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A COMPARATIVE STUDY OF WHITE CLOVER FORMS AND ACCESSIONS

The objective was to evaluate and compare accessions of white clover (Trifolium repens L.) forms hollandicum, giganteum, hollandicum x giganteum and silvestre for herbage yield, quality and bio-morphological properties. White clover varied with and within forms according to their morphological structure and biological properties: winter damage, time of flowering, profuseness of flowers, height of plants and other. Among the white clover forms, accessions of hollandicum x giganteum and giganteum produced the highest herbage DM yield. Data suggest that clover forms only slightly differed in the composition of the main components defining feeding value of herbage. Varieties and breeding lines differed more according to the concentration of toxic compounds – cyanogenic glycosides (HCN). The averaged data suggest that the accessions of hollandicum x giganteum and silvestre form had the lowest concentration of HCN in herbage (284 and 307 mg kg^{-1} respectively), while those of hollandicum – had the highest content (484 mg kg^{-1}). Relationships between the values of herbage quality components and some bio-morphological properties were calculated. Concentrations of HCN correlated positively with plant winter damage, DM digestibility (DMD)- with time of flowering, modified acid detergent fibre (MADF)- with profuseness of flowers.

Emphasis on environmentally sustainable development with the use of renewable resources moves us to pay special attention to decrease amounts of N fertilisers through the biological nitrogen fixation. White clover (*Trifolium repens* L.) is one of the most important legume components in pastures of the 250-300 species in the genus *Trifolium* and can contribute significantly to N fixation in a sward. The interest in incorporation of clovers in the nutrition chain of animals is based on their good feeding value: white clover, in comparison with grasses, contains more protein, ash, less fibre and is characterised by higher intake (Dewhurst et al., 2003), though poisonous compounds – cyanogenic glycosides are commonly found in *T. repens*. White clover populations exhibit high genetic and clonal diversity (Gustine, Elwinger, 2003). Accessions

differ in both morphological and physiological properties and they are classified arbitrarily according to plant size (Sareen, 2003; Sprainaitis, Paplauskienė, 2002). The selection of varieties for hay and pasture is an important decision requiring knowledge of both agronomic characteristics and potential feeding value of forage plants.

Materials and methods. The genetic collection of white clover of the Lithuanian Institute of Agriculture (LIA) including varieties, wild populations and breeding lines was assessed for quality over the period 2003-2007 on a sod gleyic, medium heavy, drained loam soil with a pH value in the arable layer varying from 6.4 to 7.2 and a humus content from 19 to 22 g kg^{-1}. The white clover populations were sown on 10.0–12.5 m^{-2} plots in the first half of June without a cover crop. The clover was tested for morphological or biological traits according to standard methods (IPGRI, 1992; UPOV, 1985). The assessment is based on a 1-9 or a 3-7 point system, 1-3 being very low and low value of the trait, 5-medium, 7-9-high and very high value of the character. For chemical analyses composite samples were formed at grass heading stage of the first cut. Dried and ground by a mill with 1 mm sieve samples were analysed for crude protein (CP), modified acid detergent fibre (MADF), pepsine-cellulase DM digestibility (DMD) and water soluble carbohydrates (WSC) by near infrared spectroscopy and for cyanogenic glucosides (HCN) by mercurometric method.

Results and discussion. Study results revealed that white clover forms differed more in DM yield than in quality, and that there existed a variation within each form. The statistical mean over four years, range of values and coefficient of variation (CV%) of dry matter yield and concentrations of quality components in herbage DM of white clover forms are presented in Table 1. According to averaged data clover forms differed slightly in the composition of the main components defining feeding value, i.e. CP, MADF, WSC and DMD.

The WSC concentration was found to be the most variable parameter among the rehearsed ones, except HCN: the variation coefficient was as high as 20.29-26.07 %. The herbage of the first cut of all white clover varieties tested contained quite high concentrations of CP (167-261 g kg^{-1}) and were characterised by good DMD (683-908 g kg^{-1}), but some varieties accumulated high contents of harmful substances – cyanogenic glycosides (HCN). The variation coefficient of HCN content ranged from 28.70 % for *hollandicum x giganteum* to 64.56 % for *silvestre* accessions. Averaged data indicate that the lowest content of HCN was accumulated by the individuals of *hollandicum x giganteum* and *silvestre* forms (284 and 307 mg kg^{-1} respectively), the highest – by those of *hollandicum* form (484 mg kg^{-1}).

1. Mean and variation of herbage dry matter yield and quality of white clover

Indicator of		Systematic form			
Quality	Statistics	*hollandicum* n=22	*giganteum* n=12	*hollandicum x giganteum* *n*=12	*silvestre* n=33
Crude protein (CP)	Mean g kg^{-1} Range g kg^{-1} CV %	205 182-260 10.30	203 176-257 11.33	208 188-261 11.88	209 167-258 11.51
Modified acid detergent fibre (MADF)	Mean g kg^{-1} Range g kg^{-1} CV %	196 164-267 13.26	197 175-254 10.89	191 167-216 9.14	199 161-326 19.22
Dry matter digestibility (DMD)	Mean g kg^{-1} Range g kg^{-1} CV %	833 688-904 6.54	835 759-871 4.03	843 777-902 4.22	829 683-908 5.80
Water soluble carbohydrates (WSC)	Mean g kg^{-1} Range g kg^{-1} CV %	156 77-200 22.22	164 93-199 20.29	158 78-197 22.90	135 75-185 26.07
Cyanogenic glycosides (HCN)	Mean mg kg^{-1} Range mg kg^{-1} CV %	484 268-981 61.81	416 220-736 42.59	284 224-457 28.70	307 160-1055 64.56
Dry matter (DM) yield	Mean t ha^{-1} Range t ha^{-1} CV %	7.07 6.13-7.63 7.14	8.07 7.03-9.04 6.68	7.66 7.10-8.54 5.50	6.65 5.74-7.82 11.29

Concentration of these compounds tends to increase with the cut (Figure 1). According to averaged data lower contents of HCN were accumulated in the herbage of the 1st cut (300 mg kg^{-1}), whereas the highest contents were identified the herbage of the 4th cut (424 mg kg^{-1}).

Large-leafed *giganteum* form was characterised by the highest DM yield of two cuts with mean 8.07 t ha^{-1} and a range 7.03-9.04 t ha^{-1}. Accessions of s*ilvestre* were noted for the lowest average DM yield and in individual samples it ranged from 5.74 to 7.82 t ha^{-1}. The medium leaf-sized *hollandicum* form of white clover is most common in Lithuania. It is less-yielding, with a lower competitive power, however, it is noted for higher growth rate, profuseness of flowers and higher seed set than *giganteum* (Butkute et al., 2007). Fine-leafed s*ilvestre* form, exhibiting good over winter survival, re-growth and the highest profuseness of flowers, is designed for intensive grazing. Some *silvestre* accessions are ornamental which makes it possible to breed them for amenity purposes. This is especially relevant seeking to reduce nitrogen fertilizer use. Research into morphological and biological traits of white clover forms suggests a great diversity not only between the forms but also within the forms.

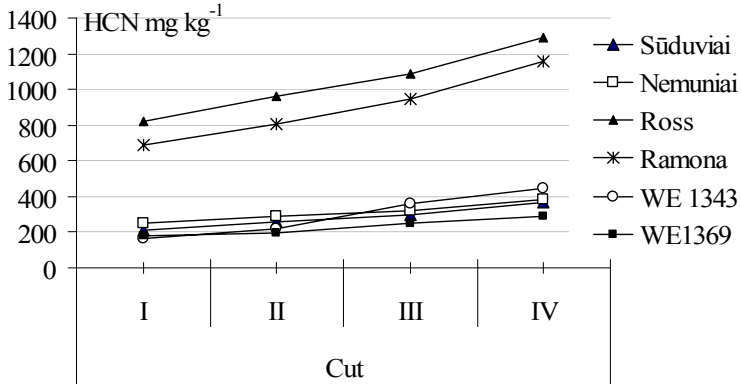

Figure 1. HCN concentrations in the herbage of different cuts of white clover varieties and wild ecotypes (WE)

White clover yield is determined by some morphological traits (Collins et al., 1991). Among the many factors that affect clover herbage yield are growth rate, plant height, bunch diameter, and flowering time (Table 2). The relation of these traits to the yield is defined by respective correlation coefficients r 0.544, 0.495, 0.487 and 0.461, $P < 0.01$. Significant correlations at $P < 0.05$ were determined between individual morphological traits: less susceptible to winter damage plants start flowering earlier but form fewer inflorescences. Plant height is directly significantly related to flowering time ($r = 0.680$, $P < 0.01$). A relationship between plant earliness and abundance of inflorescences and between some quality indicators of white clover was identified: earlier flowering plants accumulate lower MADF contents and more abundantly flowering plants exhibit poorer DMD. Concentration of HCN was positively correlated with plants winter damage ($r = 0.423$, $P < 0.05$). The pairs of quality components MADF and WSC, MADF and DMD, like concentrations of CP and WSC were inversely related. Similar trends of relationships between forage quality components are well known.

Large variation in DM yield and forage quality of white clover forms which are associated with morphological and biological traits has important implications for future white clover breeding programmes, where these traits could be incorporated as selection criteria for the development of high yielding accessions, adapted to soil properties, local climatic conditions, a range of managements, and of good quality.

2. Relationship between morphological attributes, biological traits and quality parameters of white clover, n = 33

Name of a character in comparable pairs		A and B in regression equation Y = A + BX		Coefficients of correlation r
X	Y	A	B	
Winter damage	Flowers profuseness	6.719	–0.459	–0.347*
Winter damage	Time of flowering	3.754	0.494	0.401*
Plant height	Dry matter yield	1.628	0.568	0.495**
Plant height	Bunch diameter	3.754	0.281	0.344*
Plant height	Time of flowering	0.013	0.771	0.680**
Flowers profuseness	Time of flowering	7.808	–0.527	–0.566**
Dry matter yield	Growth rate	2.666	0.349	0.544**
Dry matter yield	Bunch diameter	3.706	0.347	0.487**
Dry matter yield	Time of flowering	2.422	0.456	0.461**
Bunch diameter	Time of flowering	1.376	0.617	0.444**
Winter damage	HCN	25,68	6,677	0.423*
Flowers profuseness	DMD	83.993	–0.437	–0.389*
Flowers profuseness	MADF	18.734	0.337	0.444**
Time of flowering	DMD	79.125	0.492	0.408*
Time of flowering	MADF	22.64	–0.412	–0.506**
CP	WSC	30.41	–0.678	–0.472**
DMD	MADF	66.243	–0.559	–0.829**
MADF	WSC	24.103	–0.34	–0.402*

Significant: ** at P < 0.01, * at P < 0.05

Conclusions. White clover varies with and within forms according to their herbage DM yield, morphological and biological properties, the concentration cyanogenic glycosides, but only slightly differ in the concentration of the main components of herbage quality. The morphological and biological traits well correlate with the yield, some traits – with quality indicators.

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