Agronomy Research **8** (Special Issue III), 615–624, 2010

Evaluation of *Lolium perenne* L. wild ecotypes of Ukrainian origin in Lithuania

V. Kemešytė[1], N. Lemežienė[1], J. Kanapeckas[1], E. Vilčinskas[1], L. Z. Baistruk-Glodan[2] and R. Ivanciv[2]

[1]Institute of Agriculture, Lithuanian Research Centre for Agriculture and Forestry Instituto al. 1, Akademija, Kedainiai distr., Lithuania; e-mail: vilma@lzi.lt
[2]Prikarpatjan branch of Western region Institute of Agriculture and Animal Husbandry, Ukrainian Academy of Agrarian Science., Lishnia, Lvov r., Ukraine; e-mail: d_stancija@mail.lviv.ua

Abstract. The plants of 23 wild populations of perennial ryegrass (*Lolium perenne* L.) were collected in 2006 and 2007 in the natural habitats of grasses in West Ukraine (Prikarpatja, Karpat and Zakarpatja region). These populations and 2 Ukrainian varieties were planted in 2008 and 2009 into the germplasm collection at the Lithuanian Institute of Agriculture. In the collection trials the following parameters were assessed: winter damage, beginning of heading, growth at the beginning of vegetation and re-growth after cuts, plant height, leaf colour, flag leaf length and width, dry matter yield, disease incidence and chemical composition. The Ukrainian wild populations distinguished by winter hardiness, fast spring growth, high dry matter yield and forage quality under Lithuanian conditions. The plants of the wild Ukrainian populations were shorter, with narrower and shorter flag leaves and more sensitive to crown rust and leaf spot pathogens, compared with the Lithuanian varieties. The Ukrainian varieties exhibited early heading and worse chemical composition, compared with the standard Lithuanian variety 'Žvilgė'.

Key words: ryegrass, wild population, agro-morphological trait, chemical composition

INTRODUCTION

As agriculture becomes more and more intensive, production of high-quality feed, even under unfavourable growing conditions and without any damage to surrounding ecosystems, is crucial (Harkot, 2005). Perennial grass breeders have the task of developing new varieties, characterized not only by yield but also by resistance to adverse weather conditions, diseases and pests. Wild populations of perennial grasses are still found in the natural habitats, showing high variability of agro-morphological traits and a variety of biological properties. Therefore expeditions are organized in connection with genetic resources conservation.

Recently, due to climate warming, more attention by grass breeders has been paid to the genotypes that are resistant to drought and diseases and that offer the most economically advantageous properties. Due to our neighbouring countries' natural habitats and breeding organizations' genetic resources (wild populations, breeder's lines and varieties) being the most adapted to the adverse environmental conditions, the

initial breeding material is being sought there. One of these countries is the Ukraine situated just south of Lithuania.

Lolium species are highly important forage and turf grasses in Europe and other temperate zones of the world. Perennial ryegrass (*Lolium perenne* L.) is economically the most important species within genus *Lolium* and is the main constituent of productive pastures (Humphreys et al., 2006).

Perennial ryegrass has been grown in Lithuania for a long time. It is attributed to the most valuable grasses. It has been noted for high productivity (Reheul et al., 2003) and very good forage quality (Kanapeckas et al. 1999; Aavola et al., 2003).

The aim of current study was to analyse the Ukrainian wild populations and varieties of perennial ryegrass for forage and turf under Lithuanian conditions.

MATERIALS AND METHODS

The plants of 23 wild populations of perennial ryegrass (*Lolium perenne* L.), collected in 2006 and 2007 in the natural habitats of grasses in west Ukraine (Prikarpatja, Karpat and Zakarpatja region), and 2 varieties ('Drogobičskij-16', 'Drogobičskij-2') were planted in 2008 and 2009 into the germplasm collection at the Lithuanian Institute of Agriculture. Lithuanian variety 'Žvilgė' was used as standard in 2008 and 'Veja' – in 2009.

Plants of each genotype were planted at 50 x 50 cm distances. In the autumn of each year of use phosphorus and potassium fertilisers ($P_{60}K_{90}$) were applied. Nitrogenous fertilisers (N_{150}) were applied each year of herbage use in several applications: in spring N_{60}, and N_{45} after the first and second cut.

In the collection trials the following parameters were assessed: winter damage, beginning of heading, growth at the beginning of vegetation and after cuts, plant height, leaf colour, flag leaf length and width, dry matter yield, disease incidence and chemical composition.

Re-growth, disease incidence, leaf colour and winter damage were estimated using the standard UPOV methodology (International union for the protection of new varieties of plants, 2006), where 9 scores represent very high and 1 – very low value of the trait. Leaf colour was assessed by score: 1 – very light green colour, 9 – very dark green colour.

Herbage yield was measured by weighing production per plot; dry matter content was measured in 0.5 kg samples collected at each cut. In order to determine the chemical composition, 0.5 kg samples were collected when ryegrass was at heading stage. Concentrations of crude protein (CP), water-soluble carbohydrates (WSC), crude fibre (CF) and dry matter digestibility (DMD) were evaluated using near-infrared spectrometer NIRS – 6500 (Perstorp Analytical, Silver Spring, Maryland, USA) (Butkute et al., 2003).

The winters during the experimental years were mild. Early spring of 2008 was warm and humid, therefore the conditions for rapid plant growth were favourable, but later growth was retarded due to drought in May. During summer, weather was warm and dry until the middle of June and later it was moderately wet. The spring of 2009 arrived early and was characterized by the abundance of frost, which lasted until the end of May. Changing temperatures and uneven rainfall distribution were also typical

of this period. The summer was cool and rainy and the autumn was long, rainy and warm. Statistical reliability of research data was assessed with the least significant difference ($LSD_{0,05}$). The statistical analysis computer software ANOVA adapted by P. Tarakanovas in the Visual Basic of Application as macro program to run in the EXCEL was used (Tarakanovas & Raudonius, 2003).

RESULTS AND DISCUSSION

Winter damage. Ryegrass differs from other forage grasses by low resistance to adverse conditions in the cold season. During the research period the winters were relatively mild. For this reason the damage was low in both winters: 2007–2008 – score 1.6, and in 2008–2009 – score 1.5 (Table 1).

The standard variety 'Žvilgė' and wild populations No 3705, 3709, and 3718 were the least (score 1) affected by winter conditions in 2007–2008. The post-winter damage of the other populations and varieties was higher – score 2. Wild populationsNo 3770, 3772, 3773 and 3774 were similar to the standard Lithuanian variety 'Veja' according to this feature in 2008–2009. The plants of other populations wintered worse, compared with the standard variety. The most winter damaged plants were those of the wild population No 3771 (score 3).

Beginning of heading. Plants of the standard variety 'Žvilgė' started heading on 28 May in 2008. On the same day beginning of heading was recorded for the wild populations No 3709 and 3718. The Ukrainian varieties were the earliest (23 May). The latest was the wild population No 3712. The beginning of heading for the Lithuanian variety was recorded on 26 May 2009, for the wild populations of Ukrainian origin on 25–31 May. The wild population No 3772 started heading on the same day as the standard variety, the population No 3771 by one day earlier. The latest was the wild population No 3768. The time of heading for the other populations was registered on 30 May.

Growth in spring and after cuts. The investigated wild populations grew well in spring. The average score was 8.4 in 2008 and 7.5 in 2009, the re-growth after cuts was lower – respective scores 5.7 and 4.0. The early spring growth of the Ukrainian varieties and wild populations equalled or surpassed the Lithuanian standard variety 'Žvilgė'. The Ukrainian variety 'Drogobičskij-2' and the wild populations No 3702, 3705 and 3718 grew better (score 9) compared with the standard variety (score 8). The wild population No 3717 had the best re-growth after cuts (score 9). The wild populations No 3705 and 3706 had good re-growth (score 8). The Ukrainian varieties 'Drogobičskij-16' and 'Drogobičskij-2' exhibited the worst re-growth after cut (score 3). The wild population No 3773 equalled (score 9) to the standard variety comparing the spring growth in 2009. Other populations' growth was poorer. Conversely, the majority of the wild Ukrainian populations' re-grew faster after the cuts than the Lithuanian variety. The wild populations No 3769, 3773 and 3774 equalled to the standard variety 'Veja'.

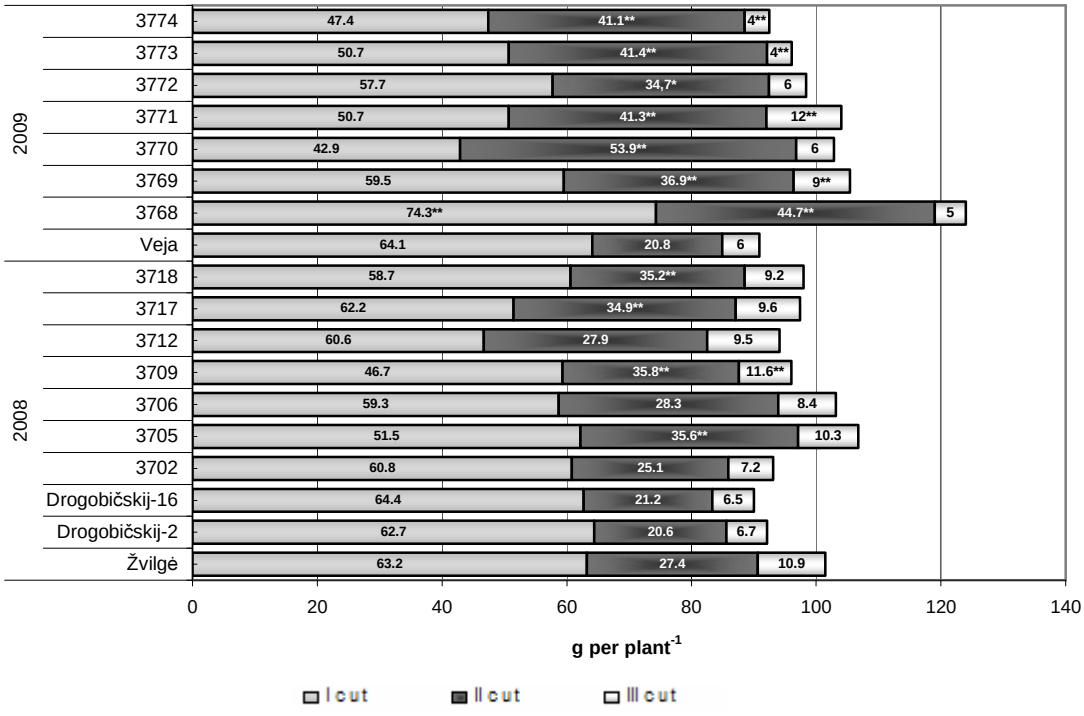
Table 1. The agro-morphological traits of the Ukrainian varieties and prominent wild populations of perennial ryegrass under Lithuanian conditions.

Variety, wild population	Winter damage (score)	Beginning of heading (date)	Growth in spring (score)	Re-growth after cuts (score)	Disease incidence (score)	
					leaf spot	crown rust
2008 (n = 16)						
Standard ‘Žvilgė‘	1	28 May	8	7	2	1
’Drogobičskij-16’	2	23 May	8	3	2	1
‘Drogobičskij-2’	2	23 May	9	3	2	1
3702	2	24 May	9	5	2	1
3705	1	03 June	9	8	1	1
3706	2	01 June	8	8	2	1
3709	1	28 May	8	5	2	1
3712	2	04 June	8	5	2	2
3717	2	02 June	8	9	1	1
3718	1	28 May	9	5	2	2
Average	1.6		8.4	5.7	1.8	1.2
2009 (n = 7)						
Standard ’Veja’	1	26 May	9	3	3	2
3768	2	31 May	7	5	3	2
3769	2	30 May	7	3	3	2
3770	1	30 May	7	5	4	3
3771	3	25 May	7	5	4	2
3772	1	26 May	7	5	4	2
3773	1	30 May	9	3	4	2
3774	1	30 May	7	3	3	2
Average	1.5		7.5	4.0	3.5	2.1

Score 9 represent very high, 1 – very low value

Disease incidence. Leaf spot pathogen (*Drechslera* spp.) caused more damage (average scores of 1.8 and 3.5 in 2008 and 2009, respectively) than the crown rust (*Puccinia coronata* Corda) pathogen (average scores of 1.2 and 2.1 in 2008 and 2009, respectively) (Table 1). There were no substantial differences in disease infection levels between the standard variety and the most Ukrainian varieties and wild populations in 2008, and only the wild populations No 3717 and 3705 were the exception. Their plants were more resistant to leaf spot (score 1). Wild populations No 3712 and No 3718 were the least resistant to rust (score 2). Four wild populations proved to be more vulnerable to the damage of leaf spot than the Lithuanian variety 'Veja' in 2009. The standard variety and the Ukrainian wild populations were equally damaged by rust (score 2). An exception was the wild population No 3770. It was the most susceptible to the pathogen.

Dry matter yield (DMY) is one of the most important characteristics describing forage grasses. It depends on many factors: genotype, weather, soil, etc. The DMY harvested from the Ukrainian varieties and wild populations in the first cut was smaller, compared with the Lithuanian variety (63.2 g plant^{-1}) in 2008 (Fig. 1). The variety 'Drogobičskij-2' (64.4 g plant^{-1}) was an exception. The yield of the second cut



*significantly different from standard at $P < 0.05$ probability level

Figure 1. Dry matter yield of the Ukrainian varieties and prominent wild populations under Lithuanian conditions in 2008–2009.

harvested from the Ukrainian varieties was smaller, compared with the standard. However, the yield of wild populations was higher. The wild populations No 3705, 3706, 3712 and 3717 had significantly ($P < 0.01$) higher yield in the second cut. The wild population No 3712 (11.6 g plant^{-1}) produced significantly ($P < 0.01$) higher third yield, the other Ukrainian wild populations and varieties lower DMY in comparison with 'Žvilgė'. The higher annual DMY, compared with the standard variety, was estimated in the wild populations 3705 (106.7 g plant^{-1}) and No 3706 (103.1 g plant^{-1}).

DMY of the first cut harvested from most of the wild populations in 2009 was significantly ($P < 0.05$) lower compared with the Lithuanian variety 'Veja' (Fig. 1). Plants of the wild population No 3768 were an exception. Their DMY (74.3 g plant^{-1}) significantly ($P < 0.01$) distinguished from the others. There were high rainfalls in June and July 2009. This had an impact on the extremely high yield from the second cut, which was significantly higher in all the populations, compared with the standard. Significantly ($P < 0.01$) higher DM yield was harvested in the third cut from the wild populations No 3769 and 3771. No 3773 and 3774 (4.0 g plant^{-1}) had significantly ($P < 0.01$) lower DMY. The second cut led to higher annual DMY in wild populations. The highest DMY was obtained from the population No 3768 – 124.0 g plant^{-1}.

Chemical composition. Herbage quality is becoming increasingly important in milk and meat production. The quality is determined by various factors: genotype, growing technology, weather conditions, cutting time and plant growth stage (Alibegovic-Grbic & Civic, 2004; Harkot, 2005). The ryegrasses are considered to be high–quality forages and their high digestibility makes them suitable for all types of ruminants (Aganga & Omphile, 2004).

The chemical plant composition index was determined in the populations of different winter hardiness, re-growth and earliness (Table 2). CP is the only forage component that contains nitrogen and is the source of amino acids, essential for cattle growth, lactation and reproductive functions (Butkutė & Pauplauskienė, 2006). Fibre is the main compound of plant cell wall. It consists of cellulose, hemi-cellulose and lignin (Jones & Moseley, 1993). Ryegrass accumulates more water-soluble carbohydrates, other digestible compounds and less crude fibre than timothy, orchardgrass or other forage grasses. There is a great interest in combining ryegrass's ability to produce WSC with effective use of nitrogen (Wilkins et al., 2003).

The Ukrainian varieties exhibited worse herbage quality, compared with the Lithuanian standard variety 'Žvilgė' in 2008 (Table 2). The Ukrainian varieties had significantly higher CF and lowest concentrations of WSC and digestible DM, compared with the standard. The wild population No 3702 had significantly ($P < 0.05$) lower content of CP (91.8 g kg^{-1}).

The herbage quality of the wild populations was similar or higher compared with the standard variety 'Žvilgė' in contrast to the varieties (Table 2). The wild population No 3706 had the highest chemical content: CP – 125.0 g kg^{-1}, CF – 197.0 g kg^{-1}, WSC – 317.0 g kg^{-1}, DMD – 785.0 g kg^{-1}.

The wild populations distinguished by higher herbage quality compared with the Lithuanian variety 'Veja' in 2009. Their plants had significantly ($P < 0.05$) higher CP,

Table 2. Chemical composition of the Ukrainian wild populations and varieties under Lithuanian conditions in 2008 and 2009.

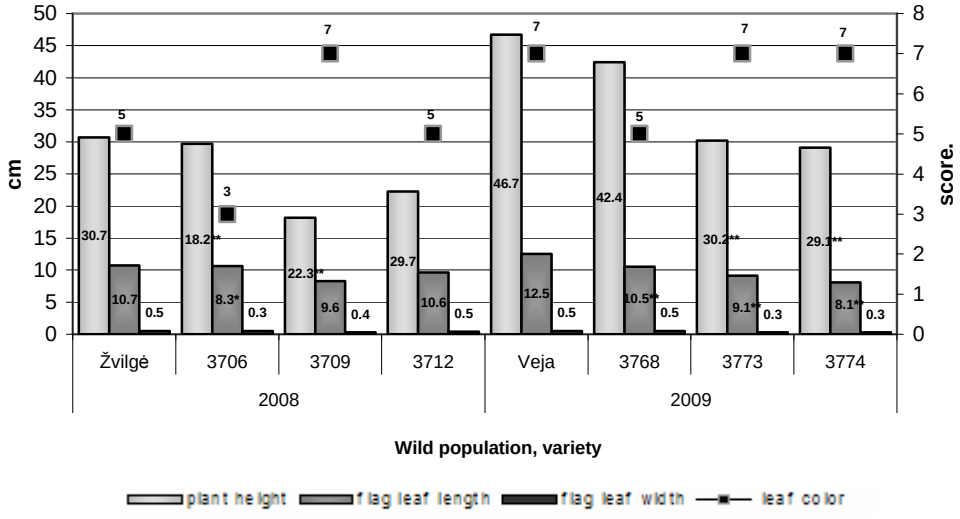
Variety, wild population	CP, G kg^{-1}	CF, g kg^{-1}	WSC, g kg^{-1}	DMD, g kg^{-1}
		2008		
Žvilgė	105.0	213.0	312.0	744.0
Drogobičskij-2	108.0	270.0	236.0	622.0
Drogobičskij-16	99.5	273.0	240.0	613.0
3702	91.8	258.0	256.0	617.0
3706	125.0	197.0	317.0	785.0
3717	113.0	206.0	307.0	771.0
LSD_{05}	1.02	1.01	1.38	1.03
		2009		
Veja	93.2	258.0	271.0	643.0
3768	127.0	215.0	298.0	762.0
3770	130.0	221.0	245.0	715.0
3771	112.0	234.0	261.0	670.0
LSD_{05}	1.01	0.23	0.98	1.93

CP – crude protein, CF – crude fibre, WSC – water soluble carbohydrate, DMD – dry matter digestibility.

DMD and WSC (except for No 3770 and 3771) and lower CF. The wild population No 3770 had the highest content of CP (130.0 g kg^{-1}). Its higher CF and lower WSC concentrations led to lower DMD (715.0 g kg^{-1}), compared with wild population No 3768. This population distinguished by higher chemical content.

The attention was paid to such morphological characteristics as plant height, leaf colour, leaf length and width, which are important for turfgrass breeding. The most prominent populations are presented in the Fig. 2.

Plant height. The height of perennial ryegrass varies from 30 to 70 cm (Nekrošas, 2000). The examined plants of all wild populations were shorter compared with the Lithuanian varieties (Fig. 2). The height of the examined varieties and populations varied from 18.2 to 30.7 cm in the droughty 2008. The plants of the standard variety were taller (30.7 cm). The small height was typical of the wild populations No 3709 (18.2 cm) and 3712 (22.3 cm). The height of the plants of the examined populations varied from 29.1 to 42.4 cm in the rainy 2009. The plants of the populations No 3774 (29.1 cm) and 3773 (30.2 cm) were significantly shorter, compared with the standard variety (46.7 cm). The plants of the No 3768 were the tallest (42.4 cm) among the populations.



*significantly different from standard at $P < 0.05$ probability level
Score 3 represents light green, 5 – medium green, 7 – dark green leaf colour

Figure 2. Plant height (cm), flag leaf length and width (cm) and leaf colour (score) of the Ukrainian ryegrass (turfgrass type) wild populations under Lithuanian conditions in 2008–2009.

Flag leaf length and width. Flag leaf width of forage-type ryegrasses ranges from 0.5 to 1.1 cm (Nekrošas, 2000). The turfgrasses should have narrow leaves. This parameter varied from 0.3 to 0.5 cm in the populations of the plants studied (Fig. 2). The wild populations No 3709, 3773 and 3774 were characterized by the narrowest leaves. The leaves of forage-type ryegrasses are longer than those of the turf-type. Short flag leaves were common to the wild populations No 3709 (8.3 cm) and 3774 (8.1 cm). The populations of the Ukrainian origin had shorter (0.1–2.4 cm) and also narrower (0.1–0.2 cm) flag leaves.

Leaf colour. Medium green leaf colour was characteristic to 22 of the examined populations and varieties. The Lithuanian variety 'Veja' and Ukrainian wild populations No 3709, 3773 and 3774 had dark green leaf colour (score 7). The plants of wild population No 3706 had light green colour (score 3).

The long-term investigation of Lithuanian perennial forage grass wild populations revealed that they are low–yielding (Lemežienė & Kanapeckas, 2008). The West Ukraine region is 1000 km to the south from Lithuania, the climate is warmer and more humid there, so it was assumed that perennial ryegrass populations, collected in West Ukraine natural habitats, would be high–yielding and early–flowering, but would exhibit low winter–hardiness under Lithuanian weather conditions. But the experiment showed only 2 out of 23 wild populations to be very high–yielding (higher yielding than the registered Lithuanian varieties 'Veja' and 'Žvilgė') and could be used as germplasm in the perennial ryegrass breeding programme. One of these high-yielding populations No 3768 was found in Rakhiv district, near Yasinia town, on the bank of river Chorna Tysa. Another population No 3705 was found in the meadow near Brody

town, Brody district. These two populations overwintered well in 2007–2008 and 2008–2009, because the winters were mild. Unlike it was expected, the Ukrainian wild ryegrass populations were not early flowering.

A part of wild populations (No 3709, 3773 and 3774) belong to turfgrass type according to such morphological characteristics as plant height, leaf colour, leaf length and width. The seeds of the three most prominent populations were collected on the dry meadow hill slopes (Mukachevo district and Rovno region).

CONCLUSIONS

1. Part of the Ukrainian wild populations of perennial ryegrass was distinguished by winter hardiness, fast spring growth, high DM yields and forage quality under Lithuanian conditions.
2. The plants of the Ukrainian wild populations were shorter and more sensitive to rust and leaf spot pathogens, part of them with narrower and shorter flag leaves compared with the Lithuanian varieties.
3. The Ukrainian varieties exhibited early heading and worse herbage quality compared with the Lithuanian standard variety ‘Žvilgė‘.
4. The most promising genetic material for perennial ryegrass breeding for forage production are the Ukrainian wild populations No 3768 and 3705, and for turfgrass breeding No 3709, 3773 and 3774.

ACKNOWLEDGEMENTS. We gratefully acknowledge the support provided by the State Science and Studies Foundation for the Lithuanian – Ukrainian bilateral research cooperation programme’s Project “Investigation of the most important biological and agronomic traits of forage grasses”.

REFERENCES

Aavola, R., Van Loo, E.N. & Boucoiran, C.F. 2003. Forage quality improvement in perennial ryegrass offspring relative to their genetically distant parents. *Czech J. Genet. Plant* **39**, 91–94.

Aganga, A.A. & Omphile, U.J. 2004. Chemical Composition of Ryegrass (*Lolium multiflorum*) at Different Stages of Growth and Ryegrass Silages with Additives. *Jour. of Biol. Sci*. **4 (5)**, 645–649.

Alibegovic-Grbic, S. & Civic, H. 2004. Effect of weather conditions, stage of plant growth and N application on yield and quality of grassland in Bosnia and Herzegovina. *Grassland Sci. Eur*. **9**, 897–899.

Butkutė, B. & Paplauskienė, V. 2006. Forage quality potential of perennial grasses. *Zemdirbyste/Agriculture* **93 (3)**, 172–187 (in Lithuanian, Abstract in English).

Butkutė, B., Mašauskienė, A. & Paplauskienė, V. 2003. Database collecting and development of calibration equations for the evaluation of chemical composition of grasses by a NIR spectrophotometer. *Zemdirbyste/Agriculture* **82**, 157–168 (in Lithuanian, Abstract in English).

Harkot, W. 2005. Differences in the phenologic development of forage on mineral and organic soil. *Grassland Sci. Eur*. **10**, 251–254.

Humphreys, M.W., Yadav, R.S., Cairns, A.J., Turner, L.B., Humphreys J. & Skøt, L. 2006. A changing climate for grassland research. *New Phytologist.* **169**, 9–26.

International union for the protection of new varieties of plants (UPOV). 2006. Guidelines for the conduct of tests for distinctness, uniformity and stability. *Ryegrass*. pp. 1–26.

Jones, D.I.H. & Moseley, G. 1993. Laboratory methods for estimating nutritive quality. In: Davies, A., Baker, R.D., Grant, S.A. & Laidlaw, A.S. (eds.). *Sward Measurement Handbook*. 2nd ed. British Grassland Soc., Reading, Berks. pp. 265–283.

Kanapeckas, J., Lemežienė, N. & Tarakanovas, P. 1999. Yield comparison of perennial grasses at the heading stage**.** *Žemės ūkio mokslai* **1**, 10–16 (in Lithuanian).

Lemežienė, N. & Kanapeckas, J. **2008.** Efficiency of wild ecotypes for forage and turf grasss breeding. *Biologija* **54** (**2**), 125–128.

Nekrošas, S. 2000. *Perennial ryegrass*. Akademija, Kėdainiai distr., 27 pp. (in Lithuanian)

Reheul, D., Baert, J. & Chesquiere, A. 2003. In search of tetraploid ryegrass with higher dry matter content. *Czech J. Genet. Plant* **39**, 54–56.

Tarakanovas, P. & Raudonius, S. 2003. *The program package „Selekcija“ for processing statistical data*. Akademija. 56. p. (in Lithuanian).

Wilkins, P.W., Lovatt, J.A. & Jones, M.L. 2003. Improving annual yield of sugars and crude protein by recurrent selection within diploid ryegrass breeding populations, followed by chromosome doubling and hybridization. *Czech J. Genet. Plant* **39**, 95–99.