effect on re-growth of perennial plants *Heracleum sosnowskyi*. This peculiarity of perennial plants of *H. sosnowskyi* can be use for current control of Hogweed in the places where chemical treatment it is impossible used. Results of analysis of soil seed bank demonstrate the significant differences between *Heracleum* ssp. seeds density depending on soil structure and soil layer. Because in samples from floodplain soil, where were sown grass mixtures, was counted a higher number of seeds of *Heracleum* ssp. in comparison with plots without grass stand, sowing grasses mixtures on floodplain soil should be not recommended.

Acknowledgement

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EARLY SUCCESSION OF GROUND VEGETATION AFTER CLEAR-CUTTINGS IN SPRUCE FORESTS IN A BOREONEMORAL ZONE, LITHUANIA

Vitas Marozas

Marozas V. 2005. Early succession of ground vegetation after clear-cuttings in spruce forests in a boreonemoral zone, Lithuania. *Acta Biol. Univ. Daugavp.*, 5 (2): 127 - 136.

The aim of the investigations was to determinate early dynamics of ground vegetation after clear-cuttings in spruce forests on fresh fertile soils in a boreonemoral zone in Lithuania. 50 plots (10x10m) were selected in the clear-cuttings of the spruce stands of different age (1-10 year). The species composition and projection cover (in per cent) of undergrowth, herbs and mosses were recorded in twenty 1x1 m subplots. Mean percentage cover was calculated for each plot. To find the relation between species composition and clear-cutting age canonical correspondence and regression analyses were performed. It was determined, that age of the clear-cutting had an influence on the species composition and cover of undergrowth, herbs and mosses. Species richness was lowest in the fresh clear-cuts and slightly increased in the older ones. Cover of herb layer increased from the fresh clear-cuts up to 4-5 years age cuttings, and then it started to decrease. Cover of moss layer was highest in the fresh clear-cuttings and decreased in the older ones. Late successional species were recorded in the fresh clear-cuttings and they disappeared in the older clear-cuttings. Early successional species predominated in middle aged and old clear-cuttings.

Keywords: clear-cuttings, cover, dynamics of vegetation, herbs, mosses, undergrowth.

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Introduction

The structure of forests is determined by climate, geomorphology, soils, historical factors and different kind of disturbances (Kuuluvainen, 2002). Natural or anthropogenetic disturbances initiate secondary forest ecosystem succession. In natural forest ecosystems the prevailing disturbance factors are fire, wind, insects, pathogens, snow and animals. In managed forests the main disturbance factor is cuttings. Clear cuttings are the most important and have the greatest impact on forest ecosys-

tems. Clear cuttings are the most common type of commercial timber harvesting in Lithuania. After clear-cuttings rapid changes of forest vegetation occurs over short time period.

Tree layer density and composition affect composition of the ground vegetation. Abiotic and biotic conditions such as microclimatic variables (light intensity and duration, relative humidity, air temperature) and soil factors (pH, organic carbon, total nitrogen, available phosphorus, soil moisture and temperature) change after cuttings. Temperature and light are among the

most important environmental variables (Geiger, 1965; Matlack, 1993; Perry, 1994). Changes in tree layer also lead to the changes in the ground vegetation. Clear-cuttings have the greatest potential impact on vegetation. Early successional species dominate and some late successional plants become extinct after clear-cuttings (Hannerz & Hanell, 1997).

Effect of cuttings on ground vegetation was studied mostly in boreal forests (Brumelis & Carleton, 1989; Ulanova & Maslov, 1989; Gromtsev, 1992; Nieppola, 1992; Zobel, 1993; Obydennikov & Tibukov, 1996. Hannerz & Hanell, 1997; Brakenhielm & Liu, 1998; Grandpré et al., 2000; Bergstedt & Milberg, 2001; Selmants & Knight, 2003; Pykälä, 2004). More complete knowledge is needed regarding the ground vegetation dynamics after clear cuttings in boreonemoral spruce forests. The aim of the investigations was to determinate early dynamics of ground vegetation after clear-cuttings in spruce forests on fresh fertile soils in a boreonemoral zone in Lithuania.

Methods and material

Study area

The study area is located in the middle plane of Lithuania (55° 32' – 55° 55' N, 24° 39' – 24° 51' E). The study area falls in the transitional deciduous coniferous mixed forest boreonemoral zone of Europe (Ahti et al., 1968). Plain landscape and luvisols soils prevail.

Annual mean temperature ranges from +5.9 to +6.2° C and temperatures of the warmest and coldest months (July and January) are from +16.6 to +17.1 and from –5.7 to –5.4° C respectively. The annual mean precipitation is between 520 and 620 mm. Period with snow continues from 85 to 100 days (Bukantis, 1994).

The most frequent tree species are: *Picea abies*, *Pinus sylvestris*, *Betula pendula*, *Betula pubescens*, *Populus tremula*, *Quercus robur*.

Corylus avellana and *Lonicera xylosteum* predominate in undergrowth. *Oxalis acetosella*, *Maianthemum bifolium*, *Stellaria holostea* and *Trientalis europea* are dominant species in herb layer (Karazija 1988).

Sampling

The chronosequence approach was used to describe the succession by taking sites in similar environments but of different time periods after cuttings (Pickett, 1989). 50 plots (10x10m) were selected in the clear-cuttings of the spruce stands of different age (1-10 year). The resulting vegetation sequence was assumed to be the successional sequence.

The species composition and projection cover (in per cent) of undergrowth, herbs and mosses were recorded in twenty 1x1 m subplots. Overall shrub, herbs and mosses cover was also estimated. Vegetation sampling was conducted during July and August of 2001

Data analysis

Mean percentage cover was calculated for each species in the plot. The age relation with overall herbaceous species composition in spruce forest clear-cuts was evaluated by Canonical Correspondence Analysis (CCA) (Jongman et al., 1987) using the program CANOCO for Windows (ter Braak & J. Milauer, 1998).

To find the relation between species and clear-cutting age regression analyses were performed for most abundant species.

Results

CCA biplot shows the relationship of overall species composition in spruce forests clear-cuts to age of clear-cut (Fig. 1). Correlation of overall species composition with age of clear-cut was 0.912, Monte Carlo test of significance was $F=8.713$, $p=0.002$. Percentage variance of species data explained by an age was 16.8. Species on

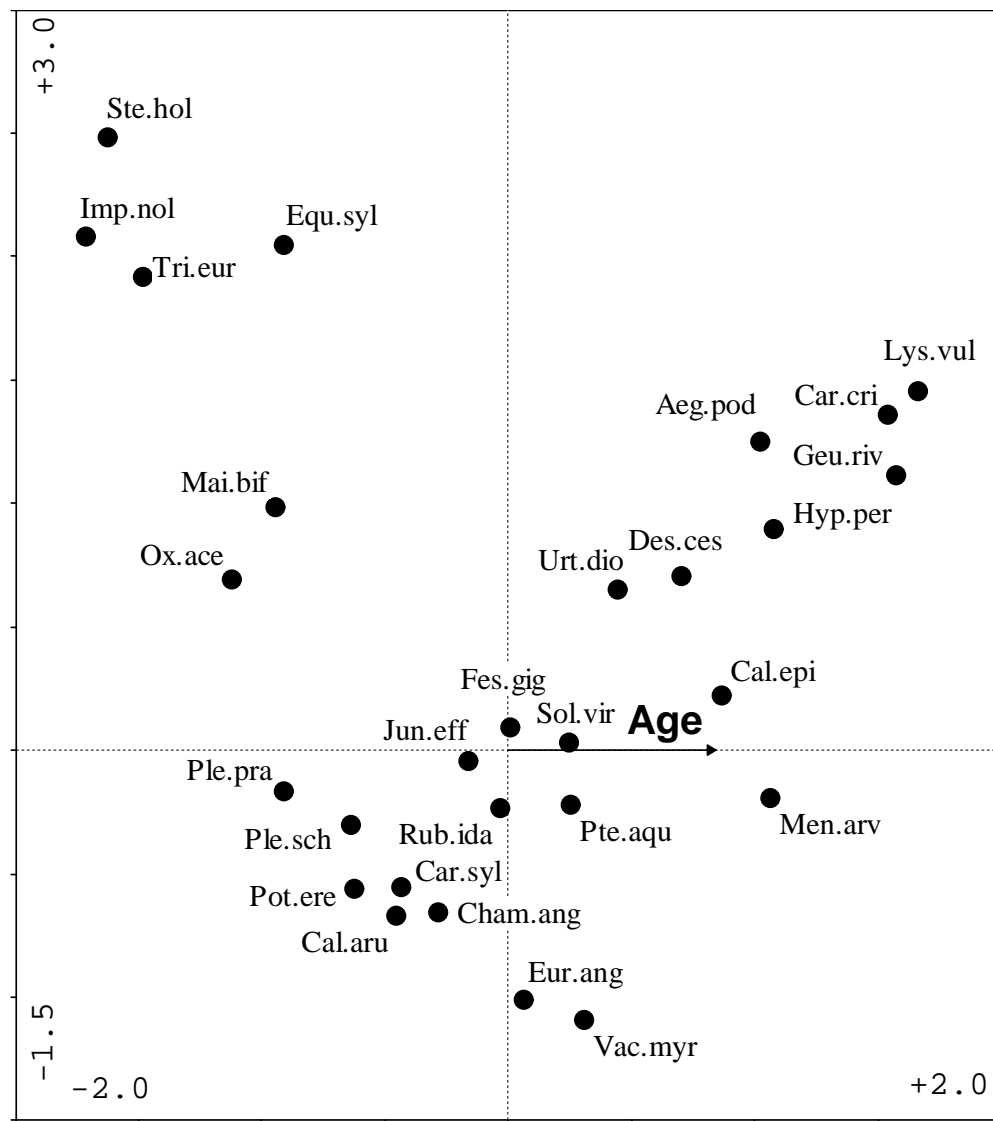


Fig. 1. Biplot of canonical correspondence analysis of herbs and mosses in spruce forest clear-cuttings

the left side of the biplot were associated with fresh clear-cuttings and species on the right part of the biplot were preferential to old clear-cuttings.

Species richness was lowest in the fresh clear-cuts and slightly increased in the older ones (Fig. 2). We found the highest species richness in the oldest clear-cuts (10 years old). Cover of

herb layer increased from the fresh clear-cuts up to 4-5 years age cuttings, and then it started to decrease. Cover of moss layer was highest in the fresh clear-cuttings and decreased in the older ones (Fig. 2).

In undergrowth *Populus tremula* predominated in the fresh clear cuttings, while *Betula pendula* and *Corylus avellana* in the older ones.

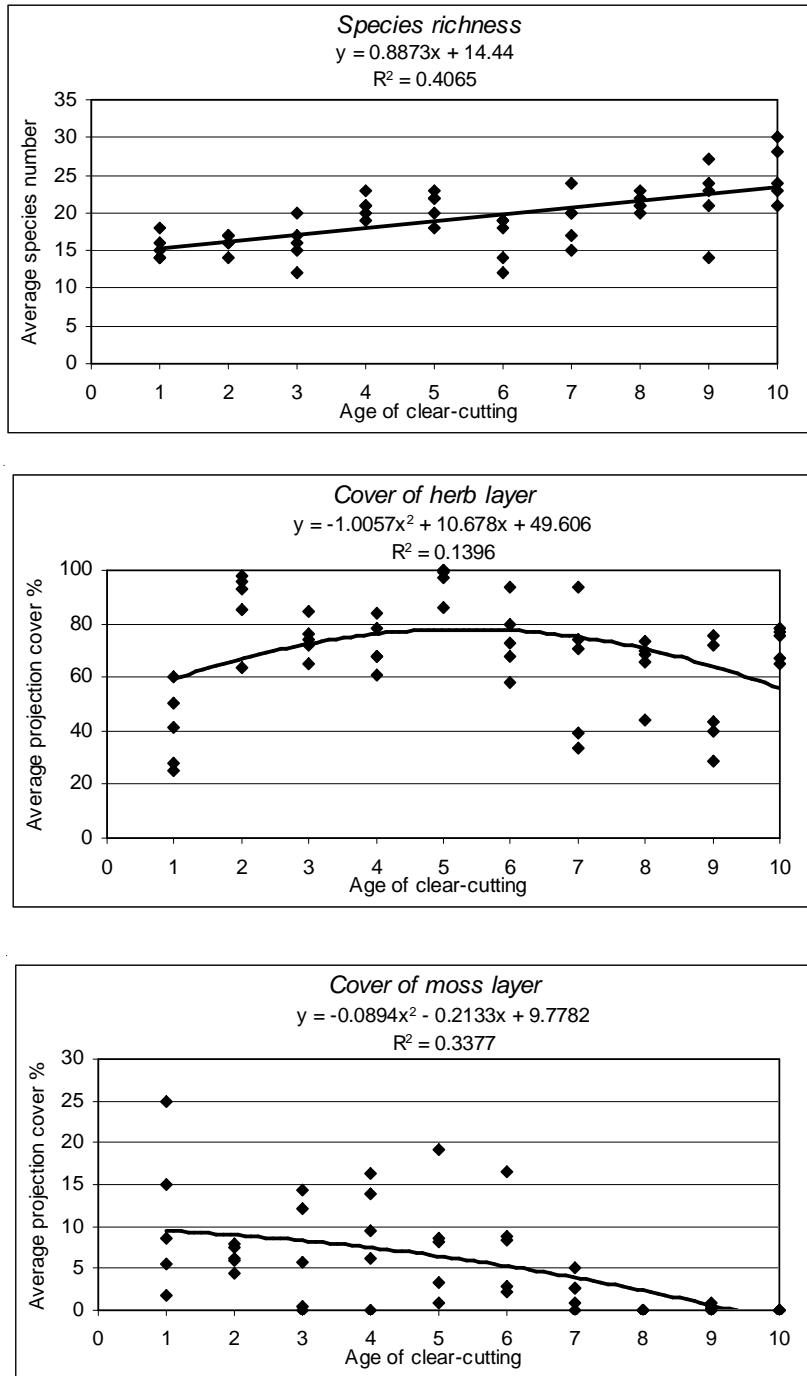


Fig.2. Dependence of species richness, the cover of herb and moss layers from the age of clear-cuts

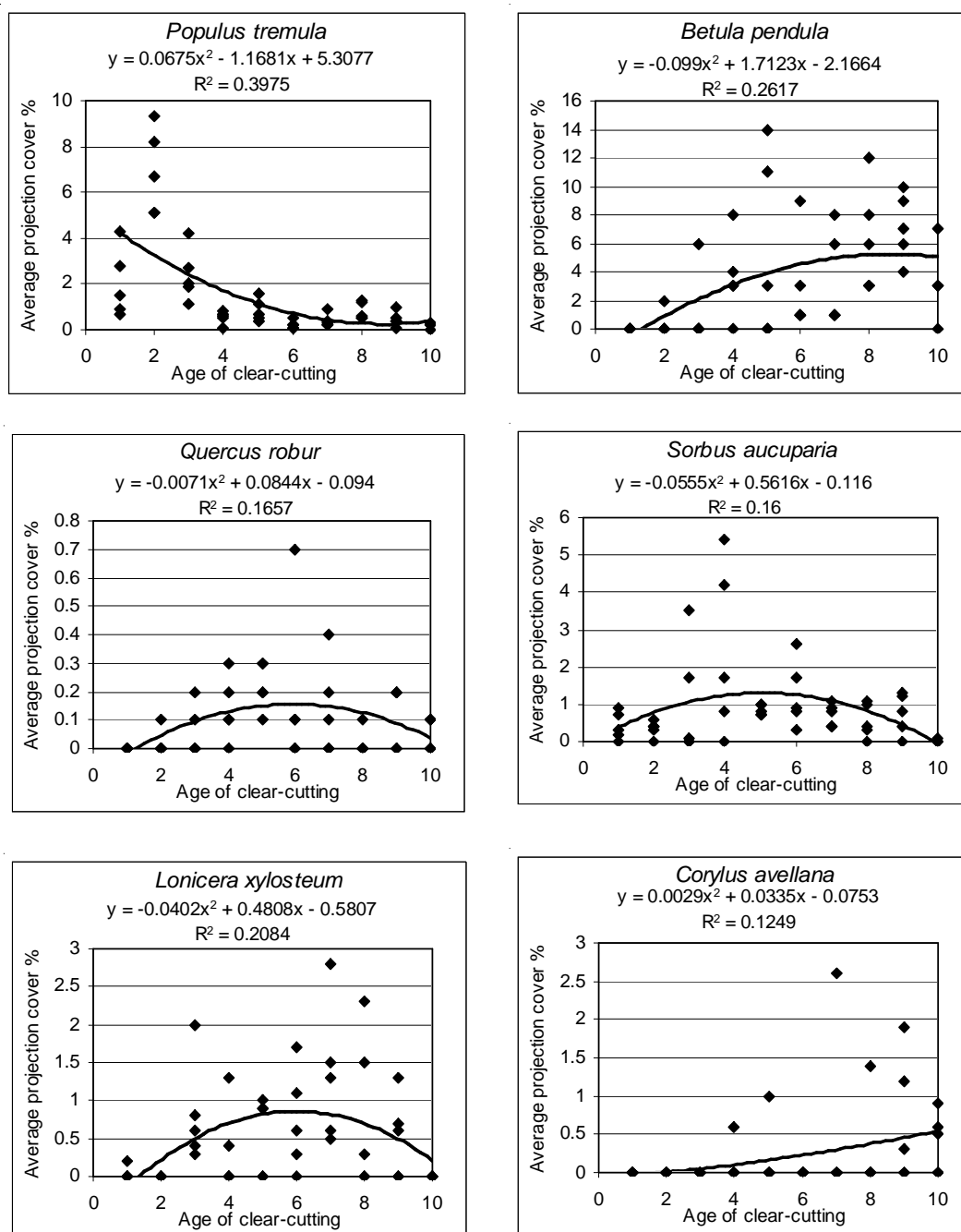


Fig.3. Dependence of undergrowth species projection cover from the age of clear-cuts

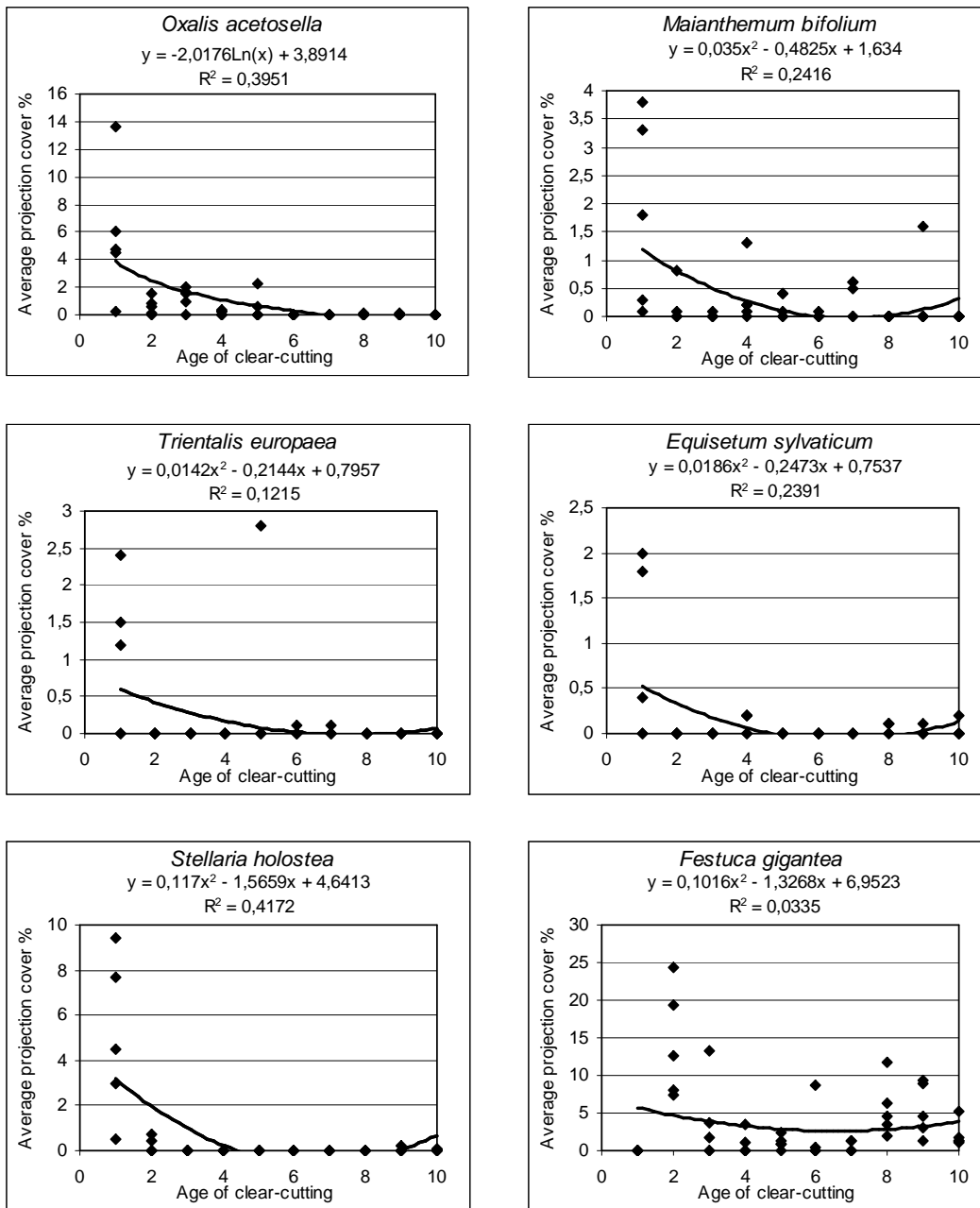


Fig.4. Dependence of late successional species projection cover from the age of clear-cuts

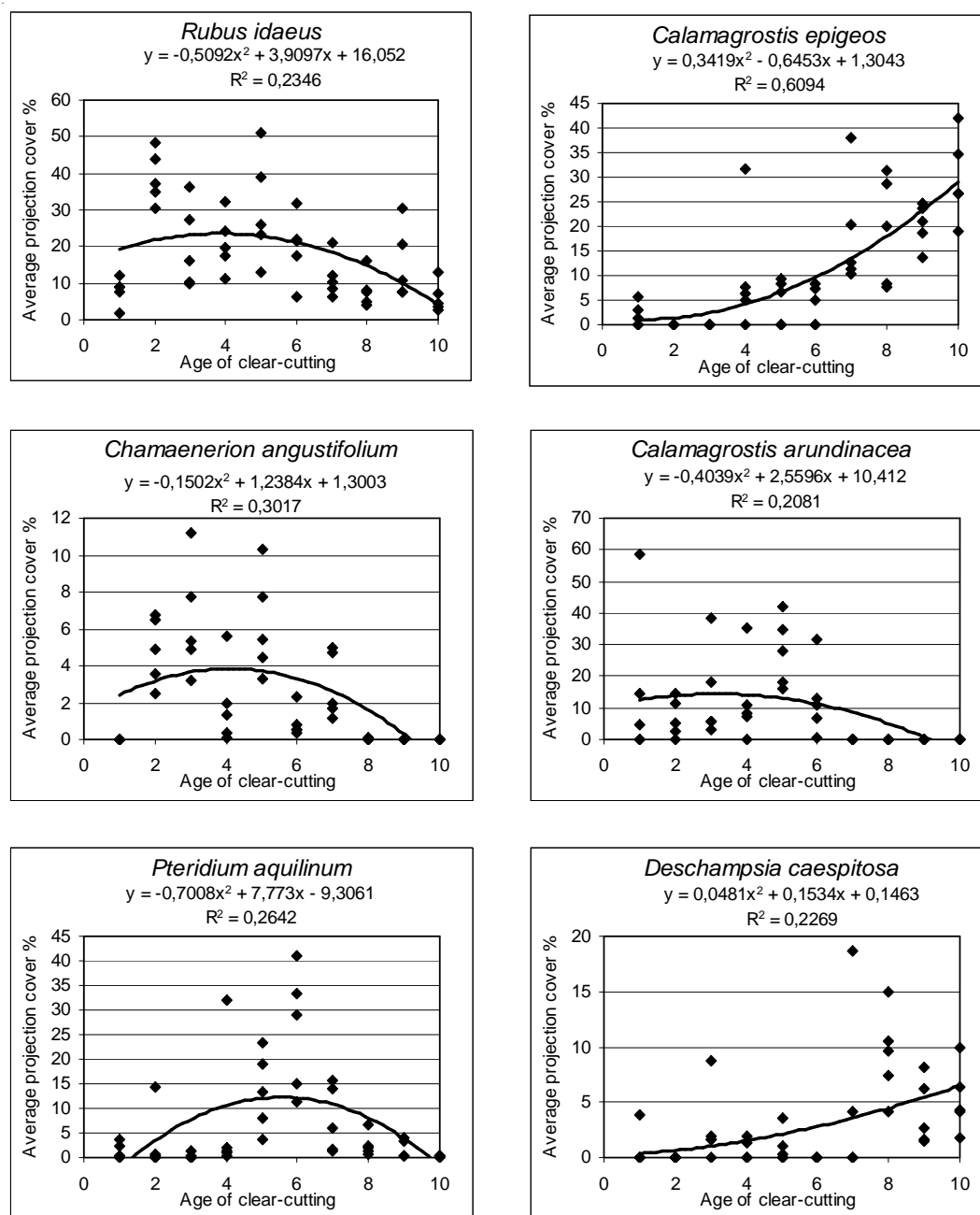


Fig.5. Dependence of early successional species projection cover from the age of clear-cuts

Quercus robur, *Sorbus aucuparia* and *Lonicera xylosteum* occurred more frequently in the middle aged clear-cuttings (Fig. 3).

Late successional species (*Festuca gigantea*, *Equisetum sylvaticum*, *Majanthemum bifolium*, *Oxalis acetosella*, *Stellaria holostea*, *Trientalis europaea*) were recorded in the fresh clear-cuttings and they disappeared in the older clear-cuttings (Fig 4).

Early successional species (*Calamagrostis arundinacea*, *Chamaenerion angustifolium*, *Pteridium aquilinum*, *Rubus idaeus*) predominated in the middle aged clear-cuttings, while *Calamagrostis epigeos*, *Deschampsia caespitosa* in the older ones (Fig. 5).

Discussion

This study showed increasing species number in clear-cuttings. This tendency can be explained by adding new non forests species, which emerged from the seeds pool when forests conditions were cut out and microsites have changed. Many studies have reported higher species richness in clear-cuttings. Pykälä (2004) found the number of vascular plant species almost two times higher in clear-cut areas (1-2 years after cutting) compared to mature forest. Most of them belonged to the native early boreal forest succesional species. Selman and Knight (2003) found higher species richness in the post-harvest stands after 30-50 years than in then adjacent mature stands.

Clear-cuttings changed microclimate and soil conditions. Due to this reason the succession of vegetation starts after clear-cuttings. Late successional species (*Majanthemum bifolium*, *Oxalis acetosella*, *Stellaria holostea*, *Trientalis europaea*) almost disappeared just in 1-2 years after cuttings. Early successional species (*Calamagrostis arundinacea*, *Calamagrostis epigeos*, *Chamaenerion angustifolium*) got favourable conditions for developing and wide-spread in clear-cuttings. In the old clear-cuttings cover of undergrowth (*Betula pendula*) became

dense and abundance of early successional species started to decrease. It is interesting that *Populus tremula* was more abundant in the fresh clear-cuttings. The reason could be that *Populus tremula* sprout up from the shoots of the roots.

Developing of undergrowth mostly had influence on the overall cover of herb layer, which started to decrease in the older clear-cuttings. Mosses were associated with late forest stages and gradually decreased and disappeared after cutting. Other studies have also reported similar changes in moss and herb layer after clear-cuttings (Kholopova et al., 1985; Nikolaeva, 1995; Karazija, 2002, 2003). Kholopova et al. found a greater mass of herbaceous plants and their higher species diversity on the cleared area comparing with the adjacent forests. Nikolaeva (1995) reported that the cover of moss layer declined and cover of herb layer increased after cuttings.

Conclusions

This study confirmed the large changes of vegetation species composition after clear-cuttings in spruce forests in boreonemoral zone. Late successional species were recorded in the fresh clear-cuttings and they disappeared in the older clear-cuttings. Early successional species predominated in the middle aged and old clear-cuttings.

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<i>Accepted: 01.12.2005.</i> |

COMMON AND ANIMAL HELMINTHS MOST WIDELY SPREAD IN DAUGAVPILS REGION (LATVIA)

Valentina Kokina

Kokina V. 2005. Common and animal helminths most widely spread in Daugavpils Region (Latvia). *Acta Biol. Univ. Daugavp.*, 5 (2): 155 - 163.

Helminths, mainly nematodes, dwell parasitically in the organisms of many people and animals. In a human, particularly child, organism pinworm *Enterobius vermicularis* is very often encountered, whereas the round worm *Ascaris lumbricoides* and *Trichocephalus trichiurus* are relatively rare. Animals are mainly infected with nematodes; among them *Strongylus equines* which is found in the organisms of horses, cattle, sheep, goats and pigs, is most often met. It is not rarely that *Ascaris suum* is found in pigs, but most dangerous is *Trichinella spiralis*. Boars are most often infected with it, and people can sometimes get infested if they consume the meat of infected animals. Among tapeworms, *Fasciola hepatica* prevails, it particularly afflicts sheep and cattle. This is caused by frequent flooding of pastures.

Key words: helminths, nematodes, *Enterobius vermicularis*, *Ascaris lumbricoides*, *Trichinella spiralis*, *Strongylus equines*, *Trichocephalus trichiurus* (*Trichuris trichiura*)

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Introduction

Helminths are parasitic worms inhabiting human and animal organisms and causing various diseases, helminthiases. They are endoparasites of the hosts and lead not only to the suffering of the latter, but also to their death. People often get infected from animals, but it is not a rare case when the causes of infection are people themselves who do not observe the rules of personal hygiene. We people most often suffer from nematodes.

One of the most widely spread nematodes is the pinworm *Enterobius vermicularis*. *Enterobius vermicularis* is a small (1 cm in length) white

threadlike worm inhabiting the cecum and adjacent gut (Fig. 1). A gravid female worm contains an average 11 000 ova and has a life span of 11 to 35 days. The gravid females migrate at night to the perianal and perineal regions for oviposition; ova are infrequently laid in the intestines. The stimulus to oviposition seems to be a subclinical temperature and an aerobic environment. Ova are laid either in clusters or in a stream but are not widely distributed in the perianal region. *Enterobius* ova are ovoid but flattened on one side and measure approximately 56x27 µm. The eggs embryonate within 6 hours and are transferred from the perianal region to night clothes, beddings, and dust and air. The most common mode of transmission, however, is via the hands

of the patient, particularly beneath the fingernails, through scratching or handling clothes and bed linen. On ingestion, the embryos hatch in the duodenum, molt twice, and develop into adult worms in 36 to 53 days (Mandell et al. 2000).

Occasionally, people suffer from the whipworm *Trichocephalus (Trichuris) trihiurus*. It is also a relatively small worm. A male worm is 30-45mm long, a female worm is 35-55mm long. They have an interesting threadlike form that reminds of a hair (Fig. 2).

Humans are the principal hosts for the *Trichocephalus trihiurus* and infection is transmitted directly. The mean expected life span of adult worms is 1 year, and during this period each female worm produces 5 000 to 20 000 eggs/day. The ova (50 to 54x22 mkm) are barrel-shaped, with a thick shell and translucent polar prominences, and are unsegmented at oviposition. After excretion in the feces, embryonic development takes place under optimal conditions of moisture and shade in 2 to 4 weeks. When the embryonated egg is ingested by humans, the larva escapes from the shell in the upper small intestine. These larval worms penetrate the intestinal villi, where

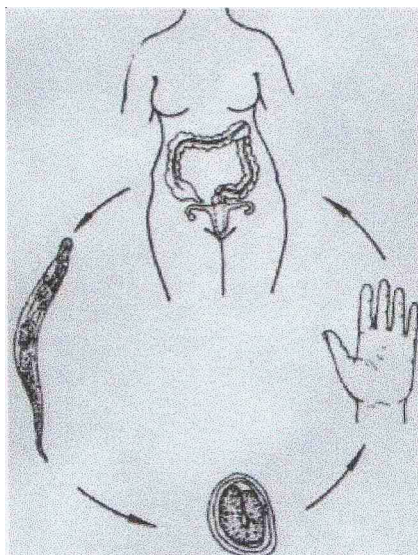


Fig.1. *Enterobius vermicularis* transmission scheme (Mandell et. al. 2000)

they lie for 3 to 10 days before slowly moving downward into the lumen of the cecum, where the anterior three quarters of the worms remain within the superficial mucosa and the short posterior end is free in the lumen. Worms develop to mature ovipositing adults in 1 to 3 months (Mandell et al. 2000).

Common round worms *Ascaris lumbricoides* are spread in many countries of the world including Latvia. This nematode is impressively long measuring 15 to 45 cm (Fig. 3).

Ascaris lumbricoides is a parasite of humans; adult worms inhabit the lumen of the small intestine and have a life span of approximately 10 to 24 months. Each female worm produces a daily output of 200 000 ova. After their passage with feces and under favorable environmental conditions, fully developed infective embryos are formed within the eggs in 5 to 10 days. When ingested by humans, they hatch in the small intestine; the embryos penetrate the intestinal wall and migrate via venous blood to the heart and reach the lungs, where they break into the alveoli and pass up through the bronchi and trachea. They are then swallowed to return to the intestines and become mature worms. The time required to produce a gravid female has been estimated to be 2 months (Blumenthal & Schultz 1975).

Looking like a common round worm, another type of it, *Ascaris suum*, causes ascariasis in pigs,

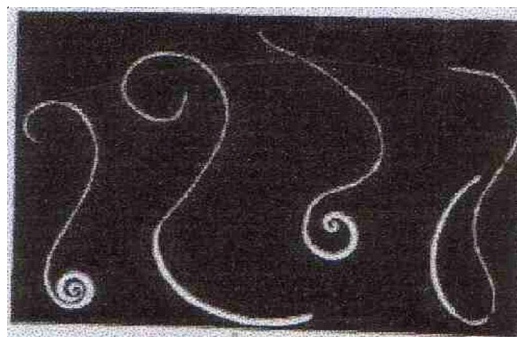


Fig.2. *Trichocephalus trichiurus* (Nesaule & Jurevica 1996)

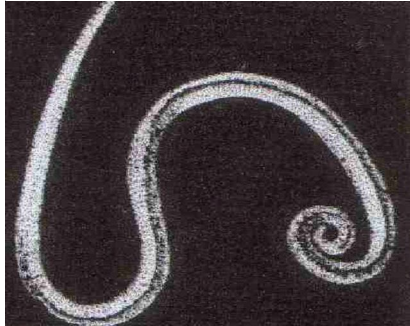
and is the most usual helminth of pigs. The major parasites are the migrating larva which may inhabit a nonspecific host, a human, and inflict a disease called *Larva migrans* causing mechanical, toxic and traumatic afflictions to the organisms (Nesaulė & Jurevica 1996).

Among the helminths causing human and animal diseases, trichinellae are most dangerous. Their name comes from the Greek word *thrix* which means 'hair'. Trichinella looks like a small threadlike helminth; a female worm measures 3-4 mm, a male worm is 1.5-2 mm long. A characteristic feature of this worm is an unpaired genital tube and viviparity (Fig. 4).

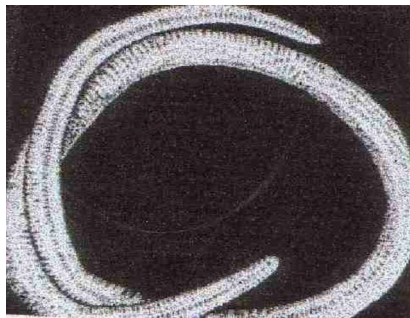
Trichinella spiralis are distributed throughout the world and are widely spread in nature among a large number of carnivorous animals, humans being an incidental host. Most human infections are due to *Trichinella spiralis*, a few are due to

Trichinella britovi, *Trichinella nativa*, *Trichinella nelsoni*. Only one case of human infection with *Trichinella pseudospiralis* has been reported (Booth M. et al. 1998). *Trichinella spiralis* is the only species with good infectivity for swine and rats. The vast majority of swine are fed with grain and are generally uninfected. The small proportion fed with garbage may become infected when given uncooked trichinous scraps, usually pig meat, or when the carcasses of infected wild animals such as rats are eaten. In Europe, the fox is the primary reservoir of the sylvatic cycle of *Trichinella*, and human infections usually occur in rural areas where traditional swine-rearing practices are used (Drugs for Parasitic Inf. 1998).

Infection of people with *Trichinella spiralis* happens when undercooked meat is consumed (salting and smoking of meat does not protect from infection). With muscles of infected animal, swung in a spiral and encapsulated larvae get into the organism of a host. In case of trichinella, one and the same organism is first a host (sexually mature forms in the intestines) and then an intermediary host (larva in muscles). While in the intestine, the larvae escape from their shells, turn into sexually mature adult worms, and females give birth to living larvae within 30 to 45 days. The larvae are distributed by the flow of blood and lymph all over the organism, but they get stuck in the diaphragm, intercostal and manductory muscles. There they swing into spirals and get encapsulated. They can stay alive in this position for 20 to 25 years. For further reproduction, they must get into a new host; a human is obviously a biological dead lock for them (Ejlskov 2002; Mandell et al. 2000). Lethal cases from trichinellosis in humans amount to 1.5%, the major reason for death being myocarditis, pneumonia, meningoencephalitis (Björger u.c. 1977). Many animals are sometimes infected with strongylates *Strongylus equinus*. Adult individuals inhabit the intestine whereas the larvae penetrate other organs. Strongylates (15-45 mm long) develop from eggs turning into the larvae which grow, moult and reach sexual maturity in the intestine. Both larvae and adult individuals dam-



1 - male



2 - female

Fig.3. *Ascaris lumbricoides* (? evcovs et. al. 1984)

age the organs and allow bacterial infections. Animals are infected with the strongylates eggs mainly in the pastures.

Among trematodes, *Fasciola hepatica* or liver fluke is most often encountered. Mainly animals (cattle, sheep) suffer from these worms, but they can occasionally inhabit human organisms. For the full cycle of its development, this parasite needs an intermediary host, i.e. a small pond snail (Fig. 5) (Urkhart 1992).

The aim of the present research was to identify the major types of helminths inhabiting humans and animals in Daugavpils region, particularly those which most often cause diseases.

Materials and methods

In order to investigate the animal tissue specimen and find out if the animals were infected with trichinelliasis, the methods of muscle biopsy and muscle digestion were used. For these, 2 tissue specimens (50g of delta-shaped muscle each) of every pig carcass were taken. From each specimen, 24 pieces were further cut the size of an oat

corn each, with the help of manicure scissors. The pieces were placed between two thick glass plates (compressorium) and squeezed to the extent that one could read a newspaper through them. The obtained 24 samples were then thoroughly examined at a low magnification under the microscope or trichinelloscope.

The method of muscle digestion is more effective. Finely cut pieces of muscle tissue were soaked in the artificial gastric juice (1.0% solution of pepsin in the 0.7% solution of hydrochloric acid) and placed in the thermostat at 37 °C for 12-16 hours. The amount of gastric juice should be 15-20 times more than that of the muscle. After digestion, the sediment was placed on the microscopic slide glass by a pipette and examined under the microscope. *Trichinella* larvae escaped from their capsules were found in the sediment (??? ? ä? . 2002).

For diagnosing enterobiasis, a method of sticky tape was used. A strip of transparent sticky tape 4-5 cm long was placed the sticky layer down across the anal opening towards the perianal folds. It was then quickly removed, stuck to the

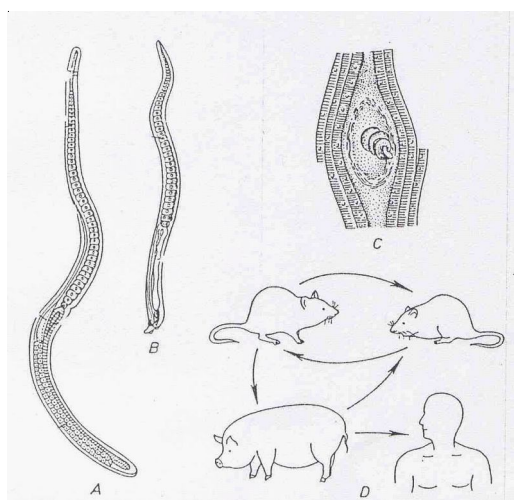


Fig.4. *Trichinella spiralis*: A – female; B – male; C – larva encapsulated in a muscle; D – major path of trichinella circulation in nature (Dogels 1986)

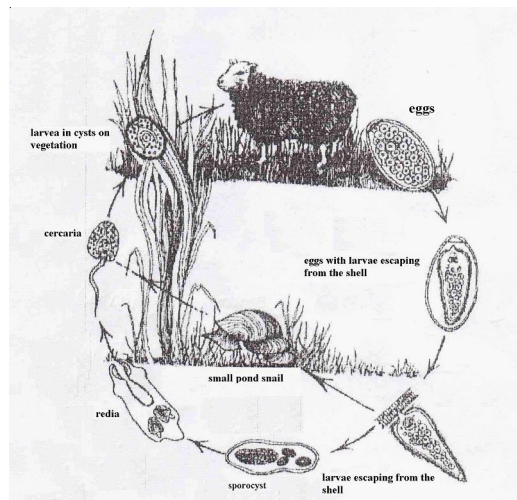


Fig.5. *Fasciola hepatica* development scheme (Urkhart 1992)

microscopic slide, and studied under the microscope.

A method of Filiborn (Devcovs u.c. 1984) was employed for identifying the make use of the eggs of ascaris, whipworm and fasciola. This method is based on the fact that helminth eggs float in the saturate solution of NaCl with a high relative density. To make use of the fin method of Filiborn, 400 g of NaCl were dissolved in 1l of boiling water; the solution was then filtered through gauze and cooled down. After cooling, salt crystals appeared on the bottom, which proved that the solution was correctly made. It was used no sooner than in 24 hours.

The research method: a sample of excrement (approximately 3 g) was placed in a plastic, polysterene or glass container with the cubic capacity of 75-100 ml. While stirring with a glass stick, 50-75 ml of the saturate solution of NaCl were slowly poured into the container, and the particles of excrement were thoroughly squeezed. The bigger floating particles were then carefully

removed with a strip of paper. The suspension was filtered through a sieve (the size of holes is 0.3-0.5 mm). After 45-60 min of settling, 3 drops of the surface film were collected with the help of a wire loop (1cm in diameter) and placed on the microscopic slide (it is possible to add 1 drop of 50% water solution of glycerine). The substance was studied under the microscope at a slight magnification, without covering it with a microscopic slide. The eggs of each helminth discovered in these 3 drops, were countered down. After examining each sample, the wire loop was processed with fire least the eggs were transferred into the following sample. Additionally, 2-4 preparations taken from the sediment were examined. Those were collected with the help of a pipette and placed on 2 microscopic slides. Nematode eggs were identified by their morphological features.

The most precise method is that of identifying helminth after the death of animals when in course of dissection the worms are found out, and pathological anatomic deformations of animal organs

Table 1. Helminths that are more often found in the organisms of domestic animals in Daugavpils region

Species	Animals infected with helminths	Cases of infection		
		2000	2001	2003
<i>Fasciola hepatica</i>	Cattle, sheep	27	21	5
<i>Trichocephalus suis</i>	Domestic pigs, sheep	14	0	0
<i>Trichinella spiralis</i>	Domestic pigs	2	1	1
<i>Strongyloides ransomi</i>	Cattle, domestic pigs	6	0	11
<i>Strongylus equines</i>	Horses, cattle, sheep, pigs	597	74	6
<i>Ascaris suum</i>	Domestic pigs	46	3	25

Table 2 Results of finding *Trichinella spiralis* in Daugavpils region

Year	Number of examined boars	Number of wild boars infected with trichinella	Extensiveness percentage
2002	115	6	5.2
2003	203	3	1.5
2004	205	4	1.9
2005 (9 months)	103	1	0.97

and tissue are identified. This method allows counting down all helminths, although for a full account all organs must be examined, which takes a lot of time, that is why in some cases only the intestines and liver of the killed animals were examined. The contents of the intestines were washed with water and filtered through a sieve with the size of holes from 0.1 to 10 mm. Then the found parasites were extracted with the help of pincers or a preparation needle and placed in the conservation solution. Since the precise number of helminths is impossible to get, the information about human helminths was obtained from the cases of disease using the data provided by the staff of the Daugavpils branch of the State society health agency. The intensity coefficient (IC) was worked out according to the following formula:

$$IC = \frac{\text{Number of disease cases} \times 100,000}{\text{Number of inhabitants in a town}}$$

The number of inhabitants in Daugavpils on January 1, 2005 was 110,581.

The extensiveness coefficient (EC) for animals infected with helminths was worked out according to the following formula:

$$EC = \frac{\text{Number of positive samples} \times 100}{\text{Total number of examined samples}}$$

More accurate data concerning the examined samples were obtained from the specialists of the Daugavpils branch of Latgale regional veterinary laboratory.

Results and discussion

During the period from 2000 to 2004, a general human morbidity caused by helminths was high, particularly many cases (361) were registered in 2000 and 2003, the intensity coefficients being 313.5% and 320.58% respectively. The most widely spread among helminths is the pinworm *Enterobius vermicularis*. Especially many cases of disease caused by it (351 with the intensity coefficient 304.8%) were registered in 2000. In comparison with the intensity coefficient of enterobiasis in Latvia which is 359,3%, it is lower, but nevertheless quite substantial. Enterobiasis

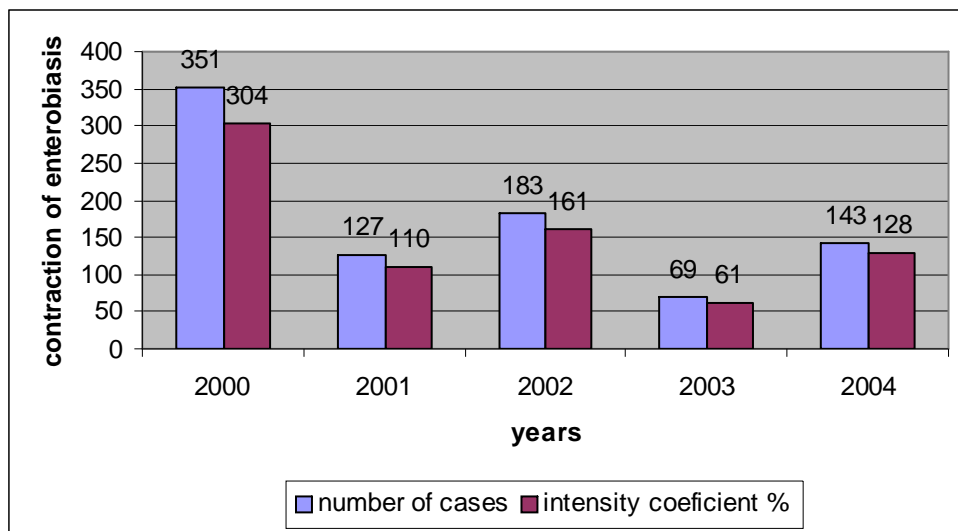


Fig.6. Dynamics of contracting enterobiasis caused by *Enterobius vermicularis* among inhabitants of Daugavpils

was mainly found in children up to 18 years of age, young children from 1 to 6 years old being most vulnerable; 164 cases of invasion were registered among the latter, which makes over 46% (Fig. 6)

The fact that the number of cases falls down does not testify to a satisfactory situation because due to the lack of necessary funding, preventive measures are hardly undertaken, i.e. children of pre-school and early-school ages are not examined, *Enterobius vermicularis* is looked for either on medical request or while examining contact people, which is not enough in order to timely identify this parasite and prevent its transmission. It must be stated that the disease caused by *Trihocephalus trihiurus* is not actual in Daugavpils region, as in the period from 2000 to 2004 this parasite was found out in only one person, which makes the intensity coefficient 0.89%. *Ascaris lumbricoides* was most often found out in Latvia in 2000 when 368 cases of disease were registered, with the intensity coefficient 15%. The situation has been improving since, and in the 6 months of 2005, 84 cases were registered, with the intensity coefficient 3.62%.

In Daugavpils, *Ascaris lumbricoides* have been more often discovered in the recent years than in the whole of Latvia. Although the cases of disease have not been numerous, their number per 100 000 of inhabitants is bigger than in the republic in general (Fig. 7)

All the people who contracted this disease in 2003 were under 18 years of age, mainly children aged 7-14, which testifies to the fact that sanitary norms such as, first of all, washing hands before eating are not observed.

The study of the spreading of helminths in animals showed that the latter are mainly infected with nematodes (94%) and only in 6% of cases with flat worms. The most often encountered nematode is *Strongylus equines*, which is found in the organisms of cattle, sheep, goats and pigs. It is followed by *Ascaris suum* found in pigs. Unfortunately it is no longer possible to obtain precise data either about ascariasis or about other helminths because the owners can not afford to pay for the examination of their animals and for the analyses. They prefer to treat their animals for helminths themselves when needed. Only large pig farms carry out the treatment of

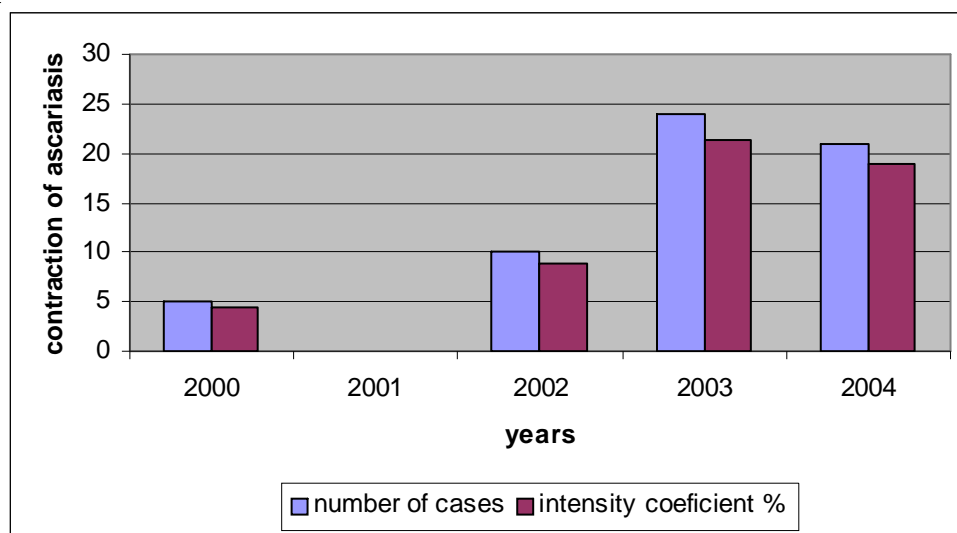


Fig.7. Dynamics of contracting ascariasis caused by *Ascaris lumbricoides* among inhabitants of Daugavpils

animals on the permanent basis. Thus 6 713 pigs were treated for helminths in Daugavpils region in 2004.

Trichocephalus suis was found in the organisms of pigs and sheep; *Strongyloides ransomi* was located in the organisms of the cattle, sheep and pigs; *Strongylus equines* in the organisms of the cattle and horses. *Fasciola hepatica* which is prevailing among tapeworms, is often found in the organisms of sheep and cattle (Table 1).

The main reason for contracting fascioliasis is the pasture meadows which are flooded in spring time and sometimes in summer and where the small pond snail dwells. Unfortunately, there is a limited number of effective means of fighting this parasite.

Trichinella spiralis has become an object of serious study in recent years, because this nematode does not escape attention once having penetrated the human organism. Latest diseases of people in Latvia, in Daugavpils particularly, testify to an unfavourable situation, because in 2005, 19 people were infected with trichinellosis in Riga, while in 2003, 8 people from Daugavpils region were taken to hospital after having eaten pork shashliks. The main sources of infection are the wild boars that are in their turn infected with trichinella when eating mice. This food chain ultimately leads to people who consume the meat that is not checked for trichinella. In the majority of cases, though, the meat of wild boars is checked for trichinella, and hunters have become more attentive to such checks (Table 2).

As the table shows, the highest number of wild boars infected with trichinella was registered in 2002, which might have been the reason why hunters changed their attitude to the sanitary checks; as a result, the number of checked game increased. The neighbouring hunting societies in Kraslava region have become more thoughtful as to the checks for trichinellosis; from 94 wild boars checked in 2004 one was infected; there were 2 infected wild boars among the 37 that were checked in 2005, which means that the extensiveness is quite high, 5.4%. Wild boars do

not know borders, and the infected animals can come to Daugavpils forests. It is not possible to register the number of invasive animals, as not all of those that were killed undergo the examination in the laboratory. They have lately begun to check horses for trichinellosis, and all the 15 checked animals were not infected.

Conclusions

It follows from the above said that the measures aimed at preventing the spread of helminths consist of strict observation of sanitary and hygienic norms, which will exclude the contamination of soil, water, and other objects around us with the eggs of helminths. In order to avoid contracting a disease caused by helminths, people must maintain cleanliness not only of their hands, and consume the meat of animals that underwent a sanitary check and adequate thermal processing. Carcasses of animals infected with trichinella must be extinguished; it can by no means be used for feeding other animals.

Since the spread of liver fluke is directly connected with the presence of small pond snails in the standing water, they should be annihilated, and pastures must be drained. It is strictly forbidden for people to drink unboiled water from such standing water reservoirs, just as it is forbidden to eat the sheep sorrel collected in such meadows. Great attention must be paid to fighting the rodent in stock-raising farms and treating animals for helminths.

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SOME ASPECTS OF INDUCING CALLUS CULTURE AND SUBSEQUENT PLANT REGENERATION OF RED CLOVER (*TRIFOLIUM PRATENSE* L.)

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Kokina I., Grauda D., Jermaļonoka M., Rashal I. 2005. Some aspects of inducing callus culture and subsequent plant regeneration of red clover (*Trifolium pratense* L.). *Acta Biol. Univ. Daugavp.*, 5 (2): 165 - 168.

Elite material of the Latvian red clover variety 'Agra' was included in the experiment for observation of calli formation and their regeneration. Leaves, stem segments and leaf petiole segments were used as explants. Different frequency of calli formation was observed depending from the explants type. Most frequently calli were formed on leaf segments. The size of calli varied depending from the size of explants and was, in general, independent from the type of explant. The largest calli were developed from the smaller (2 mm) leaf petiole segments. Different types of the calli regeneration were observed. Organogenesis was characteristic feature of regeneration from calli induced on stem segments, rhysogenesis – on leaf segments. Calli induced on leaf petiole segments formed somatic embryos and their posterior regeneration in the whole plant was observed. Embryogenesis was obtained from 12.5% of calli induced on leaf petiole segments.

Key words: red clover, callus, explants, organogenesis, rhysogenesis, embryogenesis, regenerants

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Introduction

Red clover (*Trifolium pratense* L.) is a most popular fodder legume suitable for growing in the Latvian conditions (Jansone, 1999). Traditional methods of obtaining of red clover initial breeding material by crossing are time and handwork consuming (Grauda et al., 2004). Therefore it is very important to use *in vitro* methods in red clover breeding programs.

Calli cultures induced from different parts of plants are very interesting also from the theoretical point of view to study processes of morphogenesis and embryogenesis. Combinations of phytohormones (especially auxines and cytokinines) and their concentrations in the medium can determine different types of morphogenesis. Sometime, cultivated cells form roots or shoots. It is possible also observe in the calli culture embryo formation and their posterior re-

generation in a whole plant (Halperin, 1986). The phenomenon of somaclonal variation in red clover can appear during the regeneration from callus via somatic embryogenesis (see examples for red clover: Wang, Hall, 1988; Smith, Quesenberry, 1995).

The aim of this study was to determine the best method for obtaining calli culture and plants-regenerants of the red clover variety 'Agra'. We also are discussing here particularities of callus morphology and its regeneration capacity depending of the explant type.

Material and methods

Elite material of the Latvian red clover variety 'Agra' was included in the experiment. Explants from different parts of donor plants were used for callus induction: leaves (each segment of compound leaf was cut in 2-3 parts), stem segments and leaf petiole segments with different length (2-20 mm). Explants were cultivated on the basal MS medium (Murashige, Skoog, 1962), 3% sucrose, 0.7% agar, pH 5.8 and 2 mg/l 2,4 D. Explants were cultivated under 24 °C and 16 h photoperiod. Frequency of calli formation and diameter of obtained calli were evaluated.

MS medium without phytohormones were used for regeneration, 24 °C, 16 h photoperiod, pH 5.8. Types and frequencies (%) of regeneration were recorded according of the type of explants.

Regenerated plants, which were appeared via process of somatic embryogenesis, were cultivated approximately three weeks on the MS medium without phytohormones, 24 °C 16 h photoperiod. Then regenerants were transferred to a soil and acclimatized in a growing chamber (17-20 °C, 16 h photoperiod).

Results and discussion

Data of calli formation on different explants of the red clover (*Trifolium pratense* L.) variety 'Agra' are presented in the Table 1. All leaf segment explants were formed calli. Stem segments formed calli from 87.5 of explants. Frequency of calli formation on leaf petiole segments varied depending from the length of explants: the most frequently (97.5%) calli were developed on shorter explants, the longest explants (20 mm) formed calli from 70% of explants only. Average diameter of calli on leaf and stem segments was 6.0 ± 0.3 mm and 5.5 ± 0.1 mm, respectively (difference is significant). Diameter of calli on the leaf

Table 1. Calli formation on different explants of the red clover (*Trifolium pratense* L.) variety 'Agra'

Type of explant	Total number of explants	Number of obtained calli	Frequency (%) of calli formation	Mean diameter (mm) of calli
Leaf segments	40	40	100.0	6.0 ± 0.3
Stem segments	40	35	87.5	5.5 ± 0.1
Leaf petiole segments (2 mm)	40	39	97.5	7.5 ± 0.5
Leaf petiole segments (10 mm)	40	32	80.0	6.3 ± 0.4
Leaf petiole segments (20 mm)	40	28	70.0	5.9 ± 0.3

Table 2. The type and frequency of regeneration from calli induced on different explants

Type of the explant	Type of the regeneration	Frequency of the regeneration (%)
Stem segments	Organogenesis	50.0
Leaf segments	Rhysogenesis	50.0
Leaf petiole segments	Embryogenesis	12.5

petiole segments varied depending from the length of explants. The largest calli (7.5 ± 0.5 mm) were observed on the shortest (2 mm) leaf petiole segments. On leaf petiole segments with length 10 mm and 20 mm, size of calli was 6.3 ± 0.4 mm and 5.9 ± 0.3 mm, respectively. Analysis of variance showed that calli size was significantly depended from the length of explants. In general our data proved that size of calli is depended mainly from the size of explants and is independent from the type of explants.

Two types of calli were formed on all explants: soft and watery calli, without regeneration ability and compact calli with regenerations zones. Both green meristematic and embryo structures were observed only on compact calli. On smaller morphogenetic calli formed just only one, on larger calli – 1-3 green regeneration zones.

Regeneration from calli induced on different explants was observed on the MS medium without phytohormones. The type and frequency of regeneration depended from the type of explants (Table 2). Calli from the stem and leaf segments showed significant regeneration ability – 50% of calli regenerated by organogenesis and 50% – by rhysogenesis. Lower frequency of regeneration (12.5 %) from calli induced on leaf petiole segments were observed, regeneration type was embryogenesis. This fact demonstrated that regeneration through embryogenesis is possible, although in the lower frequency. Data proved also that realization of the potential of embryogenesis in plant cells are depended from the type of explant tissue. Nevertheless, the ability of re-

generation of somatic embryos opens new possibilities for inducing of somaclonal variability in red clover (*Trifolium pratense* L.) and obtaining plants-regenerants with high percent of variability for breeding purposes.

Several green plants-regenerants were obtained through the embryogenesis. Among the regenerants different morphological changes were observed, including the shape and size of leaf plates, shorter stem and chlorophyll abnormalities. Obtained plants-regenerants were passed to the Research Centre “Skrīveri” of the Latvia University of Agriculture for further evaluation and involving in the breeding process.

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GENETIC POLYMORPHISM STUDY AND PCR DETECTION OF *BORRELIA BURGDOFERI* SENSU LATO IN *IXODES RICINUS* L. TICKS IN LITHUANIA

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Ixodes ricinus L., also known as the sheep tick or the castor bean tick, is common and widespread in Lithuania. *I. ricinus* ticks are the main vector in transmission of infection diseases like tick-borne encephalitis, Lyme borreliosis, human granulocytic ehrlichiosis. The aims of our study were to investigate genetic variability of *I. ricinus* ticks and detect ticks infected with *Borrelia burgdorferi* sensu lato. One-hundred three *I. ricinus* ticks were collected from Biržai and Vilnius districts. At first, DNA from individual ticks was extracted and ticks were analysed using PCR method for taxonomic identification. Furthermore the primers FL6 and FL7 were used for amplification the flagellin gene fragment of the spirochete genome. The mean prevalence of *B. burgdorferi* s.l. was 10,7% (11 ticks were positive) with range from 0 to 12,2 % (16,7%) at the two different locations of Lithuania. The polymorphism of *Ixodes ricinus* was investigated using random amplified polymorphic DNA (RAPD) method. Ten arbitrary sequence decamers were used as primers. Similarities between RAPD patterns were used to evaluate genetic distances among individuals of *I. ricinus* and then, these distances were used to construct a phylogenetic tree.

Key words: Tick, *Ixodes ricinus*, *Borrelia burgdorferi*, genetic polymorphism, PCR, RAPD methods

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Introduction

Ixodes ricinus L. is the most common tick in Europe. It is the European vector of Lyme borreliosis,

caused by the spirochaete *Borrelia burgdorferi* sensu lato, most prevalent arthropod-borne disease of humans in the temperate northern hemisphere. Ticks *I. ricinus* also is common and widespread in Lithuania and *B. burgdorferi* s.l. oc-

curs throughout the country. The cases of Lyme borreliosis in Lithuania are identified each year (Žygutienė, 2000).

The ixodid ticks are relatively sensitive to desiccation and are especially common in grasslands and woodlands, with each species having its own particular optimal environmental conditions and biotopes that determine the geographic distribution of the ticks. *I. ricinus*, usually feed on different host species, particularly large mammals but also small mammals and birds. Larvae and nymphs feed on rodents, insectivores, reptiles and birds; adult feed on medium to large wild and domestic animal. Generally, habitat distribution also influences host selection, because ticks that are adapted to a habitat or a vegetation type, for example woods for *I. ricinus* and *I. scapularis*, will encounter vertebrates that are adapted to the same habitat (Parola & Raoult 2001).

In Lithuania tick activity period depends on climate conditions and as parasitological investigation shows it is continue about 8 months. *I. ricinus* number in Lithuania has increased by 9,3 percent every year. According Regaliene (1985) and Žygutienė (1999) researches, the rate of average annual ticks number from 1988 till 1998 increased tenfold. It is happen because of human economical activity has improved ticks existence conditions (Bagdonas et al. 2003).

I. ricinus is a member of the *I. ricinus* species complex, which consist of 15 species which collectively have a nearly cosmopolitan distribution. Researches data on measuring the intraspecific genetic variation using enzymes, cuticular hydrocarbons and single strand conformation polymorphism (SSCP) suggested that this tick has an unusually high degree of intraspecific variations in comparison to other ticks, but similar to that of other members of the *I. ricinus* species complex. *I. ricinus* appears to have high levels of variation among populations, comparable to that of a closely related North American species, *I. scapularis*. (Ames A. et al., 2000).

Random amplified polymorphic DNA (RAPD) technique is ideal for genetic mapping, plant and animal breeding programs, and DNA fingerprinting, with particular utility in the field of population genetics. In many instance, only a small number of primers are necessary to identify polymorphism within species. A single primer may often be sufficient to distinguish all of the sampled varieties (Williams et al. 1990).

The aims of our study were to investigate genetic variability of *I. ricinus* using random amplified polymorphic DNA (RAPD) method and to determinate the prevalence of *B. burgdorferi* s. l infection by using PCR method in ticks *I. ricinus* collected from two Lithuanian districts.

Material and methods

Sample collection

Ticks were collected from their microhabitats using “flagging” standard method for collecting active ticks on vegetation. A cloth or blanket (1 m²) is drawn over the vegetation. Ticks become attached to it and can be removed periodically.

The 103 individuals of *I. ricinus* ticks were collected from Vilnius (54) and Biržai (49) districts. Unfed nymphs, females and males of *I. ricinus* were preserved in 70% ethanol until processed. All specimens were identified as *I. ricinus* - like by their morphological characteristics according Pomerancev, 1950.

DNA Extraction and molecular identification of ticks

Extraction of DNA was carried out by lysis of ticks in ammonium hydroxide (NH₄OH). All ticks were analysed individually. The ticks were taken from the ethanol solution, briefly dried, and boiled in 2.5% ammonium hydroxide (at 100°C for 25 min) to free the DNA. After brief centrifugation, the tubes were opened and incubated at 95°C to evaporate the ammonia (10-15 min.). The tick's

lysates were either used directly for PCR or stored at -20°C until use (Stańczak et al. 1999).

54 ticks from Vilnius and 49 from Birzai districts were used for molecular analysis with specific primers: Ixri-F (5' GGAAATCCC GTC GCACG 3') and Ixri-R (5' CAAACG CGC CAA CGAAC 3'). They amplify 150 bp segment of the 5.8s rRNA gene, which is specific for *I. ricinus*.

The detection of *Borrelia burgdorferi sensu lato*

The ticks were tested individually for the presence of the spirochetes using polymerase chain reaction (PCR) techniques able to identify *Borrelia burgdorferi* s. l.. PCR was performed according to Stańczak et al. (1999), using the oligonucleotide primers: FL6 (5' TTC AGG GTC TCA AGC GTC TTG GAC T 3') and FL7 (5' GCA TTT TCAATTTTA GCAAGT GAT G-3') in conserved regions of the *fla* gene of *B. burgdorferi*.

PCR protocol

PCR was performed in reaction volume of 25 μl containing 0.2 μl *Taq* DNA polymerase (stock 5 U/ μl), 2.5 μl 10x PCR Buffer, 2 μl MgCl₂ (stock 25mM), 2.5 μl dNTPs mixture (stock 2.5 mM) (MBI Fermentas, Lithuania), 1.5 μl FL6 (stock 10 pmol/ μl), 1.5 μl FL7 (stock 10 pmol/ μl) (Roth, Germany), 10.8 μl double distilled water and 4 μl of the processed tick sample. In each PCR run we used positive and negative controls. All reactions were carried out in Eppendorf PCR system "Mastercycler personal" thermal cycler. Samples were initially denatured for 1 min at 94°C . Subsequent cycles were at 94°C for 30 sec (denaturation), 55°C for 30 sec (annealing), and 72°C for 1 min (extension). The forty cycles were performed (Ambrasienė D. et al. 2004).

Agarose gel electrophoresis

For the analysis of PCR amplification products, 10 μl aliquots of reaction mixtures were applied to 2 % agarose gels (MBI Fermentas, Lithuania) with Tris-Borate-EDTA (pH 8.2) as running buffer

and electrophoresed for 1 h at 75 V. DNA bands were stained with ethidium bromide and visualized by UV transillumination (EASY Win32, Herolab, Germany) (Ambrasienė D. et al. 2004).

RAPD-PCR amplification

The polymorphism of *I. ricinus* was analyzed using random amplified polymorphic DNA method. The 47 DNR samples of *I. ricinus* ticks were used for RAPD reaction

At first, 10 different random oligonucleotide primers, synthesized in MBI Fermentas, Lithuania was tested for their usefulness for the typing of *I. ricinus* species (Table 1)

Table 1. Primer names and sequences amplified by RAPD-PCR for individuals of *I. ricinus*

Primer	Sequence (5' to 3')
OPA-01	5'-CAGGCCCTT-<C>-3'
OPA-02	5'-AATCGGGCT-<G>-3'
OPA-05	5'-AGGGGTCTT-<G>-3'
OPA-07	5'-GAAACGGGT-<G>-3'
OPA-08	5'-GTGACGTAG-<G>-3'
OPA-09	5'-GGGTAACGC-<C>-3'
OPA-10	5'-GTGATCGCA-<C>-3'
OPA-11	5'-CAATCGCCG-<C>-3'
OPA-17	5'-GGTCCCTGA-<G>-3'
OPB-11	5'-GTAGACCCG-<T>-3'

PCR was performed in reaction volume of 25 containing 12,5 μl 2X PCR Master Mix (MBI Fermentas, Lithuania), 2 μl 10- oligonucleotide primer (stock 10 pmol/ μl) (MBI Fermentas, Lithuania), 6,5 μl ddH₂O and 4 μl DNR of tick sample. All reactions were carried out in Eppendorf PCR system "Mastercycler personal" thermal cycler. Samples were initially denatured for 1 min at 94°C . Subsequent cycles were at 94°C for 30 sec (denaturation), 35°C for 30 sec (primers annealing), and 72°C for 1 min (extension). Forty cycles were performed. And final extending 72°C for 3 min..

RAPD fingerprinting analysis. For the analysis of PCR amplification products, 15 µl aliquots of reaction mixtures were applied to 1,7% agarose gels (MBI Fermentas, Lithuania) with Tris-Borate-EDTA (pH 8.2) as running buffer and electrophoresed for 2,5 h at 120 V. The DNA bands were stained with ethidium bromide and visualized by UV transillumination (EASY Win32, Herolab, Germany).

DNA fragments sizes were assessed by comparison with GeneRuler™ 100bp DNA Ladder Plus (MBI Fermentas, Lithuania).

In order to detect the genetic homogeneity as well as diversity of the *I. ricinus* individuals that were examined, the RAPD fingerprints were analyzed. Interpretation of the patterns was based on the size and on the presence or absence of amplified DNA bands.

The TREECON program (TREECOM for Windows (Van de Peer, De Wachter 1994)) was used to draw a dendrogram and evaluate genetic distance among individuals. Genetic distances were evaluated using Nei and Li formula (Nei & Li, 1979) and dendrogram was drawn using UPGMA (Unweighted Pair Group with Arithmetic Mean) method.

Results and discussion

Taxonomical identification. In total, 103 (3 nymphs, 27 male and 73 female) *I. ricinus* were

tested using PCR techniques for taxonomic identification. All of them reproduced 150bp patterns (Fig. 1). Analysis results confirmed morphometric data.

Molecular identification method was performed to identify collected ticks as *I. ricinus*. Other species of *Ixodid* ticks *I. persulcatus* is common in neighbourhood countries as Latvia, Byelorussia and Russia. It was found only ones in north part of Lithuania in 1972. According molecular phylogenetic analysis of these two species they not closely relatives, but differences in morphology are very small (Turcinaviciene et al., 2005). In our research one of analysed district was Biržai, which situated near the Latvian borderline. And we didn't know if we have these two sympatric species in Lithuania. PCR analysis data showed that all of collected ticks were *I. ricinus*.

The prevalence of *B.burgdorferi* in collected ticks

The 54 ticks from Vilnius district Nemenčinė locality and 49 ticks from 4 localities of Biržai forest: Spalviškiai, Būginiai Tamošiunai and Latveliai were tested for the presence of the *B.burgdorferi* s.l. using PCR technique. Achieved specific product of 276 base pairs was considered as a positive result (Fig. 2).

Overall, 10,7% ticks were infected with *B.burgdorferi*. The prevalence of *B. burgdorferi*

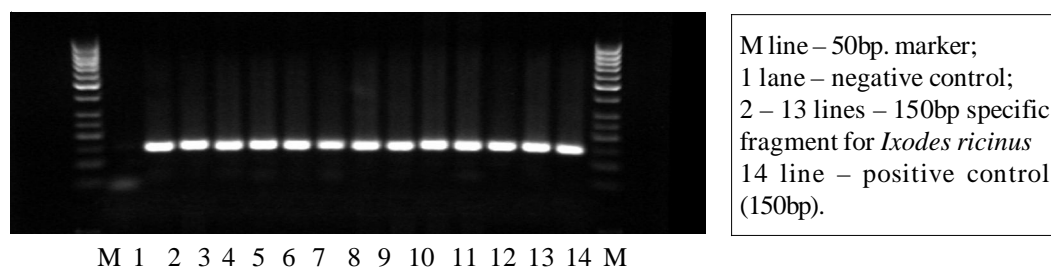


Figure 1. Results of PCR products analysis: 150bp product of the 5.8s rRNA gene, which is specific for *I. ricinus*

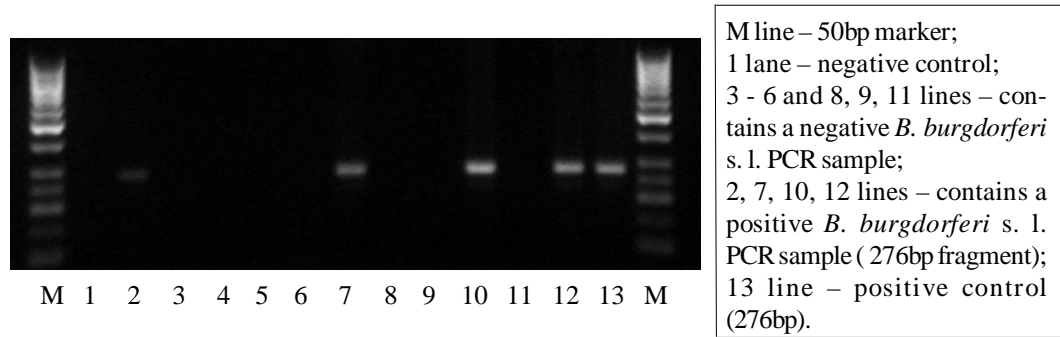


Figure 2. Results of PCR product analysis with specific oligonucleotide FL6 and FL7 primers

infection in *I. ricinus* in particular localities ranged from 0 to 16,7 % (Table 2).

The 6 of 49 ticks from Biržai district and 5 of 54 ticks from Vilnius district were infected with *B. burgdorferi* s.l. The highest proportion of infected tick – 12,7 % was noted in Biržai district (in Vilnius it was 9,3%). The rate of infection in this district was different in each of locality: Tamošiunai - 0 %; Spalviskiai- 12,9 %; Būginiai – 14,3; Latveliai – 16,7 (Table 2.).

RAPD analysis

The 47 DNR samples of *I. ricinus* ticks were used for RAPD analyzes: 27 from Biržai: 10- Spalviskiai; 6 – Būginiai; 5 – Tamošiunai; 6- Latveliai and 20 from Nemenčinė. For amplifica-

tion were chose five primers: OPA-01, OPA-02, OPA-05, OPA-07 and OPA-09, which produced more polymorphic and reproductive profiles. The each primer provided a distinct and reproducible pattern of amplified PCR fragments (Fig.3). The number of fragments and the amount of intraspecific polymorphism varied between the primers. In general, five RAPD primers produced fifty-four bands, with 6 to 18 individual bands being generated by each primer (Table 3).

Similarities between RAPD patterns were based on a fraction of identical fragment for every possible pair of the DNA samples.

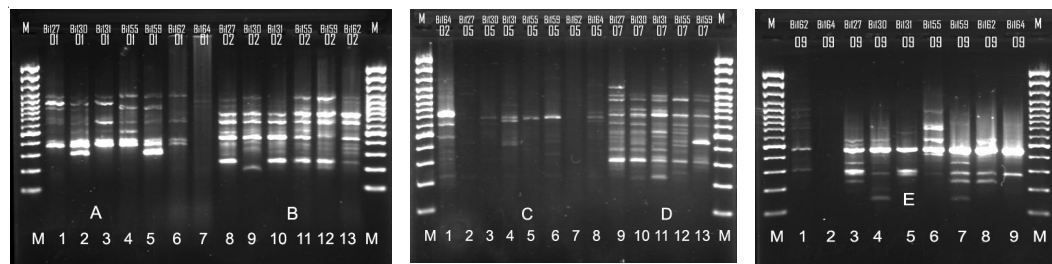
For each tick sample, the DNA fingerprinting patterns obtained with the five primers were combined. These combined patterns were used for the similarity estimation and cluster analysis.

Table 2. Infection rate of *Ixodes ricinus* ticks with *Borrelia burgdorferi* s.l.

District	Locality	Examined ticks	B. burgdorferi positive	
		n	n	%
Biržai	Spalviskiai	31	4	12,9
	Būginiai	7	1	14,3
	Tamošiunai	5	0	0
	Latveliai	6	1	16,7
Total in Biržai		49	6	12,2
Vilnius	Nemencine	54	5	9,3
Total in Biržai and Vilnius		103	11	10,7

Table 3. Primer names and sequences, number of polymorphic bands per primer and range of molecular weight in base pairs (bp) amplified by PCR for individuals of *I. ricinus*

Primer	Sequence (5' to 3')	Number of band range	Range of molecular weight (bp)
OPA-01	5'-CAGGCCCTT-<C>-3'	8	300 - 1100
OPA-02	5'-AATCGGGCT-<G>-3'	13	200-1070
OPA-05	5'-AGGGGTCTT-<G>-3'	6	300-1500
OPA-07	5'-GAAACGGGT-<G>-3'	18	300-1700
OPA-09	5'-GGGTAACGC-<C>-3'	9	250-800



M line – 100 bp marker;
1 – 13 lines – The DNA fingerprints from different sample of *I. ricinus*

Fig. 3. Reproducibility of RAPD fingerprinting. The DNA fingerprints of different sample of *I. ricinus* obtained by amplification with primers A - OPA-01; B - OPA-02; C - OPA-05; D - OPA-07; E - OPA-09.

In general were analyzed 54 loci and 53 (98,15%) of them were polymorphic.

viduals from Buginiai locality B(B)-127 and B(B)-130 – genetic distance is 0,14.

The phylogenetic tree was constructed by Treecon program using UPGMA method. Genetic distance among 47 individuals of *I. ricinus* ticks from Birzai and Vilnius populations were evaluated using Nei and Li formula (Nei & Li, 1979).

As we can see from dendrogram (Fig.3), *I. ricinus* individuals collected in Birzai and Vilnius are very polymorphic.

Some ticks from Vilnius and Birzai, according their genotypes, are similar and situated in the same clusters, but as we can see there are individuals that formed clusters according their sample places (Fig. 3. B and V). According dendrogram (Fig.3) most genetically distant individuals is sample from Birzai: B (T)-156 and B(S)-130 – genetic distance is 0,91 . Most genetically similar are indi-

Conclusion

1. Analysis data of Taxonomical identification of *I. ricinus* ticks showed that all of collected ticks were *I. ricinus*. Analysis results confirmed morphometric data.
2. According our results ticks' infection rates in surveyed areas were not so different, the percentage of infected ticks ranging from 9,3% in Vilnius district to 12,2% in Birzai. According the published data in the Lithuania in 2001-2002 (Ambrasiene et al., 2004), infection rate in Vilnius was 9,8% and in Birzai 11,5%.

3. Data of RAPD analysis showed, that *I. ricinus* individuals collected in Birzai and Vilnius district were very polymorphic. 98,15% of analysed locus were polymorphic. Reproduced RAPD pattern showed high degree of intraspecific variations. According evaluated genetic distance among individuals of *I. ricinus* we can conclude that Lithuanian *I. ricinus* ticks population is heterogenic.

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GENETIC VARIATION AND SUBSTRUCTURING OF GEOGRAPHICALLY DISTINCT BANK VOLE POPULATIONS IN LITHUANIA

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Skiriutė D., Paulauskas A., Balčiauskas L., Balčiauskienė L., Mažeikytė R., Šinkūnas R. 2005. Genetic variation and substructuring of geographically distinct bank vole populations in Lithuania. *Acta Biol. Univ. Daugavp.*, 5 (2): 177 - 185.

The bank vole (*Clethrionomys glareolus* Schreber, 1780) is the most widespread vole species in wooded habitats in Lithuania. Levels of genetic variability and possibilities of gene flow based on variation at seven polymorphic allozyme loci have been determined in this study among six disjunctive populations of bank vole. Mean heterozygosity (observed) per population was 9%. All bank vole populations exhibited heterozygote deficiency (mean $F_{IS}=0.455$) in this study. Mean value of G_{ST} for the polymorphic loci ($G_{ST}=0.521$) suggests a big genetic differentiation among geographically distinct populations. The average number of migrants per generation among those populations is estimated to be 0,06 therefore gene flow is quite weak evolutionary force among populations in this study. Differences in genetic structure of the populations depend on “strong” barrier effect rather than on isolation-by-distance.

Key words: bank vole, allozymes, genetic differentiation, isolation-by-distance, gene flow

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Introduction

Reproduction and survival of species affected by different ecological factors (habitat type, climatic conditions etc.), along with movements of individuals and gametes, leading to gene flow,

change frequencies of genes among populations (Ishibashi et al. 1997, Slatkin 1987). Substructuring of populations is dependent on territory available for movement (habitat configuration) and attributable to species dispersal and migration distances. Different types of barriers are known

to cause a geographic variation and genetic separation in species. Geographic differentiation of mtDNA haplotypes of bank vole females was observed over areas of about 8.5 km (Stacy et al. 1997). It was reported, that only 50 m wide highways caused no migration of bank voles suggesting a strong barrier effect on genetic differentiation (Gerlach & Musolf 2000). The old geographic barriers along with the most recent landscape fragmentation by roads etc. may cause a significant genetic differentiation in a species. Genetic distances among distinct bank vole populations appeared to be positively dependent on physical distances (Gebczynski et al 1993). This could lead to some geographical clusters in species genetic profile.

This study provides analysis of genetic differentiation of bank vole populations inhabiting different parts of Lithuania. The genetic profile of geographically distinct populations could therefore explain the individual phylogeographic history of populations. Present day patterns of genetic structure may often be the reflection of effects of Pleistocene glaciations and post-glacial species range expansions (Bossart & Prowell 1998).

Material and methods

Site description and trapping

The bank voles were sampled from six geographically distinct populations in Lithuania (table 1). During September of 1999–2001 bank voles were sampled from the Birzai, Kedainiai, Kaunas, Varena, Sakiai, and Zarasai districts (fig. 1). The distance between sampling sites differed from 34 to 272 km. Bank voles were captured with snap-traps. In most cases mixed deciduous forests were chosen as trapping sites except for Birzai and Zarasai in which the bank voles were captured in oak-wood and peat bog habitats respectively (table 1).

Enzyme electrophoresis and genotyping

Samples of bank vole liver tissue were homogenised and analysed using 5% and 7.5 % PAG electrophoresis as described in Skiriute et al. (2000). Four enzymes coded by seven genetic loci were analysed: (isozyme, abbreviation, E.C. number and corresponding structural gene loci in parentheses): glucose-6-phosphat dehydrogenase (Gpd1), malate dehydrogenase (Mdh1,2), malic enzyme (Me1), and non-specific proteins Np2,3,4. Gpd was resolved using Tris-EDTA-boratic buffer (pH 8,3). Staining was performed as described by Show & Prasad (1970) and Harris & Hopkinson (1977). Non-specific proteins were stained using Coomassie Brilliant Blue G-250 (Serva). Allelic variants were resolved by direct side-by-side comparison of migrating allozymes on the same gel. Unfortunately in some individuals, genotypes could not be determined for the entire set of loci due to insufficient quality of resolution.

Statistical analysis

First, loci were tested for departure from Hardy-Weinberg equilibrium (HWE) using chi-square (Weir & Cockerham 1984) and exact tests as implemented in BIOSYS-2 (Swofford & Selander 1997). Linkage disequilibrium between loci examined using GENEPOP (Raymond & Rousset 1995), with exact tests and applying Bonferroni correction. To characterize within-locality diversity we computed the mean number of alleles per locus (A) and Nei's unbiased estimates of average expected heterozygosity (H_E) (Nei 1978). Nei's gene diversity (H_T , H_S and G_{ST}) was used to evaluate the distribution of genetic diversity within and among populations (Nei 1973, 1977). The genetic structure within and among populations was also evaluated using F-statistics: F_{IT} , F_{IS} and F_{ST} , as in Weir and Cockerham (1984). and coefficients measure excesses of homozygotes or heterozygotes relative to the panmictic expectations within the entire samples and within the populations, respectively. The coefficient estimates relative population differentiation. among all localities was esti-

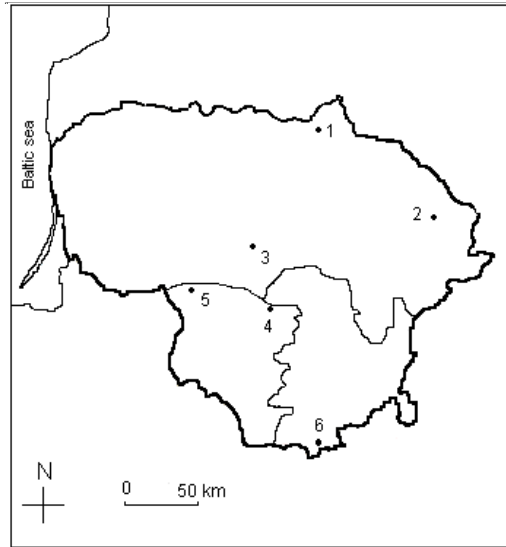


Fig. 1. Lithuanian sites, where bank voles were captured, and the river Nemunas: 1 – Birzai, 2 – Zarasai, 3 – Kedainiai, 4 – Kaunas, 5 – Sakiai, 6 – Varena

mated and tested for significance via permutation test.

Indirect estimate of gene flow (λ) – the number of migrants per generation between pairs of localities were calculated using the relationship based on (Wright 1969), which is mathematically related to gene flow (m) by the following equation: (Wright 1931).

Further in this study we examined the data for evidence of isolation-by-distance. We performed linear regression of estimates of F_{ST} between all pairs of localities against the logarithms of interpopulation map distances (in km) following Rousset (1997). In order to estimate the significance of regression there was performed Mantel test with 5000 randomisations for analysis. An additional linear regression analysis of barrier effect was performed on genetic distances vs. presence of barrier: no barrier – 0, presence of a barrier – 1.

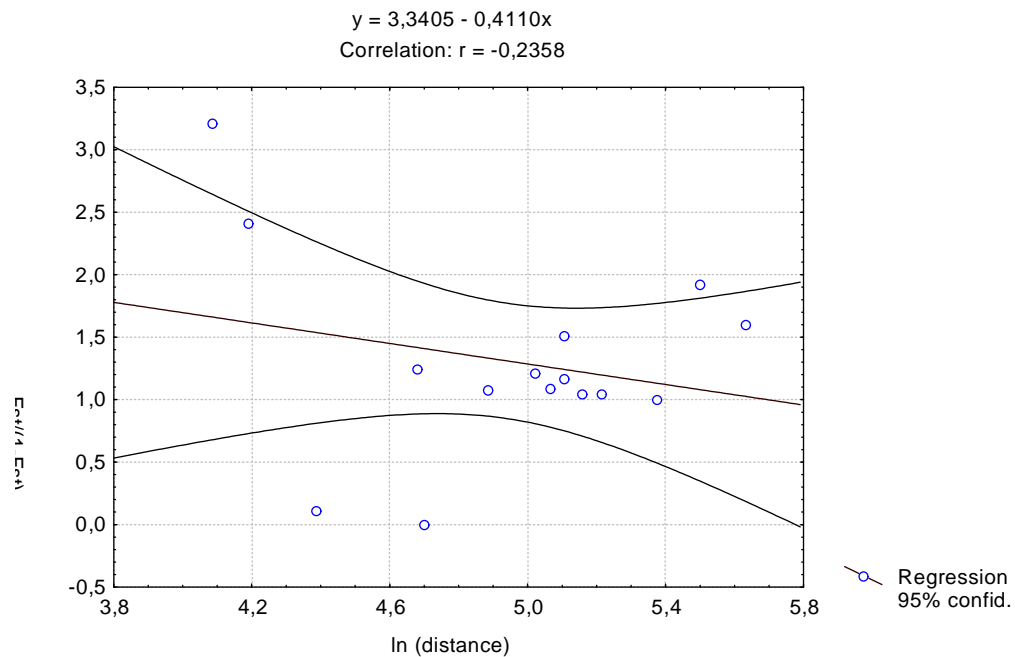


Fig. 2. Differentiation among bank vole populations in Lithuania. Multilocus estimates of pairwise differentiation are plotted against logarithm of map distances (in km). The maximum distance between two populations is 262,71 km. F_{ST} was estimated according to Weir & Cockerham (1984)

A dendrogram of Nei's genetic distances (D) between localities was constructed using unweighted pair group-method with arithmetic mean (UPGMA) (Sneath & Sokal 1973) from cophenetic matrices in STATISTICA 5.1 (Stat Soft 1998).

Results

Four polymorphic protein coding loci of six bank vole populations were studied. Np2 and Np3 loci were coded for two alleles and in most cases only one of them was found to be "private" in forming genotypes in a site. Three other loci (Gpd1, Np2, Np4) were polymorphic for two or three alleles in at least one site (table 2).

Intrapopulation genetic variability

Significant departures from HWE occurred at 5% level when 'globally' tested on a per-locus or per-locality basis, however there are cases when significant heterozygote deficiencies have been observed within populations at individual loci

(locus Gpd1 in all but Kedainiai and locus Np4 in Zarasai and Kaunas populations).

The mean number of alleles per locus was 1.62 (range 1.50 – 1.75), mean observed heterozygosity (H_o) was 0.095 (range 0.025 – 0.216) (Table 3). Allele Np2⁹⁹ is found to be rare in Birzai population ($q=0.09$) while the most frequent ($q=1$) in Kedainiai and Varena sites and absent in all the rest ones. No loci exhibited significant linkage disequilibrium at 5% level after sequential Bonferroni correction, assuming independence among loci in our data analysis. Analysis of fixation indices calculated for all polymorphic loci in each population showed deficiency of heterozygotes relative to the HWE with range from -0.903 in Zarasai to -0.158 in Kaunas populations.

Interpopulation differentiation and gene flow

On a per locus basis, the proportion of total genetic variation due to differences among populations (F_{st}) ranged from 0.069 for Gpd1 to 1.000 for Np3 with the mean of 0.521 indicating that

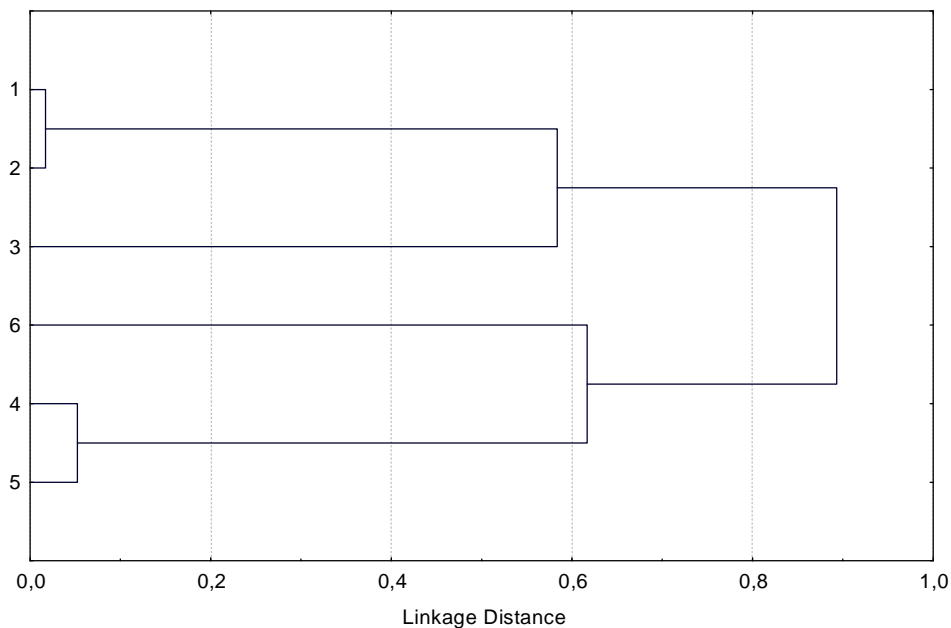


Fig. 3. A dendrogram showing the phylogenetic relationships among six populations of bank vole in Lithuania constructed using cophenetic matrices. Numbers of populations are given in Table 1 and Figure 1.

Table 1. Number of population in a study, locations, habitat type and the year of trapping

Pop number	District	Location	Habitat type	Year
1	Birzai	Draseikiai	Oak-wood	2000
2	Zarasai	Puscia	Peat bog	1999
3	Kedainiai	Liplunai	Mixed deciduous forest	2001
4	Kaunas	Dubrava	Mixed deciduous forest	2001
5	Sakiai	Gelgaudiskis	Mixed deciduous forest	2001
6	Varena	Cepkeliai reserve, Pogarenda	Mixed deciduous forest	2001

Table 2. Allele frequencies for polymorphic loci in six populations of bank vole. Locus abbreviations are given in the text; sample sizes are given in parentheses

Loci/ allele	Population					
	Birzai	Zarasai	Kedainiai	Kaunas	Sakiai	Varena
<i>Gpd1</i>	(33)	(14)	(11)	(8)	(20)	(8)
99	0.750	0.550	0.318	0.750	0.350	0.714
100	0.250	0.350	0.682	0.250	0.275	0.286
101		0.100			0.375	
<i>Mdh1</i>	(33)			(8)	(20)	
99	0.485	N/A	N/A	0.625	0.600	N/A
100	0.470			0.250	0.400	
101	0.045			0.125		
<i>Me1</i>		(13)	(8)		(20)	(7)
99	N/A	0.615	0.687	N/A	0.775	
100		0.308	0.313		0.225	0.714
101		0.077				0.286
<i>Me2</i>		(7)	(11)		(18)	
99	N/A	0.786	0.727	N/A	0.028	N/A
100		0.214	0.273		0.972	
<i>Np2</i>	(32)	(5)	(11)	(8)	(20)	(5)
99	0.094		1.000			1.000
100	0.906	1.000		1.000	1.000	
<i>Np3</i>	(33)	(5)	(11)	(8)	(20)	(8)
99	1.000	1.000	1.000			
100				1.000	1.000	1.000
<i>Np4</i>	(33)	(14)	(10)	(8)	(20)	(8)
99	0.561	0.786	0.900	0.875	0.825	0.500
100	0.439	0.214	0.100	0.125	0.175	0.500

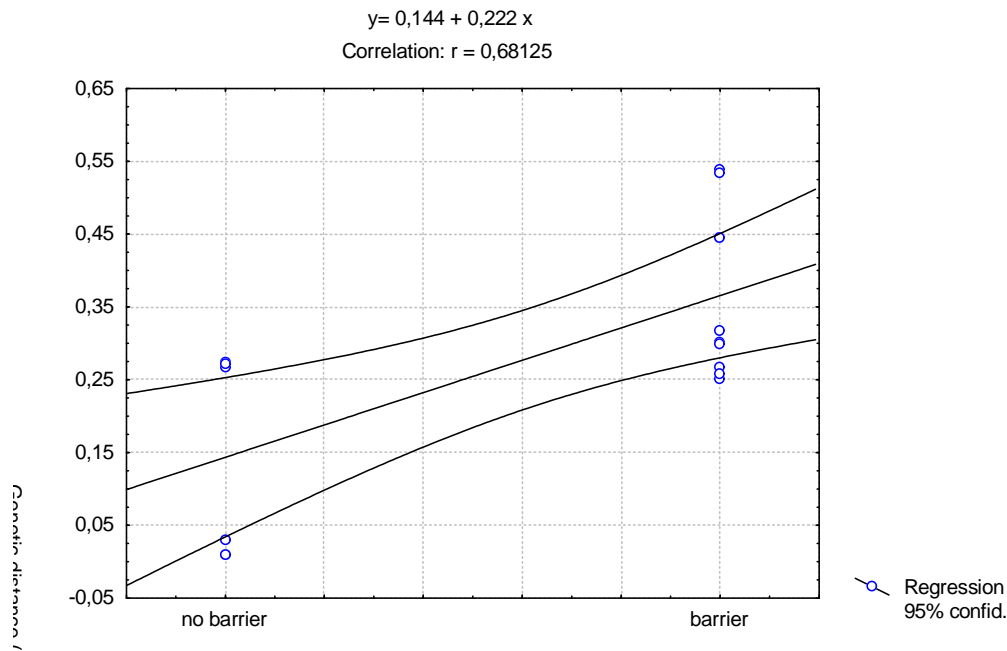


Fig. 4. Genetic differences due to “strong” barrier effect. Genetic distances (Nei, 1978) are plotted against presence or absence of a barrier

about 52% of the total allozyme variation was among populations (Table 4).

Using indirect method of estimating gene flow among populations based on , the mean number of migrants () per generation among these populations was 0.06. This value suggests that

gene flow isn't strong enough to prevent substantial differentiation due to genetic drift. In our study mean value below 1 show that populations are separate and not panmictic among localities in a study.

Table 3. Average number of alleles per locus (A), mean observed H_{ob} and expected H_{ex} heterozygosity and mean F_{IS} value for populations

Pop	N	A	Hob (SE)	Hex (SE)	F_{IS}
Birzai	33	1.75	0,215 (0,04)	0,267 (0,11)	0.203
Zarasai	14	1.75	0,025 (0,03)	0,247 (0,15)	0.903
Kedainiai	11	1.50	0,068 (0,04)	0,161 (0,12)	0.589
Kaunas	8	1.50	0,062 (0,04)	0,158 (0,09)	0.622
Sakiai	20	1.75	0,137 (0,04)	0,244 (0,16)	0.442
Varena	8	1.50	0,062 (0,05)	0,243 (0,14)	0.757
MEAN	15.7	1.62	0,095 (0,04)	0,220 (0,13)	0.586

Table 4. Total genetic diversity (H_T), genetic diversity within population (H_S), deviations of genotype frequencies from Hardy-Weinberg expectations over all populations (F_{IT}) and within individual populations (F_{IS}), and proportion of total genetic diversity partitioned among populations (G_{ST}) of *Clethrionomys glareolus*

Locus	H_T	H_S	Nem	F_{ST}	F_{IT}	G_{ST}
<i>Gpd1</i>	0.552	0.514	2.22	0.101	0.726	0.069
<i>Np2</i>	0.455	0.030	0.04	0.848	0.838	0.934
<i>Np3</i>	0.500	0.000	0.00	1.000	1.000	1.000
<i>Np4</i>	0.393	0.365	2.53	0.090	0.282	0.071
MEAN	0.475	0.227	0.06	0.538	0.748	0.521

Table 5. Genetic (Nei, 1978) and geographic distance matrix between six bank vole populations. 1 – Birzai, 2 – Zarasai, 3 – Kedainiai, 4 – Kaunas, 5 – Sakiai, 6 – Varena

pop	1	2	3	4	5	6
1	***	0.009	0.273	0.268	0.301	0.445
2	96.016	***	0.268	0.251	0.258	0.507
3	135.560	139.800	***	0.538	0.533	0.317
4	164.368	151.054	34.286	***	0.030	0.272
5	183.364	198.937	59.162	62.750	***	0.299
6	262.710	213.714	146.345	112.649	153.649	***

Isolation-by-distance

Isolation-by-distance will generate a positive correlation between geographical distances and multilocus estimates of pairwise differentiation (Raymond & Rousset 1995, Rousset 1997). For bank vole populations analysis of plotted against logarithm of map distances (km), when taken into account all populations in the study, yields a negative slope and the relationship is not significant ($r=-0.24$, $p=0.557$) as it is indicated by Mantel analysis with 5000 permutations (fig. 2). These results show that the bank vole genetic differentiation is not strongly related to the degree of geographic isolation.

Genetic distances and barrier effect

Genetic distance value estimates the number of allelic substitutions per locus that have occurred since populations diverged. Genetic distance (Nei 1978) among pairs of bank vole populations ranged from 0.009 to 0.538 when scoring from four polymorphic loci (Table 5). The similarity among the bank vole populations in Lithuania can be seen in the genetic tree in figure 3.

A linear regression analysis of a barrier effect was performed on genetic distances vs. presence of barrier (the river Nemunas). Positive significant relationship ($r=0.68$, $p=0.003$) between genetic distance and barrier (fig. 4) showed dependence of genetic differentiation on natural historical barriers.

Discussion and conclusions

All populations in the study are not in consideration with HWE because of heterozygote deficiency ($F_{IS}=0.58$). Np3 locus differs by appearing slower migrating allele Np⁹⁹ on a gel on the populations Birzai, Zarasai and Kedainiai ($q=1$) and a faster allele in Kaunas, Sakiai and Varena sites ($q=1$). Total diversity in Np3 locus between populations is $H_T=0.500$ and at intrapopulation level $H_S=0.000$ exhibits no variation at all. Overall intrapopulation diversity as indicated by H_S value ranged from 0 to 0.514 in Np3 and Gpd2 loci respectively.

The mean $F_{IS}=0.586$ and $F_{IT}=0.748$ values suggest strong population subdivision. Populations are quite differentiated in different geographic locations. 46% (mean $F_{ST}=0.54$) of the total variation in allele frequency resides inside localities and this is mostly imposed by Np2 and Np3 loci in which all genetic diversity lies among populations (54%). Overall the fixation index as a measure of differentiation supports the consideration that there is a strong barrier to gene flow between bank vole populations.

The more far from each other reside populations geographically the more distinct genetically they become. The highest genetic distance occurred in far apart inhabiting populations in most cases Birzai–Varena [262 km, $D=0.445$], Zarasai–Varena [213 km, $D=0.507$], Kaunas–Kedainiai [34 km, $D=0.538$] and Kedainiai–Sakiai [59 km, $D=0.533$] are quite close in space but different in genetic structure, suggesting that the linear geographic distance is not the only reason for preventing gene flow among studied areas. In some taxa (saddle-back tamarins) rivers form an effective barrier to dispersal (Peres et al., 1996). However in some species of small mammals like spiny rat and common shrew river doesn't reduce gene flow (Lugon-Moulin et al., 1999, Patton et al., 1994). The barrier effect in bank vole populations appears to be of high importance (barrier effect regression $r=0.68$) and possibly divides populations in to distinct genetic lineages from South and the North of Lithuania. We presume, that populations may be separated by biggest

rivers (Nemunas and Neris - South-North direction), and main roads, like Via Baltica (East-West direction). The distribution of genetic variation among populations also can be the result of a historic gene flow (Milligan et al., 1994).

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TRENDS OF CHANGES OF GENETIC STRUCTURE OF THE POPULATION OF *BLUMERIA GRAMINIS* F.SP. *HORDEI* IN THE LATGALE REGION OF LATVIA IN 2003-2004

Inese Kokina, Isaak Rashal

Kokina I., Rashal I. 2005. Trends of changes of genetic structure of the population of *Blumeria graminis* f.sp. *hordei* in the Latgale region of Latvia in 2003 - 2004. *Acta Biol. Univ. Daugavp.*, 5 (2): 187 - 192.

In 2003-2004, samples of the pathogen *Blumeria graminis* f.sp. *hordei* both in sporulation and cleistothecia phase were collected from commercial barley fields in the Latgale region of Latvia near the town Daugavpils. Tendency to increasing of frequencies of virulence genes *Va1*, *Va3* and *Va13* were detected in the population, especially in the phase of cleistothecia. During last years virulence genes *Va6*, *Va7*, *Va9*, *Va12*, *Vk* and *Vla* were presented in the Latgale region with high frequencies. In 2003-2004, tendency of decreasing of virulence frequencies, especially in the phase of sporulation, were detected for those virulence genes. Low frequencies still were detected for virulence genes corresponding to the resistance from 'Steffi', 'Goldie', and 'Meltan'. Not any isolate, which overcome barley line *SII* resistance genes, was found.

Key words: powdery mildew, virulence frequencies, barley, resistance

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Introduction

Obligate biotrophic fungi, like causal agent of barley powdery mildew *Blumeria graminis* (DC.) Golovin ex Speer f.sp. *hordei* Em. Marchal (synonym *Erysiphe graminis* DC. f.sp. *hordei* Em. Marchal), has specific "gene-for-gene" interactions with the host. Interactions are determined by unique combinations of host resistance genes, designed *Ml*, and cognate pathogen avirulence genes (Haltermann, Wise, 2004). There is a long history of using specific resistance genes to control of barley powdery mildew in Europe

(Wolfe, Schwarzbach, 1978; Rashal, Tueryapina, 1991; Wolfe et al., 1992; Müller et al., 1996; Dreiseitl, Jrgensen, 2000; Finckh et al., 2000; Czembor, Blandenopoulos, 2001). At the same time, resistance, which is based on the race-specific barley resistance genes, usually can not be effective for the long-time exploitation. The main reason of this is a high level of pathogenic variability in natural populations of *Blumeria graminis* f.sp. *hordei*. According to the "gene-for-gene" interactions, just one mutation may cause a pathogen to become virulent on a host with the matching resistance gene. Pathogen can

Table 1. Samples of *Blumeria graminis* f.sp. *hordei* collected in the Latgale region in 2003-2004

Date of collecting	Phases of the life cycle of the pathogen	Number of tested isolates
July 15	sporulation	27
August 10	cleistothecia	30
July 15	sporulation	24
August 10	cleistothecia	30
Total:		111

develop many new genotypes and spores are spread by the wind over large distances across Europe (Limpert et al., 1999; Hovmøller et al., 2000; Czembor, Blandenopoulos, 2001; Brown, Hovmøller, 2002.). That is a reason why populations of the pathogen are changeable and regular observations of local populations are necessary for understanding of trends of evolution of the pathogen. At the same time, such kind of knowledge is obligate for choosing a host breeding strategy for the resistance.

In the Latgale region of Latvia (South-eastern part of Latvia), observations of the *Blumeria graminis* f.sp. *hordei* population was done since 1995 (Rashal et al., 1997; Rashal et al., 2000 a; Rashal et al., 2000 b; Kokina, Rashal, 2001; Kokina, Rashal, 2004; Rashal et al., 2004; Kokina, Rashal, 2005). The main objective of this paper is presenting frequencies of virulence genes in the pathogen population of the region in 2003-2004 and discussing trends of changes those virulences in comparison with the previous years.

Materials and methods

In 2003-2004, samples of the pathogen were collected in sporulation and cleistothecia phases from commercial barley fields in the Latgale region of Latvia, approximately 7 km from the town Daugavpils (Table 1). Isolates were multiplied from a single pustule on the universally susceptible barley variety 'Otra'. Only first leaves of plants were used for monopustules multiplication.

For virulence testing the first leaf of the set of differentials (Table 2) was used. Inoculation of differentials was done by the microinoculation

technique (Dreiseitl, 1998). According to the method, spores of monopustule isolates were sucked into the micropipette and blown into settling tower, under which detached leaf segments of differentials were exposed on 0.004 % benzimidazole agar in a Petri plate and then incubated under 18-20°C in light with photoperiod 8-10 h.

After 8-9 days of incubation disease reaction types of differentials were scored according Torp et al. (1978). This scoring was done in a 0-4 scale and differentials were classified into susceptible or resistant groups. Leaf segments of differentials with infection types 0-3 were classified as resistant, segments that scored 4 – as susceptible. Frequencies of virulence genes in the pathogen population were calculated.

Results and discussion

Frequencies of virulence genes *Va1*, *Va3* and *Va13* in the population of the pathogen in 2003-2004 are presented in Fig. 1. For those virulence genes a medium level of virulences was detected in the population: virulence frequencies in different samples varied from 29 to 50%. For virulence genes *Va1* and *Va3* a clear tendency to increasing of virulence frequencies in the phase of cleistothecia in both years of the investigation was observed. In case of *Va13*, increasing of virulence frequencies in cleistothecia was detected in the pathogen samples in 2004 only. In 2003, virulence frequencies of *Va13* in the sporulation phase were higher in comparison with the phase of cleistothecia. During the previous years virulence frequencies of genes *Va1*, *Va3* and *Va13* were lower and varied in different samples approximately from 12 to 30% (Kokina, Rashal,

Table 2. Set of differentials used for detection of pathotypes in the population of *Blumeria graminis* f.sp. *hordei* in the Latgale region in 2003-2004

Differentials	Main resistance genes
<i>P01</i>	<i>Mla1</i>
<i>P02</i>	<i>Mla3</i>
<i>P03</i>	<i>Mla6</i>
<i>P04B</i>	<i>Mla7</i>
<i>P08B</i>	<i>Mla9</i>
<i>P10</i>	<i>Mla12</i>
<i>P11</i>	<i>Mla13</i>
<i>P17</i>	<i>Mlk</i>
<i>P23</i>	<i>MLa</i>
<i>SII</i>	<i>MI(SII)</i>
'Steffi'	<i>MI(St1)</i> , <i>MI(St2)</i>
'Goldie'	<i>Mla12</i> , <i>MLa</i> , <i>U</i>
'Meltan'	<i>Mla13</i> , <i>MI(Im9)</i> , <i>MI(Hu4)</i>

2004). Described results illustrated known fact that effectiveness of the race-specific resistance genes can be broken down in rather short time, as well as fact that increasing virulence frequencies of low-medium virulence genes is possible during the growing season. It can be explained by the recombination during the sexual spores production. Influence of spore migration during the growing season can not be also excluded because in some European countries virulence frequencies of the mentioned above virulence genes were medium-high already earlier (Hovmøller et al., 2000) because wide using of particular resistance. For example, resistance gene *Mla13*, corresponding to the virulence gene *Va13*, was used in commercial varieties in the Central and Western Europe during the 1970s, starting in Denmark and Czechoslovakia (Limpert et al., 1999). Increasing of frequencies of virulence genes *Va1*, *Va3* and *Va13* is not favourable for choosing of corresponding resistance genes *Mla1*, *Mla3* and *Mla13* for a resistance breeding programme.

High level of virulences for *Va6*, *Va7*, *Va9*, *Va12*, *Vk* and *Vla* was detected (Fig. 2), it varied from 44 to 77%. A tendency to increasing of frequencies of mentioned above virulence genes in the phase of cleistothecia was also observed. In 2003, the most considerable differences in virulence frequencies between sporulation (44%) and

cleistothecia (60%) phases were detected for *Vk* virulence. Comparison with previous results (Kokina, Rashal, 2004) shows that some decreasing of frequencies of virulence genes *Va6*, *Va7*, *Va9*, *Va12*, *Vk* and *Vla* occurred in 2003-2004, especially in the phase of sporulation. During the previous years mentioned above virulence genes were widely presented in the population (70-90%). Results suggested that significant changes of these virulences can be occurred in the population in future.

Resistance factors from 'Steffi?', 'Goldie?' and 'Meltan?' is still rather effective in the Latgale region at the moment (Fig. 3). Frequencies of corresponding virulence genes *VSt*, *VGo* and *VMe* were low and varied from 8 to 22%. Increasing of the frequency of those virulences in the phase of cleistothecia appeared only in 2004, in 2003, in opposite, frequencies of the mentioned virulences in the phase of cleistothecia were even lower, than in the sporulation phase. The most considerable changes of virulence frequencies between different phases of the life cycle were found for *VMe* (Fig. 3). Actually, *VMe* virulence corresponds to combination of resistance genes *Mla13*, *MI(Im9)* and *MI(Hu4)*. It means, that to overcome the host resistance the pathogen must accumulate several mutations.

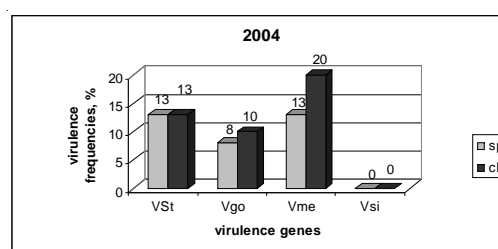
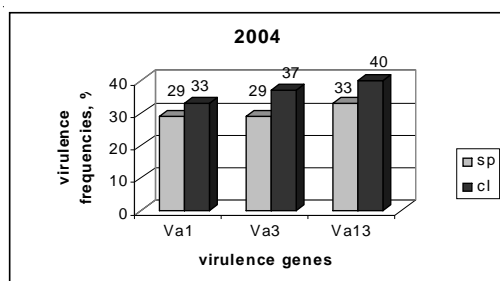
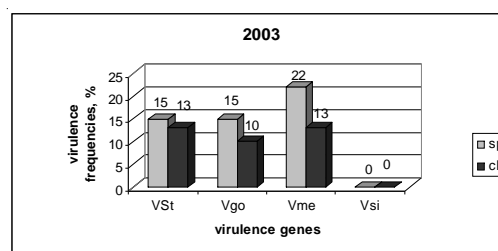
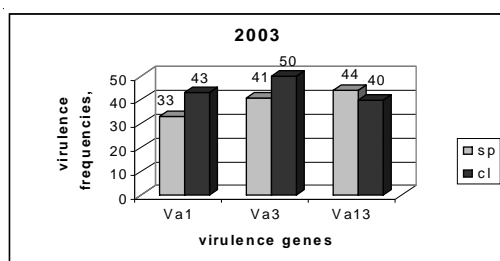


Fig. 1. Frequencies of virulence genes *Va1*, *Va3* and *Va13* in the population of *Blumeria graminis* f.sp. *hordei* in the Latgale region in 2003-2004

Fig. 3. Frequencies of virulence genes *VSt*, *VGo*, *Vme* and *Vsi* in the pathogen population of *Blumeria graminis* f.sp. *hordei* in the Latgale region in 2003-2004

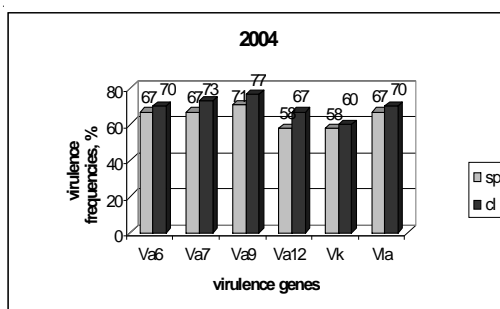
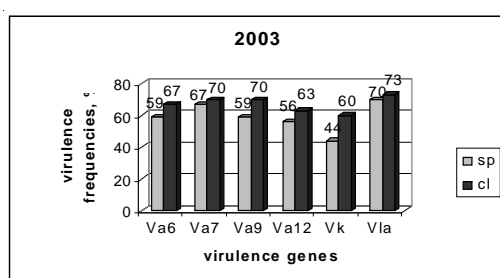


Fig. 2. Frequencies of virulence genes *Va6*, *Va7*, *Va9*, *Va12*, *Vk* and *Vla* in the population of *Blumeria graminis* f.sp. *hordei* in the Latgale region in 2003-2004

At the moment *SII* resistance factors are considered as a new resistance source for the controlling of powdery mildew in Europe (Hovm•ller et al., 2000). It is completely true also for the Latgale region: not any isolate with the correspondent virulence was detected in the pathogen population in 2003-2004.

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