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Forage Crops Basis of the Sustainable Animal Husbandry Development

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GRASSLANDS AND FORAGE CROPS IN EUROPE: CONTEXT AND STAKES. CONSEQUENCES FOR BREEDING

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Invited review paper

Abstract: In a first part, the paper will present the socio-economic context of grasslands and forage crops in Europe over the last decades. The grasslands and annual forage crops contribute a significant part of the total agricultural land, with a major share to permanent grasslands. The variation in the size of herbivore herds will be described, and especially the number of dairy cows. The structure of milk production in the various EU countries will be documented through the mean herd size and the share of milk production. The second part will present the main stakes for the future of grasslands. They are related to i) balance between income and workload for the farmers, taking into account the common agricultural policy, ii) quality of animal products in response to end-users' expectations and iii) combination of economic performance and environment preservation, with a special interest to reduction of soil and nutrient losses, reduction of fossil energy consumption and greenhouse gas emission, preservation of hosted biodiversity. Meeting these stakes assigns new goals to breeding. It first requires an increased persistence of grasslands stands which may be achieved through either more persistent plants or through exploitation of the population dynamics of swards. It also offers good prospects for mixtures of species as sources of over-yielding and more yield persistency. Finally, these stakes offer new prospects for forage legumes, source of nitrogen fertility in cropping systems and protein in animal diets with a positive effect on the grassland biodiversity.

Key words: grasslands, farming, breeding, Europe

Introduction

Breeding perennial species, such as forage grasses and legumes is a long term enterprise. Indeed, the process of plant breeding is long with more than 10 years between the initial crosses and the registration of a variety. Moreover, the turn-over of varieties is much slower than in annual crops even if the duration of the commercial life of varieties tend to decrease *(Tabel and Allerit, 2005)*. It is thus necessary to analyse the present and future stakes of the supply chain where the future varieties have to be used. These stakes must include the economic aspects of the supply chain. In the case study of the perennial forage species, the economics must include the animal production as well as the expectations of the farmers. Environment preservation has to be taken into account by all production systems, and it is especially important for the grasslands and herbivore production systems as they valorise a very large part of the arable land in Europe. Among these environmental stakes, reducing nitrogen losses, improving energy efficiency and preserving biodiversity are essential. It is also necessary to anticipate the possible long term changes. Among them, the consequences of climate change are likely to be very important for grasslands and for plant species.

The present paper will first present the economic importance and associated stakes of grasslands and related animal production in Europe, with a description of acreage, animal herds and performance. Challenges for combining economic performance and environment preservation will be presented. It will be proposed that multi-species swards combining grasses and legumes offer a high potential to meet these objectives. The adequate breeding objectives will be discussed.

Grasslands and related animal production in Europe: economic importance and stakes

Acreage of grasslands and forage crops. Grasslands and forage crops contribute a major part of agricultural and arable land in Europe (EU27). In 2007, for the EU27, permanent grasslands covered a total of 60 Mha (Eurostat) while annual forage crops and temporary grasslands covered a total of 18 Mha (Eurostat). Thus, permanent grasslands contribute more than 75% of total acreage in grasslands and forage crops in Europe. Over the last three decades, the acreage in permanent grasslands showed a strong decrease. Acreage change was different among countries, with a 25% reduction in France (Figure 1). Over the last ten years, the mean decline was not so severe. Although permanent grasslands declined by 24% in Spain between 1998 and 2008, the decline reached only 3% in France and 9% in Germany, while they remained stable in Bulgaria and Czech Republic. Over the last three decades, part of this loss led to an increase of the acreage of annual crops while another part turned into forests and bushes. In the North-Western and Nordic countries with a high annual rainfall, permanent grasslands are scattered all over the countries. In the rest of Europe, most permanent grasslands are located in mountainous areas (Massif Central, the Alps, the Pyrenees, Jura and Vosges) where nothing else could be produced, except forestry. They are a major component of the preservation of these fragile environments.

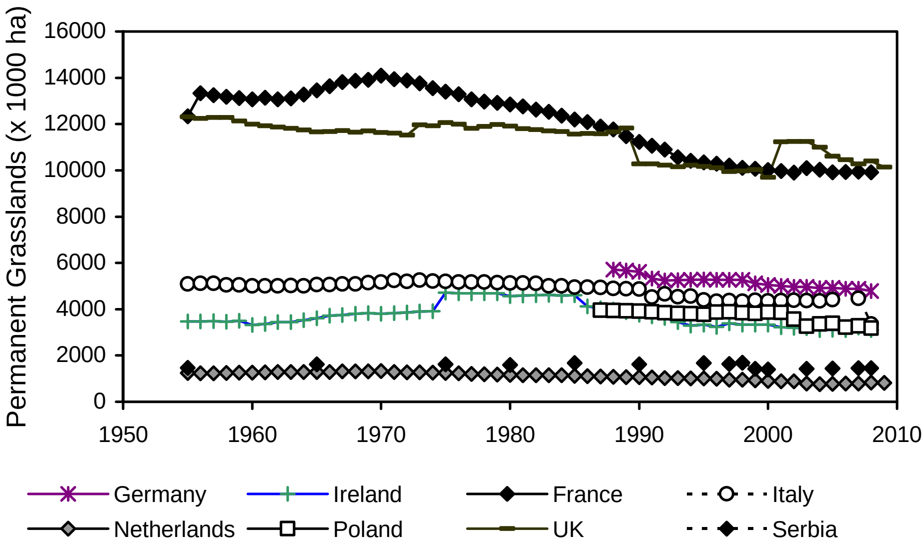


Figure 1. Changes in acreage of permanent grasslands over the last decades in several European countries. Source: Eurostat

The acreage of temporary grasslands and annual forage crops showed important variation of the last decades (Figure 2). It tended to decrease in Italy and UK, while it stayed fairly stable in France and Germany. On the opposite, it steadily increased in the Netherlands. The acreage in silage maize increases in all European regions specialised in milk production and this explains the trend in the Netherlands.

The acreage and its variation over decades in pure perennial legumes show very contrasting features among countries. Due to historical reasons, perennial forage swards with pure legumes, named artificial grasslands, were abundant in France and Tchecoslavakia in 1960 *('t Hart and Van der Molen, 1966)*. In France, acreage in pure legumes crops, mainly alfalfa, is still decreasing after a peak of 3.5 Mha including 1.2 Mha of alfalfa in 1961. The acreage of temporary grasslands slowly increases in France *(Huyghe, 2008)* as well as in the rest of Europe. Interestingly, a high percentage of the temporary grasslands are sown with mixtures of species, mainly grasses and legumes. The use of mixtures was boosted by the European regulation on the commercialisation of seed mixtures for forage production in 2000. In France, this percentage was estimated to reach 70% in 2007.

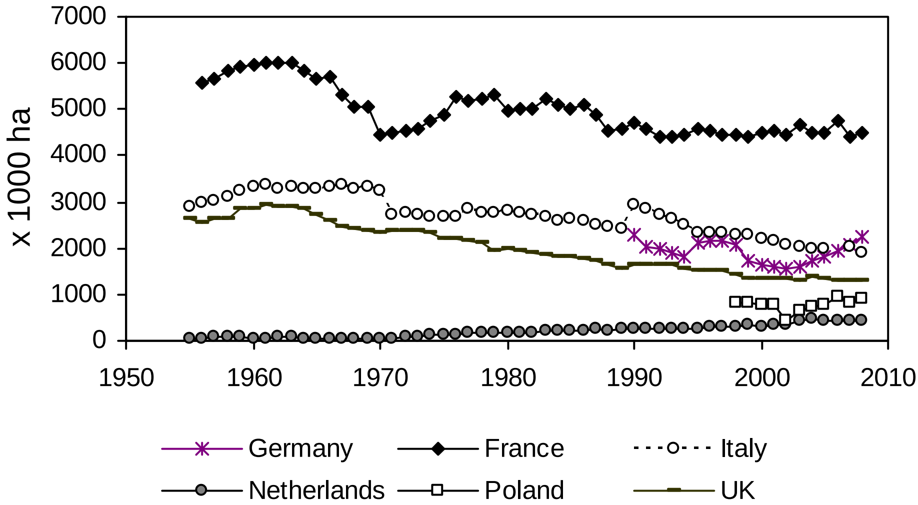


Figure 2. Changes in acreage of annual forage and temporary grasslands over the last decades in several European countries. Source: Eurostat

Animal production. Considering the same time period, the herbivore herds significantly varied. The herd of dairy goats was constant with a sharp increase in milk production per head. The number of sheep steadily decreased, this being due to a strong reduction in the number of sheep devoted to meat production. The main variations were due to the number of dairy and suckling cows. As a consequence of the milk quota established in 1984 and of the sharp increase of the milk production per head, the number of dairy cows was dramatically declined. Their number was divided by 2 in most countries (Figure 4). Dairy cows are more and more located in a limited number of lowland regions with a high animal density. West of France, Ireland and Western England, Denmark and the Netherlands, Northern Germany, Northern Italy, Poland are the main regions for milk production. Europewide, a small proportion of dairy cows are still present in the mountainous regions where the milk is mainly processed into cheeses with PDO or IGP value. The number of suckling cows showed a very contrasting situation among countries. In countries where the bovine meat consumption did not drop much during the same period of time, the reduction in the number of dairy cows opened a possibility for the development of a large herd of suckling cows. For instance in France and Ireland, the number of suckling cows slightly exceeds the number of dairy cows with 4 Millions of heads in France. On the opposite, in countries where the beef meat consumption declined, such as Germany, no significant herd of suckling cows was developed. These changes have strong consequences for grasslands. Because of the increase in milk production per dairy cow, the diets have been modified for a higher concentration in digestible energy and protein. This explains why silage maize is popular for these animals, when soil and climate are suitable. However, high milk production may also be achieved with diets based upon grazing, where it requires large biomass availability per grazing animal and adequate species composition. Suckling cows are more located in mountainous and hilly areas and efficiently valorised permanent grasslands through grazing.

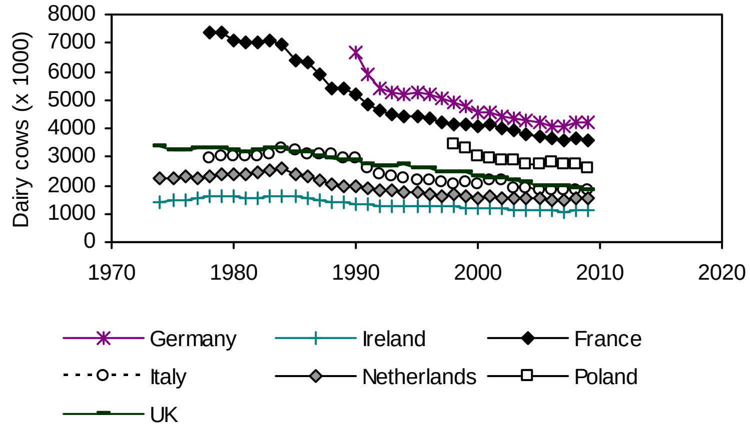


Figure 3. Changes in the number of dairy cows in some European countries over the last decades. Source: Eurostat

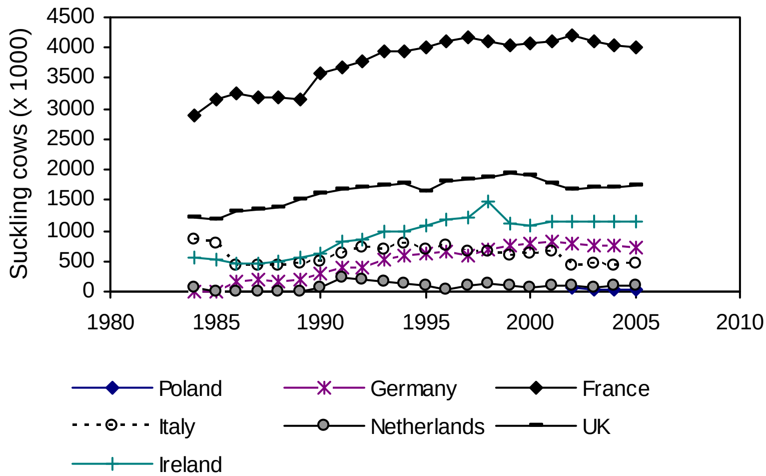


Figure 4. Changes in the number of suckling cows in some European countries over the last two decades. Source: Eurostat

It is also very important to consider the production per animal, as this has a major influence on the composition of the diet and especially the proportion of forage. There is a wide range of variation among countries with up to 7000 kg of milk per cow in Netherlands (Figure 5). It is must also be underlined that this milk production quickly increase over the last decades. In France, it was multiplied by two between 1980, while it increased by 64% in Serbia.

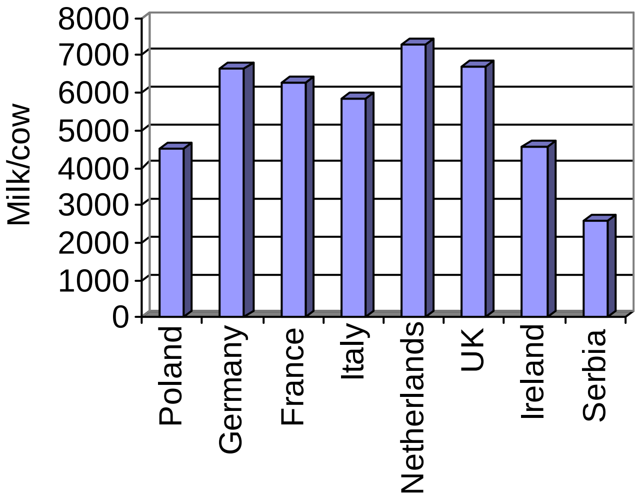
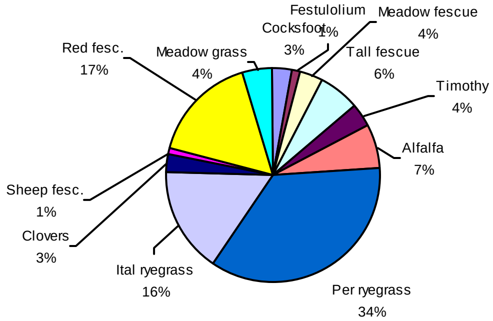


Figure 5. Annual milk production per cow in various European countries.

Forage and turf seed production in Europe. The seed production for forage and turf species is very active in Europe. The main species are Italian and perennial ryegrass and red fescue among the grass species, while alfalfa is the main legume species (Figure 6a). This is due to a large number of very active breeding companies and specialised seed producers, especially in North-West of Europe. It is also due to a wide range of climatic conditions which offers possibilities for production of a wide range of species. This economic activity shows variations among years, with a strong decline in 2007 and 2008 due to constraints on the market and competition with cereals (Figure 6b).

a)



b)

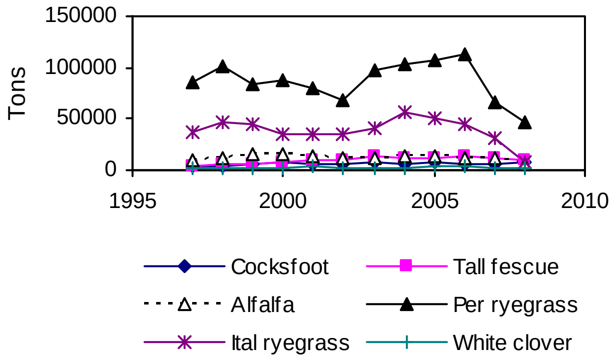


Figure 6. Forage and turf seed production in Europe: a) share of production among the various species and b) changes in seed production of some forage species in Europe.

Farms and farmers. The number of dairy farms showed a strong reduction over the last three decades in most countries. In France, this reduction reached 80%. Reducing the number of farmers was a main tool in the public policy to reduce milk production and reach the national quota. This mainly targeted the small farms. The reduction has become less pronounced since 1993. Over the last ten years, the number of farms involved in milk and beef cattle production declined at a similar rate of 5000 farms a year. Due to the variation in number of farms, the production per farm increased, even if more moderately when compared to the large average herd size observed in Denmark or in the UK. Figure 7 shows the large variation among European countries in terms of mean herd size. For most Eastern countries which joined the EU in 2004, a major increase in herd size and the subsequent reduction in number of farms is anticipated in the next decade, this change being accompanied by strong European policies and significant budgets. The increased production per farm generated a new stake for the farmers, where reducing work load and above all work uncertainty became a major objective, alongside with economic performance. This raises new challenges for grasslands whose production and quality depend more on the unpredictable weather than the forage crops which are stored as silage.

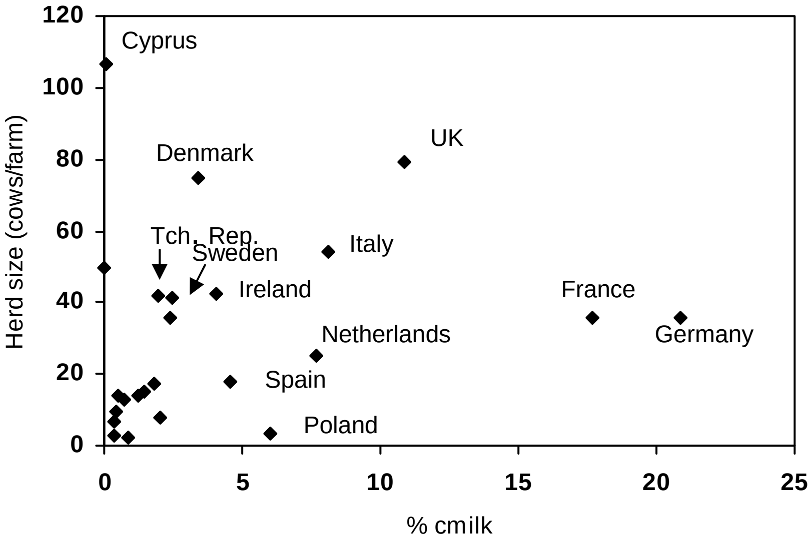


Figure 7. Relationship between milk production (in % of the whole European production) and mean dairy herd size among European countries in 2004.

Increasing environmental performance

Alongside with the economic stakes which include the main concerns for the farmers, for the industry but also for the end-users, it is necessary to consider the aspects related to preservation of environment, or the environmental performance.

Three aspects are especially relevant to grasslands and herbivore production *(Huyghe, 2009).*

Losses of nutrients and water pollution. The first aspect is related to the losses of nutrients and especially nitrate leaching, with the possible consequences on the quality of water and the subsequent pollution. Mineral nitrogen fertilisation plays an important role, as it must meet the requirement of the grassland growth. However, the species composition and especially the use of mixtures may provide an adequate answer as it may limit the use of nitrogen fertilisers and nitrate leakages. Grassland management is also a tool to monitor and limit nutrient losses. The exploitation regime (cutting and grazing) and the animal stocking rate are important. Sward persistency and renovation also have to be considered, as a significant part of the losses are likely to occur during ploughing and renovation.

Improving energy efficiency and reducing greenhouse gas emission. Fossil energy consumption and greenhouse gas emission must decrease to reduce global warming. In ruminant production, the energy efficiency is low. In cow milk production, the mean efficiency measured on numerous French farms by *Bochu (2006)* close to 1. A similar value was found by *Haas et al. (2001)* in intensive farming system in Northern Germany. Interestingly, there is a wide range of variation among dairy farms, *Bochu (2006)* reporting a variation between 0.5 and 2.5, while in *Haas et al. (2001)*, extensive and organic farming systems had efficiencies close to 2. Investigating the main energy consumptions showed that mineral nitrogen fertilisation and concentrate supplementation contribute a large part of the consumption of fossil energy in grassland production and herbivore production. Indeed, 1.8 kg equivalent oil is needed to produce 1 kg of N under ammonitrate form. The relationship between concentrate supplementation and energy efficiency was documented by *Fuchs (2009)*, unpublished data from Parmeeli project, pers. comm.) on a set of French farm (Figure 8). Species composition of grasslands, with presence of legumes thus offers an opportunity to reduce consumption of fossil energy both through reduction of nitrogen fertilisation and less need for protein supplementation.

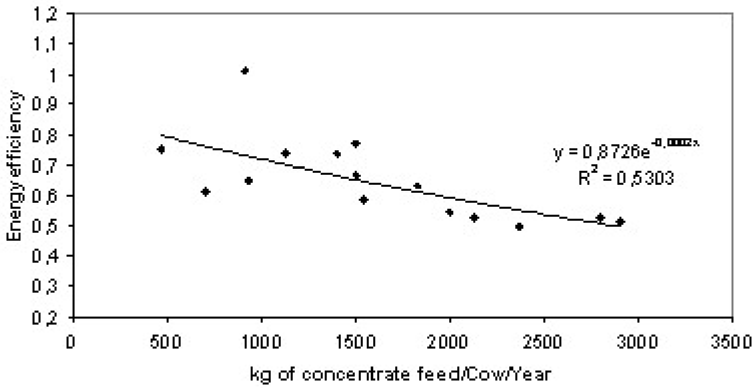


Figure 8. Relationship between the quantity of concentrate and the energy efficiency in various French dairy farms (from F. Fuchs, 2009, Parmeeli project)

This points out the importance to achieve and maintain a high proportion of legumes in swards and to preserve proteins during harvest. Moreover, legumes also limit the emission of nitrous oxide, as the emission coefficient was estimated to be null for symbiosis while its estimation is 0.7% of the applied nitrogen either through mineral or organic fertilisation. This topic is still very much under debate and requires to be extensively documented.

The drawback of grasslands regarding GHG is the methane produced by ruminants, as a consequence of the rumen functioning. *Lassey (2007)* estimated that 6% of the carbon in the diet was lost as methane. It was suggested by *Martin et al. (2006)* that the presence of tanniferous legumes species in the diet was likely to modify the bacterial population in the rumen and may reduce methane emission. It is also suggested that the presence of saponins could also influence the composition of the ruminal flora and significantly reduce methane emission *(Holtshausen et al., 2009; Wang et al., 2009)*. Although these preliminary research works were performed with saponins extracted from yucca species, saponins are also produced by some forage species. Even though this has still to be documented, it would support the idea to promote temporary grasslands with a large number of species.

Preserving biodiversity. The last possible contribution of grasslands to environmental performance is the preservation of biodiversity. It was shown on the basis of many studies that the hosted diversity increases with the number of plant species, of functional groups and with the functional diversity in the grasslands *(Lavorel and Garnier, 2002)*. This is true for instance for the number of fungi *(Fischer et al., 2008)*, but we will get back to this item later in the paper. It is also true for the number of pollinating species, and especially wild bees. The abundance of pollinators also increases in grasslands because of the presence of hedges. The management is obviously critical for preserving a rich hosted biodiversity.

Adequate sward composition and varieties to meet the stakes

Multi-species swards offer promising options. All these elements lead to the definition of a sward ideotype and support the hypothesis that temporary grasslands must be increasingly sown with mixtures of species, and especially with mixtures of grasses and legumes. A sward ideotype must have a high persistency as ploughing temporary grassland is a major perturbation generating possible nitrogen leakages and deeply modifying the ecosystem. It must also have a high feed quality, through a high quality of each component of the sward.

Mixtures of species may lead to over-yielding (the mixtures on average produce more aerial biomass than the individual species in pure swards) as documented by several authors, a major study being published by *Hector et al., (1999)*, following the seminal work of *Sinclair (1826) (Hector and Hooper, 2002)*.

Similarly, over-yielding was also observed when the functional diversity (number of functional groups) increased *(Kirwan et al., 2007)*.

Over-yielding mechanisms in multi-species swards. Three physiological processes mainly explained the over-yielding features. It may first be explained by either competition where the most competitive species or functional groups are also the most productive ones *(Tilman, 1997)*. The importance of competition was clearly shown by *Korner et al. (2008)* as being a key element during the establishment phase. The second process is complementarity. Complementarity among species means that these species are able to uptake and use different resources or to use the same resources but at different times or locations in order to avoid competition. This is clearly the case when grasses uptake mineral nitrogen when legumes symbiotically fix nitrogen. This process was described by *Gross et al. (2007)* as a key mechanism of co-existence of various functional groups in a grassland sward. It could be the basis of positive effects on biomass production, especially in environment with intermediate fertility levels. The relative abundance is crucial to achieve complementarity. The last process is facilitation which is a fairly recent hypothesis. It was described by *Bruno et al. (2003)*. According to this hypothesis, facilitation makes it possible for a given species to expand the ecological niche actually explored beyond the fundamental niche. This is due to the action of the other species present in the sward and may be due to an increased access to resources, a limitation of defoliation or predation, an improvement of the environment and improved conditions for seedling recruitment (Figure 9).

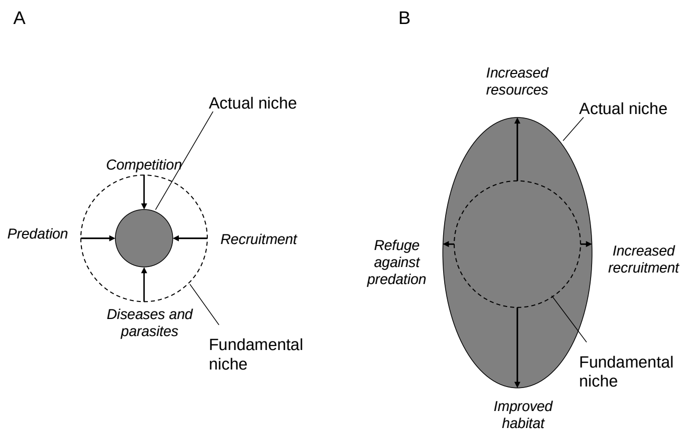


Figure 9 . Effects of competition (A) and facilitation (B) on the size of the actual niche comparted to the fundamental niche. Adapted from Bruno et al (2003)

A recent synthesis by *Brooker et al. (2008)* underlines the need to further investigate the various aspects of facilitation and mentions a possible importance of indirect facilitation between two partner species through the action of a third one.

In grasslands, two examples of facilitation may be documented. The first one is related to the foliar diseases.

This was documented on a large ecological experimental design set at Jena (Germany). It was shown by *Fischer et al. (2008)* that when increasing the number of species or of functional groups in a sward, the number of fungi species increased. But, the damages on each individual plant decreased (Figure 10). This feature may partly explain the over-yielding.

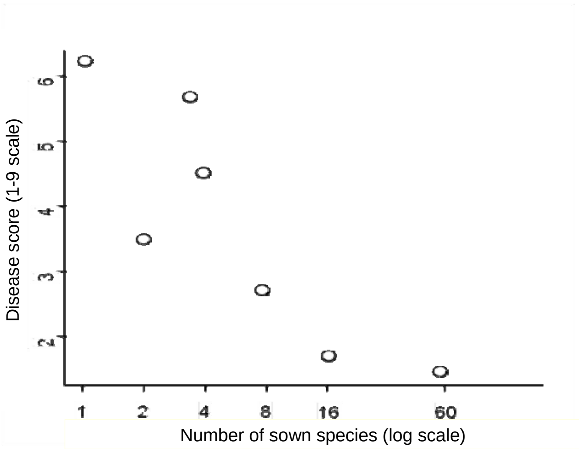


Figure 10. Relationship between the number of plant species sown in a grassland sward and the disease score (adapted from Fischer et al., 2008)

A second example is the process of nitrogen transfer from legume species to grass species in a grassland sward. Nitrogen may be transferred through senescence of leaves and roots. But the major mechanism of transfer could well be rhizodeposition. This mechanism is the secretion of organic compounds into the rhizosphere, making their uptake possible for the other species of the sward. This was documented by Hogh-Jensen and Schloerring in 2001 in pure swards and mixtures of red clover, white clover and perennial ryegrass. *Hogh-Jensen and Schloerring (2001)* showed that rhizodeposition contributed 80% of the nitrogen transferred from legumes to grasses over the two years of their study, most of this nitrogen originating from symbiotic fixation. During their two years study, the total rhizodeposition was up to 890 and 320 kg N ha^{-1} for red clover and white clover respectively. This process is very little documented for the various legumes species. But it is likely that large differences exist among species both for the intensity and the timing of rhizodeposition.

Management practices must be adapted either to preserve plant species diversity or to facilitate the species replacement over time in the swards. If mixtures of grasses and legumes offer an adequate answer, it is however necessary to define the optimum species composition. Indeed, over-yielding also depends on species identity *(Sanderson et al., 2004; Kirwan et al., 2007).*

Adequate breeding to exploit potential of multi-species swards

Even though temporary grasslands with a large species diversity offers promising prospects, it opens several questions to breeding and breeders.

Breeding the right species. An important question is the share of research between the various forage species. In Europe, more breeding efforts are devoted to forage grasses than to forage legumes. In view of the environmental stakes as well as the economic aspects, this feature is not fully relevant and more emphasis should be given to legumes.

Varieties with the adequate morphology and competitiveness. One key difficult of multi-species swards is to stabilize, monitor and predict the dynamics of swards over time. This point is important, as the unpredictable species composition is not relevant with the farmers' expectation of less uncertainty. This was already pointed out by *Blaser et al. (1952)* who declared that complex mixtures of grasses and legumes would be unsuccessful because of the unpredictable and uncontrolled competition among seedlings during the establishment phase or later among plants!! It is necessary to understand and model the mechanisms underlying the dynamics of these swards and it is very unlikely that it will be possible to prevent any change. However, it would be very important to be able to predict to some extent the future of a sward sown with many species, and especially the installation period, as it was shown by *Korner et al. (2008)* that this stage was critical. Reduce sowing density and implement adequate seed technology would offer possibilities for a more predictable species composition.

This is a key challenge, especially for most legumes which show a slow establishment with a long period of severe competition by companion forage grasses or by weeds.

Maximising rhizodeposition from legumes to grasses. It is also necessary to investigate whether there is a range of variation among legume species and within species for their ability to transfer nitrogen to companion grasses. If there is a within-species variation, it is likely to exist among genotypes within- population. This within genetic variance will be difficult to identify as adequate technologies have to be implemented. Moreover, if breeding is to be undertaken, the objective will not be to maximise the transfer, as it would be detrimental to the legumes species but to finely tune it and adapt it. The optimum level of nitrogen transfer will have first to be defined through modelling and simulation, using 3D explicit modelling as performed by *Verdenal et al. (2008)* on aerial morphogenesis of grassland swards but implemented to plant nitrogen status.

Disease resistance. It is also necessary to revise the main traits of the varieties to be sown in mixtures. The hierarchy of traits and thus the prevalence in selection index (or registration index) may be different.

This situation may be illustrated by the importance of disease resistance. In variety selection and registration for use in pure swards, a heavy weight was given to disease resistance. This was very relevant because of the losses in biomass production and quality that may be due to fungal symptoms. However, when growing species in mixtures, the pattern of damages due to leaf pathogens may become very different as shown above, based upon the results from *Fischer et al. (2007)*. This means that the level of disease resistance of varieties may be lower. And, with a constant research investment, this may offer possibilities to have more genetic gains on other traits.

Improving feed value and exploiting feed interactions. Improving the feed value is also a major issue. Improving feeding value requires improving the quality of each component. No positive diversity effect was observed for the main components of feed value such as protein content and dry matter digestibility *(Huyghe et al., 2008)*. So, it is important to improve quality of each component. For the grass to be used in grass – legume mixtures, it seems important to target high content in water soluble carbohydrates (WSC). Indeed, high WSC content will improve animal use of the high protein content of such mixtures. It will also improve their ensilability when relevant. This would be very important for some grass species with low WSC content, such as cocksfoot or tall fescue.

For legumes, especially alfalfa and to a lesser extent red clover, it is necessary to improve the digestibility. Even if the high fibre content of these legumes is useful to avoid acidosis in diet with a high energy content, their low digestibility per se is a diluting factor of diet. As a consequence, farmers may decide to discard them from their diet. Significant differences among varieties have been identified and may be exploit, while preserving their beneficial effect in controlling acidosis.

The context of multispecies swards offers the possibility to exploit the feed interactions, i.e. where a component of one species interacts with a component of another species to improve its valorisation. This could be the case for tannins and polyphenol oxydase (PPO) *(Lee et al., 2004)*, both produced by legumes species which may reduce the protein degradability of the whole diet. These interactions have been mainly documented for tannins *(Julier et al., 2002)*. These possible interactions would suggest that it would make sense to increase the tannins content and the PPO activity to a level which would not make sense for a pure crop.

Conclusion

There are numerous new stakes for the grasslands and herbivores supply chain, especially due to the need to combine economic and environmental performance. Because it is possible to use a wide range of species and to combine species in multi-species swards, forage species offer a wide range of answers. Breeding new varieties as well as registration of these varieties have to take into account this context, exploiting all available tools and concepts from a wide range of disciplines including ecology and population genetics. This must include new tools from molecular genetics and possibly transgenesis when relevant and when this does not induce risks of dissemination towards environment and local plant populations.

Travnjaci i krmni usevi u Evropi: kontekst i ulozi. Uticaj na oplemenjivanje

C. Huyghe

Rezime

U prvom delu rada je prikazan socio-ekonomski kontekst travnjaka i krmnih useva u Evropi u poslednjoj deceniji. Pašnjaci i jednogodišnji krmni usevi pokrivaju značajan deo ukupnog poljoprivrednog zemljišta, sa najvećim udelom stalnih pašnjaka. Veličina krda preživara, a posebno broj mlečnih krava su diskutovani. Struktura mlečne proizvodnje u ražličitim zemljama EU je pokazana kroz srednju veličinu krda i udeo u ukupnoj proizvodnji mleka. U drugom delu rada su prikazani glavni pravci u iskorišćavanju travnjaka u budućnosti. Oni su povezani sa i) ravnotežom između prihoda i rashoda farmera, uzimajući u obzir uobičajenu poljoprivednu praksu, ii) kvalitetom animalnih proizvoda u odnosu na očekivanja krajnjih potrošača i iii) kombinacijom ekonomičnosti i očuvanja životne sredine, sa posebnim osvrtom na redukciju gubitaka nutrijenata u zemljištu, smanjenje potrošnje energije iz fosilnih goriva i efekta staklene bašte i očuvanje biodiverziteta. Usvajanje ovih pravaca određuje nove ciljeve oplemenjivanja. Prvi

cilj zahteva povećanu otpornost travnjaka, koja se može postići kroz korišćenje otpornijih biljaka ili iskorišćavanja dinamike populacije samih travnjaka. Takođe, korišćenje smeša krmnih biljaka predstavlja izvor povećanja i stabilnosti prinosa. Konačno, ove smernice nude nove perspektive za krmne leguminoze kao izvor azota u sistemima za gajenje i proteina u ishrani životinja, sa pozitivnim efektom na biodiverzitet travnjaka.

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PRESENT SITUATION IN LIVESTOCK PRODUCTION IN REPUBLIC OF SERBIA

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Invited review paper

Abstract: Livestock production is important branch of agriculture in Republic of Serbia. Over 700.000 households are engaged in this production, which is over 55% of total number of households. Livestock production provides necessary products (milk, meat, eggs) for nutrition of domestic population. Also, livestock production provides raw materials for food industry (dairy plants, slaughterhouses, meat industry, conditory industry and leather industry). Livestock production is expected to provide high quality products for export, primarily beef and lamb meat. Chance/opportunity for export exists also for cheeses of high quality (especially sheep and goat cheeses) of defined origin and quality.

Based on available data, current situation in livestock production is assessed as inviolable. Number of heads of all species of domestic animals and poultry has been constantly decreasing over the period of last 10 years (2000 to 2009). During this period, number of cattle decreased by 21%, of pigs by 11%, sheep by 17%, number of goats by 22%, horses by 62% and number of poultry increased by 12%. So, by the end of 2009, number of cattle was 1.000.200, pigs 3.631.000, sheep 1.504.000, goats 143.000, horses 14.000 and poultry 22.821.000.

Republic of Serbia will in its near future become member of European Union (EU) and World Trade Organization (WTO), which means that livestock production should prepare for competition on unique developed market, without any state trade barriers. Serbia has been preparing for this since 2006 when it signed bilateral agreement on free trade with neighbouring countries - Central European Free Trade Agreement (CEFTA) and in this way became part of the market and accepted the competition rules on the free market. Accession to EU and WTO includes liberalization of trade in livestock products, low possibility for import protection, implementation of quality standards (HACCP; ISO, Global GAP), reduction of the level of domestic support, discontinuing of export subsidies, increase of profitability and ability to be competitive on the international market.

Key words: livestock production, number of livestock and poultry, breeds, production of livestock products, international market

Introduction

Animal husbandry, together with crop farming, is the major branch of agriculture. Harmonious relationship and coordinated development of these two branches is a prerequisite for developing agriculture and successful accomplishing of tasks relating to human nutrition, development of processing industry and export of agricultural products. Serbia has considerable nature resources (agricultural land, climate, water and vegetable etc.) and very significant capacities and potentials (farmers, livestock fund, production and processing structures and techniques, etc.).

The Republic of Serbia has 5.1 million ha of agricultural land, 4.2 million ha of arable land and fields and 0.83 million ha grassland, approx. 0.6 ha of agricultural and 0.5 ha of arable land per capita. The quality of soil is best in low lands and moderately good in hilly and mountain regions.

Over 700.000 households are engaged in livestock production, which is over 55% of total number of households, and it is important branch of economy which participates in the gross domestic product with 42%. Livestock production provides necessary products (milk, meat, eggs) for nutrition of domestic population. Also, livestock production provides raw materials for food industry (dairy plants, slaughterhouses, meat industry, conditory industry and leather industry). Livestock production is expected to provide high quality products for export, primarily beef and lamb meat. Chance/opportunity for export exists also for cheeses of high quality (especially sheep and goat cheeses) of defined origin and quality.

Present situation in livestock production in Serbia

In Table 1 data are presented relating to number of heads of domestic animals according to species in the period of last 12 years *(Republic Bureau of statistics, 2000-2009)*.

Table 1. Number of livestock and poultry in Serbia in last 12 years in 000

Year	Cattle	*Index*	Pigs	Index	Sheep	Index	***Goats***	Index	*Horses*	*Index*	Poultry	Index
2000	1272	100	4066	100	1611	100	183	100	37	100	20372	100
2001	1162	91	3615	89	1490	92	180	98	30	81	19290	95
2002	1128	89	3587	88	1448	94	164	90	29	81	18804	92
2003	1102	87	3439	85	1586	98	156	85	27	73	16062	79
2004	1079	85	3165	78	1576	98	152	83	24	65	16631	82
2005	1096	86	3212	79	1609	100	138	75	19	51	17905	88
2006	1106	87	3999	98	1556	97	162	90	22	59	16595	81
2007	1087	85	3832	94	1606	100	149	81	18	49	16422	81
2008	1057	83	3594	88	1605	100	154	85	17	48	17188	84
2009	1002	79	3631	89	1504	93	143	78	14	38	22821	112

Based on available data, current situation in livestock production is assessed as inviolable. Number of heads of all species of domestic animals and poultry has been constantly decreasing over the period of last 12 years. The greatest decrease of number of heads of livestock was recorded in year 2004 and it continued to 2009, in all species of domestic animals. During this period, number of cattle decreased by 21%, of pigs by 11%, sheep by 17%, number of goats by 22%, horses by 62%, whereas the number of poultry increased by 12%. So, by the end of 2009, number of cattle was 1.000.200, pigs 3.631.000, sheep 1.504.000, goats 143.000, horses 14.000 and poultry 22.821.000.

Production of milk in Serbia is one of the major livestock production in regard to number of agricultural households engaged in this production as well as in value of this production which is additionally increased (added value) by processing of milk into dairy products. Production of milk, in spite of decrease of number of heads, is constant and at the same level since year 2000 (table 2). This can be explained by increased production of milk per cow. Genetic progress in milk traits was achieved by use of progeny tested bulls and increase of use of artificial insemination of cows and heifers and through import of high quality pregnant heifers *(Aleksić et al., 2007)*.

Table 2. Production of milk, eggs and wool in Serbia

Year	Milk in million litres				Hen eggs in mill.	***Index***	Wool tons	***Index***
	Cow milk	***Index***	Sheep milk	***Index***				
2000	1566	100	19	100	1374	100	2264	100
2003	1576		13		1421		2322	
2005	1602		14		1476		2527	
2006	1587		15		1456		2493	
2007	1549		14		1364		2499	
2009	1478	94	13	68	1026	75	2403	106

Data in table 2 indicate slight decrease in production of cow milk whereas production of sheep milk decreased by 32%. Production of eggs in observed period was relatively stable. Because of the import of genetic material, protein feeds, additives and medicines, this production is to great extent import dependant.

Drastic decrease of number of livestock in the period from 2000 of all species of domestic animals has reflected on decrease in production of meat of all types, and especially in 2009 significant decline was recorded. As orientation for production results year 2003 was taken when number of domestic animals had stabilized. Since 2003, total meat production increased (10%), but insufficiently from the aspect of existing capacities and potential export opportunities. Export of beef was in expansion until Italy (1974) and Greece (1980) became members of the European Economical Community. For instance, in 1974, 50.500t/year was exported to Italy, and in 1980 51.310 t *(Aleksić et al., 2005; Aleksić et al, 2007)*.

Table 3. Production of meat in Serbia in 000 tons

Year	Total	Index	Beef meat	***Index***	***Pork meat***	*Index*	Sheep meat	Index	Poultry meat	Index
2000	518		104		283		19		67	
2003	430	100	95	100	258	100	18	100	59	100
2005	431	100,2	90	94,7	253	98,1	21	116,7	67	113,5
2006	433	100,7	83	87,4	255	98,8	20	111,1	75	127,1
2007	474	110,2	95	100,0	289	112,0	20	111,1	70	118,6
2009	314	73	74	78	139	54	21	116	80	136

Production of pork increased in the period from 2003 to 2009. Problem was that the structure of slaughtered pigs was not good. In total number of slaughtered pigs one third were piglets. Serbia has tradition in production and consumption of pork. Existing production of pork is sufficient to satisfy domestic needs and one part can be exported in form of durable products and cans.

Similar situation is in the production of mutton. Of total production of mutton approx. 70% is meat of young lambs at the age of 90 days. Considering that lamb meat represents strategic product, this production should be restored on private farms owned by individual agricultural producers.

Production of poultry meat is relatively stable. In Serbia, annually 80.000 tons of poultry meat is produced and mainly used for nutrition of domestic population. Because of short production cycle this production is easier for restoration and is less of a problem compared to other branches of livestock production *(Cmiljanić et al., 2006)*.

Breed composition

Cattle breeding. Dominant breeds are Domestic Spotted and Simmental cattle, participating by about 70% in total number, then crossbreds of Domestic Spotted and Simmental and Busha cattle participating with about 25%, and about 5% are Black and Red-White cattle of European White-Black and Holstein breeds.

Sheep breeding. Sheep breed composition is unfavourable since 80% of animals belong to Pramenka, with most represented Pirot, Svrljig and Sjenica strains. Tsigai sheep participates with about 5%, and 15% are crossbreds of Pramenka and imported foreign breeds, among which the most often is Württemberg sheep.

Goat breeding. Balkan goat living in low land regions is represented by about 40%, and goat living in higher regions 15%. The crossbreds between Balkan and other breeds make about 35%, domestic Saanen goat 5% and the Alpine and Saanen goat about 5%.

Pig breeding. Among swine meat species the most often is Swedish Landrace and Great Yorkshire, while Dutch, German and Belgian Landrace, Domestic meaty pig, Hampshire, Duroc and Pietrain are present in a small number (less than 1% individually). Crossbreds from various combinations of cross-breeding are frequent, being in sows on larger farms about 60%, where most frequent is the F1 generation between Great Yorkshire and Swedish Landrace. Private producers with small number of pigs most often grow crossbreds obtained by unplanned cross-breeding.

Poultry breeding. Market production of eggs and broilers is based on the hybrids of light and heavy types.

Future directions of development of livestock production in Serbia

Republic of Serbia will in its near future become member of European Union (EU) and World Trade Organization (WTO), which means that livestock production should prepare for competition on unique developed market, without any state trade barriers. Serbia has been preparing for this since 2006 when it signed bilateral agreement on free trade with neighbouring countries - Central European Free Trade Agreement (CEFTA) and in this way became part of the market and accepted the competition rules on the free market. Accession to EU and WTO includes liberalization of trade in livestock products, low possibility for import protection, implementation of quality standards (HACCP; ISO, Global GAP), reduction of the level of domestic support, discontinuing of export subsidies, increase of profitability and ability to be competitive on the international market.

Developed countries are already facing the trend of increased production of food, and at the same time population of consumers is decreasing which is reflected on the market by permanent decrease of prices. Based on existing situation in livestock production, as well as previous domestic and international practice, a quick and efficient transformation of livestock production is necessary in order to be competitive on the international market *(Aleksić et al., 2005; Aleksić et al., 2007, 2009)*. Therefore, it is necessary first of all to stop further decrease of number of certain species of domestic animals.

It is necessary to improve the production potential of certain species and breeds of domestic animals using genetic-selection measures. Beside standard selection methods, today methods of molecular genetic are used more and more. All this knowledge should be used when developing breeding programs.

By application of new technologies in livestock production and processing a higher level of production and improved quality of livestock products will be ensured. By introduction of new technologies the efficiency and competitiveness of this production will be improved on the global market *(Petrović et al., 2005)*. Future livestock production will be based on private farm production with market orientation. By specializing production higher profitability will be ensured, and in this way competitiveness on foreign market. It is necessary to associate market oriented producers into associations which would contribute to more rational utilization of available assets. Specialized farms for production of milk have objective to produce high quality milk which is in compliance with standards in regard to % of milk fat, % of milk proteins and especially bacteriological safety of milk. On specialized farms a system of certification and registration should be introduced since in this way the value of product is increased *(Petrović et al., 2002, 2003)*. Milk produced in this way can be validated on the market through special dairy products, promotion of brands and commercial – trade marks (hard cheeses, semi-hard cheeses, white cheeses, milk beverages, etc.). Also, specialized farms for production of meat have objective which is production for domestic needs and export. Future of Serbian export of meat is in production of young beef and lamb meat of such quality which is in compliance with demands of the adequate market. One of the ways for efficient improvement of production of high quality meat is crossing for the purpose of realization of better gain, carcass quality and meat quality *(Čobić et al., 1990; Aleksić et al., 1995,1998; Petrović et al., 2004)*.

In livestock production it is necessary to provide certain quality of products. This quality has to be monitored constantly using modern control methods (HACCP and GAP, etc.). It is necessary to provide the principle of traceability, which means that certain product can be traced to the primary production, through processing to ultimate consumer. Adjustment/harmonization of regulations and practice to those which are implemented in EU, so called Acquis Communautaire is necessary, and if these standards are accepted export opportunities for livestock products will be greater

For improvement of livestock production in Serbia it is necessary to provide indispensable quantities of high quality livestock food (forage and concentrated). Food is one of the most important para-genetic factors in modern livestock production. By providing high quality feed we provide not only higher level of livestock production but also better quality of product *(Cmiljanić et al., 2006)*.

Promotions and marketing are also important in realization of greater export of livestock products. For realization of greater export, besides incentives provided by government, it is necessary to have products which comply with standards of the global market. Greater export will have stimulation effect on improvement of livestock production in Serbia.

Measures of the agricultural policy should provide incentives to market oriented producers to produce more efficiently and to produce products of better quality. Credits for livestock producers under more favourable conditions (lower interest rates and longer grace period) also contribute to faster development of livestock production in Serbia.

All afore mentioned measures should be long term because of the long production cycle in livestock breeding, since this is the only way to provide progress in livestock production.

Conclusion

Condition in livestock production was assessed as inviolable. Number of heads of all species of domestic animals is decreasing constantly. Decrease is especially noticeable and negative in production of certain types of meat where significant decline in production has been recorded. Contrary to production of meat, production of milk and eggs was relatively stable.

Long term implementation of recommended measures for restoring and development of livestock production should stop further decrease of number of livestock, provide increase of profitability and competitiveness of livestock production and especially improve the quality of livestock products.

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Stanje stočarske proizvodnje u Republici Srbiji

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Stočarska proizvodnja je značajna grana poljoprivrede u Republici Srbiji. Ovom proizvodnjom se bavi preko 700.000 domaćinstava što čini 55% od ukupnog broja domaćinstava. Stočarskom proizvodnjom se obezbedjuju neophodni proizvodi (mleko, meso, jaja) za ishranu domaćeg stanovništva. Pored toga stočarstvo obezbeđuje sirovine za prehrambenu industriju (mlekare, klanice, konditorske industrije i industrija prerade kože). Od stočarske proizvodnje se očekuje da obezbedi kvalitetne proizvode za izvoz, pre svega juneće i jagnjeće

meso. Šansu za izvoz imaju i kvalitetni sirevi (posebno ovčiji i koziji) sa definisanim poreklom i kvalitetom.

Na osnovu raspoloživih podataka stanje stočarstva u Srbiji se ocenjuje kao nepovoljno. Broj grla kod svih vrsta domaćih životinja i živine u poslednjih deset godina (2000.-2009. godine) konstantno opada. U ovom periodu broj goveda je smanjen za 21%, broj svinja za 11%, broj ovaca za 17%, broj koza za 22% broj konja za 62% dok broj živine raste za 12,0%. Tako da je na kraju 2009. godine broj grla goveda 1.000.200, 3.631.000 grla svinja, 1.504.600 grla ovaca, koza 143.000, 14.000 grla konja i 22.821.000 živine.

Republika Srbija u bližoj budućnosti postaće član Evropske unije (EU) i Svetske trgovinske organizacije (STO), što znači da se stočarska proizvodnja mora pripremiti za konkurenciju na jedinstvenom razvijenom tržištu, na kome neće postojati državne trgovinske barijere. Srbija na tom putu se priprema već od 2006. godine potpisivanjem bilateralnog sporazuma o slobodnoj trgovini sa susednim zemljama (Central European Free Trade Agreement-CEFTA) i samim tim postala deo tržišta i prihvatila pravila konkurencije na slobodnom tržištu. Pristupanje u EU i STO podrazumeva veću liberalizaciju trgovine stočarskim proizvodima, mala mogućnost zaštite od uvoza, implementaciju standarda kvaliteta (HACCP, ISO, Global GAP), smanjenje nivoa domaće podrške, ukidanje izvoznih subvencija, povećanje profitabilnosti i sposobnost da se izdrži konkurencija na međunarodnom tržištu.

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THE STATE OF FORAGE CROPS PRODUCTION IN SERBIA

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Abstract: The climate and soil characteristics as the main prerequisite for forage crops production will be presented in this paper. Although Serbia is small country, climate conditions are significantly different due to various relief characteristics. The average level of precipitation varies between 540mm on the northern and eastern regions to over 1000mm in some mountain regions. Vegetation seasons in mountain regions of Serbia are much shorter than in other areas, because of large differences between average year temperatures. Also, this article contains basic characteristics of forage crops production in different geographic regions. The largest part of arable forage crops is grown in valleys, which have both favourable climate and edaphic conditions for high production potential. Two thirds of total arable land (446.000 ha) are under alfalfa and red clover, while the rest in under bird's foot trefoil and annual legumes (forage pea, vetch), sorghum, Sudan grass and other crops. Sown (artificial) grasslands (grown on 155.000 ha) are very important source of livestock feed in Serbia. The production of arable forage crops decreases in higher regions, so in some mountain regions the only source of livestock feed are natural grasslands (1.454.000 ha). The research activities and the main results of scientific work on forage crops are, also, presented in this paper. During several decades, a lot of cultivars are created characterized by high DMY and a number of other important traits. There are many new production technologies which provide high DMY in different agro ecological conditions. Despite of these facts, average yields achieved in practice are unacceptably low.

Key words: forage crops, forage production

Introduction

The animal husbandry is the most important and most complex agricultural branch since it provides crude material for human food production. Beside that, its influence on the other segments of agriculture is very high. Livestock feed makes the main costs in ruminant production. Therefore, it's very important to produce enough of cheap and quality fodder and make it available during the year *(Đorđević and Dinić, 2007).*

Predominant climate in Serbia is temperate-continental and continental. Many factors, especially altitude, can affect weather conditions. There are also different types of soil with different production possibilities. Regardless of this variety, we can, certainly, conclude that the climate and edaphic conditions are favourable for forage crops growing. In lowlands, the nutrition of ruminant, cattle especially, is based on arable forage crops production. The perennial legumes (alfalfa, red clover, bird's foot trefoil and white clover), perennial grasses (orchard grass, timothy, Italian ryegrass, meadow fescue, tall fescue, red fescue, tall oat grass and perennial ryegrass), annual legumes (vetch and forage pea), sorghum and Sudan grass are the economically most important forage crops in Serbia. The total arable forage crops area in 2008 was 466.000 ha, i.e. 14.11% of arable land. Alfalfa and red clover are grown in monoculture on 312.000ha that represent nearly 70% total forage crops area. The other forage crops such as bird's foot trefoil, sorghum, Sudan grass and annual forage legumes are grown on 154.000 ha. Perennial legumes, especially red clover, bird's foot trefoil and white clover and perennial grasses are used as components of grass legume mixtures, grown on 150.000ha.

The natural grasslands that are very important source of fodder, especially in highland regions, spread on 1.454.000 ha. They are not only important as an economic objective, but also from the ecological and biodiversity point for environment preservation and protection of the soil from erosion. Investigation and classification of natural plant association as well as consumption of mineral fertilizers were the topics of many scientific studies in the last 40 years (*Mijatovic, 1973; Stosic, 1974; Lazarevic, 1991; Mrfat-Vukelic 1987; Vuckovic et al., 2002; Vuckovic et al., 2005).*

It should be mentioned that natural grasslands in lowlands on unfavourable soil, have important role in animal husbandry. Their performances mainly depend on soil characteristics, precipitations and management (*Erić, 2005).*

Climatic conditions for forage crops production

Serbia is situated between 32°15' and 46°11' N and 18°49' and 23°01' E and 80% of its territory is in South Europe region, while 20% (Pannonian lowlands) is in Middle Europe region. The central part of territory have temperate continental climate, while Panonian lowlands (Vojvodina) has continental climate with strong influence of humid and cold winds from north and north-west during winter. The south part of Serbia (Kosovo and Metohija) is influenced by Mediterranean climate with long and warm summers. Winters are cold and with high snowfalls, especially in mountain regions. Big rivers (Danube, Sava, Morava, Drina) and mountains areas (Stara Planina, Suva Planina, Šar Planina, Prokletije, Kopaonik) have strong impact on climate characteristic.

Considering the most important climatic factors (temperature, precipitation, and number of sunny days and vegetation period), it can be concluded that there are favourable conditions for forage crops production, but their utilization is not satisfactory, *(Lazarević, 2006).* Average annual temperatures mostly depend on altitude. In Vojvodina and central parts of Serbia up to 300 m altitude, they range from 10,5°C at Palić to over 11°C in Niš and Belgrade. Average annual temperatures decrease with altitude, so at the mountains (over 1000m a.s.l.) they range from 6 °C at Pešter plateau to 2.7°C at Kopaonik. The coldest months are December, January and February (in lowlands from -1°C to 1.8°C, and -1.5°C to -5.6°C in mountain regions). Long-term average values show that in lowlands and hilly regions the temperatures are over 5°C in March, while they are over 11°C in April. In higher mountain regions at altitude about 1000m the average monthly temperatures above 10°C are in May, while above 1.500m such temperatures occur in June and July. The highest average temperatures on the whole territory of Serbia are in July an August and they range from 20°C to 22°C (up to 400 m), while at the higher altitude they range from 11°C to 16°C. Spring temperatures in March and April are similar to average monthly temperatures in November and October.

Precipitations are mostly influenced relief, altitude and geographic position. The average annual precipitations, over a few decades in Serbia are between 540mm and 970mm.

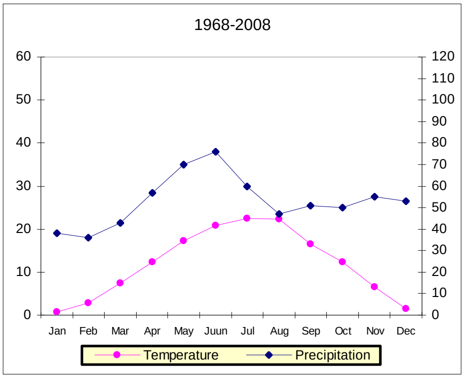

Figure 1. Climagraph by Walter for Serbia (1968-2008)

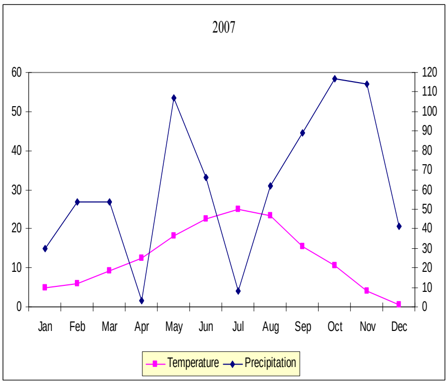

Figure 2. Climagraph by Walter for Kruševac (2007)

The lowest long-term average precipitations occur on the North (539.2 mm), North-East and South-East (610.5 mm). In these regions precipitations are the most frequent in May, June and November. In central parts of Serbia (Šumadija and Pomoravlje) with temperate continental climate, precipitations are higher and they range from 630 mm-700 mm. The western parts of Serbia are characterized with higher average annual precipitations and they range from 700 mm to above 800 mm, while the average precipitations in mountain regions, at altitude higher than 1000 m range from 800 mm to 1000 mm. Obtained results show that the total precipitations in our country isn't low, but their distribution during the year is irregular. So it is very often that during summer months (July and August) dry periods may occur, which are unfavourable for forage crops production (Figure1).

It's very important to say that extremely dry years aren't rare in Serbia, such was the case in 2000 and 2007 when the annual precipitations ranged from 400 mm to 500mm, with distinct dry periods and extremely high temperatures (over 40 ^{o}C), (Figure2). Agro ecological conditions like these can sometimes cause significant oscillations in forage crops production, which is unfavourable for animal husbandry. Considering the fact that, only 2% of arable land is irrigated, and that the forage crops are irrigated even less, it is necessary to contribute to stabilise production of forage crops with a good choice of crops and an adequate management.

Soil fitness for forage crop production

In regard to relief, i.e. altitude, several geo-morphological entities can be distinguished. The lowest terrains are mainly present on the territory of Panonian lowland (Vojvodina) and in the valleys of large rivers. Beside river valleys there are vast lowlands of Vojvodina, Mačva and Stig. Above them there are hilly terrains of Central, East and South Serbia. In these regions, soil is favourable for forage crops production. Above altitude 500 m, the share of arable land and productive capacity of soil decreases. On altitudes greater than 1000 m shallow soils are predominant. Decrease of production capacity of these soils is also influenced by shortening of vegetation period and limiting climatic conditions.

In regard to physical and chemical properties, Serbia, and especially central part, is considered as real mosaic of types of soils *(Maksimović et al. 2009).* Appropriately, the soil fertility also varieties, so for field crop production the best soils are located in lowland regions of Serbia (Vojvodina, Mačva, Stig ravine and valleys of the South, West and Great Morava rivers). With the increase of altitude, in hilly regions soils of poorer quality are predominant (brown forest soil, pseudogley, luvisol…) with distinct limitations in regard to field crop production.

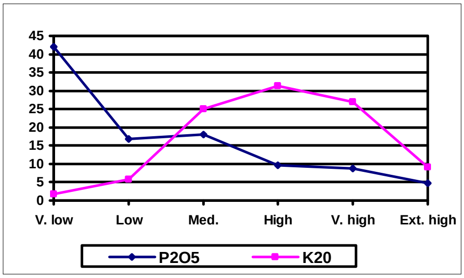

Figure 3. The rate of available P_2O_5 and K_2O in soil (%).

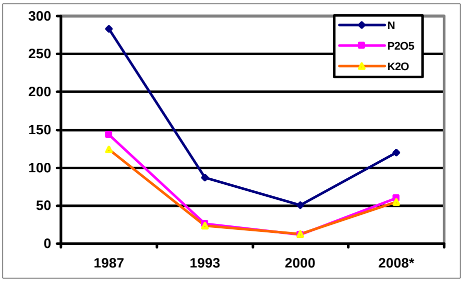

Figure 4. Total applied mineral fertilisers 000t (SAR 2002) * rating data for 2008.

Significant trait of these soils, as well as soils in mountainous regions and highlands, is distinct process of superficial erosion. It is estimated that 75% of Central Serbia is susceptible to water erosion and this is huge problem on light and loose soils on greater slopes.

Problems to be solved

One of important traits of soils in Serbia is the lack of the available form of phosphorus (P) in soil. (Figure 3). According to results obtained by *Čakmak et al. (2009)* as much as 56% of agricultural land area has very low P content, and only 20% of soils have satisfying content of this element. Situation with potassium content (K) is significantly better. Over 70% of agricultural land has high or very high content, whereas only 12% has low content of this element.

Application of mineral fertilizers in Serbia is in correlation with their price and economic position of agricultural production. The greatest consumption of mineral fertilizers was during eighties of the last century (Figure 4), when total of 283.000 t N, 144.000 t P_2O_5 and 124.00 t K_20 (SGS, 2002) were applied. Calculated per hectare, approximately 60 kg ha^{-1} N and close to 30 kg ha^{-1} of P and K were applied. With the economic crisis and economic sanctions imposed on our country in the last decade of previous century, consumption of mineral fertilizers declined drastically to only 18 kg ha-^{1} N and 5 kg ha^{-1} P and K in 2000. In the last 10 years application of mineral fertilizers has been increasing and according to estimation in 2009 it was about 40 kg ha^{-1}N and approx. 20-25 kg ha^{-1} P and K.

Numerous authors pointed out that specific problem in Serbia are presence of large area of acid soils. This problem is especially expressed in Central Serbia where 60% of soils are very acidic or medium acidic, whereas 22% is low acidic (pH 5.5-6.5) and only 18% is neutral and alkali *(Čakmak et al. 2009).* Acid soils are present not only on acidic substrates, but also in agro-ecological conditions with distinct degradation processes. Such soils are of poorer mechanical and physical composition, as well as water-air regime and decreased microbial activity.

Therefore, for the purpose of improvement of characteristics and production capacities of these soils, it is necessary to apply melioration measures *(Dugalić, 1994; Jarak et al. 2003; Stević et al. 2007)*. In solving of the soil fertility and acidity problems also the government is included through Agricultural extension services *(Official journal of RS 33/04, 2004)*.

Investigation of large number of samples of Central Serbia has shown that in addition to geological substrate, also the anthropogenic factor, (industrial zones, mines, melting plants, fossil fuel consumption, etc.) have great impact on content of heavy metals (As, Cd, Hg, Ni, Pb, Cu and Zn) in soils *(Mrvić et al., 2009)*. In spite of higher concentrations of certain heavy metals close to great industrial plants, it can be concluded that the greatest part of arable land in Serbia is not endangered by presence of heavy metals. However, *Stevanović et al. (2009)* states that great danger for soil and water courses are pollution by heavy metals (Cr, Cd and Sr) and radioactive uranium due to increased import of some mineral fertilizers.

One of the greatest problems facing the domestic agriculture is small area per farm, which results in low economical production efficiency and small number of livestock heads per farm. About 77% of total agricultural holdings dispose with up to 5 ha of land, 17% with 5 to 8ha, 8% with up to 15 ha, and only 2% of farms own land property over 15 ha (Figure 5). This situation represents significant limiting factor for agricultural production improvement, expending of the land property and incentive to market producers.

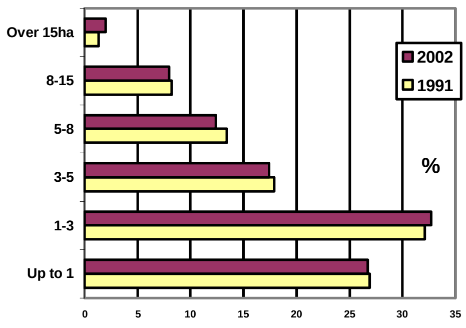

Figure 5. Structure of farm size (%)

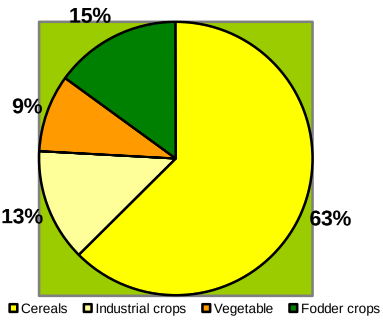

Figure 6. Structure of arable land (%)

The fact that in the last 20 years the number of agricultural holdings with land property of over 15 hectares has increased is encouraging, as well as interest of farmers to lease land owned by the state.

Forage production in lowland

Altitude has a large impact on the forage yield and quality also (*Vučković, 1999*). Lowland regions of Serbia, up to 300 m a.s.l., are characterized with soils of great production capacity, better climatic conditions and longer growing season for intensive forage production. Most of arable forage crops are produced in this region with minor production in mountainous areas.

Total agricultural land in Serbia extends on 5.093.000 hectares, of which area arable land takes about 3.302.000 ha. In the last 40 years, there is significant decline of arable land, caused by construction of infrastructures, expansion of industrial zones and housing settlements. Also, area under pastures is decreased, whereas area under meadows has increased (Table 1.). In this period, as significant resources for livestock food production are sown meadows spread on approx. 150.000 ha. On the other hand, due to distinct migration flows of population from rural regions to cities, abandoned and uncultivated fields are also increased and today they spread to over 200.000 ha, as well as abandoned areas on higher altitudes which are subject to spontaneous forestation.

Table 1. Agricultural land structure in Serbia 1970-2008 (000 ha)

Year	Total agricultural land	Arable land	Pastures	Meadows	Alfalfa	Red clover
1970	5849	3838	1015	607	190	122
1980	5786	3735	999	650	192	122
1990	5715	3666	1000	668	188	121
2004	5075	3344	823	598	186	119
2008	5093	3302	833	621	192	120

Dominant characteristic livestock feed production in lowland region is great presence of corn on over 1.200.000 ha and corn grain production of approximately 4.500.000 tons annually. Most of the corn grain produced (80%) as well as significant part of products and semi-products of soybean and sunflower are used for livestock nutrition. In addition, most of produced barley and even of some wheat grain and triticale are used as livestock feed *(Denčić and Kobiljski, 2004)*.

Production of forage livestock feed is carried out on 466.000 ha, which makes 9% of total or approx. 15% of arable agricultural areas (Figure 6). According to assessments, in order to provide adequate quantities of forage feed, are under arable forage crops should take 20% of arable land. However, this unfavourable situation is mitigated by the fact that two of the most important and most productive perennial forage legumes, alfalfa and red clover, take 67% of total area under arable forage crops *(Stošić and Lazarević, 2007)*. Beside these crops, economically important perennial legumes in Serbia are bird’s foot trefoil and white clover in grass-legume mixtures, as well as annual forage crops (fodder peas, vetches, fodder sorghum, Sudan grass and fodder beat).

Alfalfa (*Medicago sativa* L.) is the most important perennial forage legume and is grown on 192.000 ha. Depending on the soil type and climate, alfalfa, without irrigation, in establishment year, can provide 6 t ha^{-1} to 9 t ha^{-1}, and in the second year 14-19 t ha^{-1} of hay. The average content of crude protein from four cuts of alfalfa is 217 g kg^{-1} and annual yield of crude proteins is 3.12 t ha^{-1} *(Radović et al. 2007)*. The same author stated that in the second year, most of the annual yield is realized in the first cut (38%) and that it decreases with subsequent cuts to 16% in the fourth cut. Main limiting factor in growing of this crop is the soil acidity, and this is why for growing of this crop it is very important to adequately select the terrain *(Mijatović et al 1988)*. Melioration measures application gives very good results in reducing the effect of soil acidity (*Katić et al. 2007; Stevović et al. 2004*).

Alfalfa is mainly grown as monoculture, although it can also be component of grass-legume mixtures for hay production *(Lazarević et al., 1999)*. It is used for livestock nutrition in various forms, most often as hay. Results show that by ensiling of alfalfa, with addition of carbohydrates, it is possible to produce silage of good quality with minimum loss of nutritive *(Dinić et al. 1999)*. In this way problems occurring in conservation of the fist and the last cut of alfalfa in time of greater precipitations, can be avoided. Alfalfa forage is rich in proteins of favourable amino-acid composition, high digestibility if it was cut in adequate stage *(Ignjatović et al 1998)*.

Red clover (*Trifolium pratense* L.) is grown in Serbia in monoculture on 120.000 ha and it can successfully substitute alfalfa on soils of increased acidity in lowland and hilly regions. Thank to developed root and intensive biological nitrogen fixation and favourable effect on soil, it is very important in crop rotation *(Lugić et al. 1998)*. Due to low tolerance to dry conditions, period of the utilization of this plant is short, only 2 to 3 years and it is mostly grown in more humid hilly regions of West Serbia. In conditions without irrigation in the second year it gives high yields of hay (14-18 t ha^{-1}), with high crude protein (18-22%) and mineral content *(Lugić et al., 2002; Lugić et al., 2006)*. Alfalfa and red clover in lowland and hilly regions of Serbia annually provide approx. 1.500.000 tons of high quality hay without which the breeding of ruminants would be unconceivable (AJS, 2009). In recent years, high quality silage of balanced quality in regard to protein and carbohydrate content is produced by combining biomass of this crop with perennial grasses and ground corn *(Dinić et al. 2000; Jatkauskas and Vrotniakiene, 2005)*.

On lower quality soils, bird's foot trefoil (*Lotus corniculatus* L.) is very important in ensuring protein component for livestock feed. Even though there is no economical justification, in some regions on soils of increased acidity this crop is traditionally grown in monoculture. Due to its good adaptability, it is grown in different agro-ecological conditions, even on higher altitudes *(Steiner, 1999)*. Hay made from bird's foot trefoil is of excellent quality with high content of crude protein *(Radović et al., 2003)* and enough carotene and vitamins.

White clover (*Trifolium repens* L.) represents very important perennial forage legume in production of livestock feed, worldwide, and especially in some West European countries, *(Frame et al. 1997)*. It can realize high yields of excellent quality, but due to low tolerance to drought, this crop is not extensively grown in Serbia and is mainly used in clover-grass mixtures for grazing *(Krstić et al., 1995; Lazarević et al. 1999; Lugić et al., 2004)*.

Annual forage crops have long tradition of production in Serbia and they represent important source of forage and concentrated livestock food. The most important annual leguminous crops are fodder pea (*Pisum sativum* L.) and common vetch (*Vicia sativa* L.) grown on 30.000 to 35.000 hectares and used in different ways (*Mihailović et al. 2007*). In agro-ecological conditions of our country these crops provide high yields of biomass of excellent quality *(Mihailović et al. 2004a, Mihailović et al. 2005a, and Mihailović et al. 2006c)*. Target created cultivars of fodder peas with changed stem architecture, in broad production, can give grain yield of 3-5 t ha^{-1} *(Mihailović et al. 2004)*. Peas for production of grain are becoming increasingly important source of protein, because it is not necessary to heat treat the grain and it can be directly ground and used for feeds. They are sown early in spring and they utilize the winter moisture reserves and spring precipitation well, and therefore average yields are very stable and with less oscillating compared to soybean. In addition to high yields and forage quality, these forage crops are very important for improvement of the structure and fertility of soil and its more efficient utilization and exploitation. Sowing in intercropping, farmers continuously provide livestock feed from smaller land areas which is main goal *(Ostojić et al. 1996; Terzić et al. 2007)*. Also, fast development and high yields of green forage are recommended as good green manure *(Erić et al. 2000; Ćupina et al. 2004)*. On small and medium farms, which are dominant in Serbia, during the eighties of the last century, systems of livestock feed production were created in which these forage crops had very important place *(Mijatović, 1975; Erić et al. 1998)*.

However, domination of corn in lowland region, simplifying of the production process, reduction to less cultures and increase of the energy cost caused decline in presence of these crops. But, in recent years, with introduction of adequate agricultural mechanization and adoption of conservation technologies, these crops are grown more with grain crops and are used for production of hay, haulage and silage *(Dinić et al., 1998)*.

Production of livestock feed in hilly-mountainous regions

Dominant sources of livestock feed in hilly-mountainous regions are grasslands, primarily natural ones, whose share in structure of agricultural land increases with increase of altitude. Grasslands (natural and sown) take almost one third of agricultural land (Tab. 1). Depending on the degree of climatic and anthropogenic influences numerous dynamic associations are formed, except primary associations (climax associations). Their production potentials are different and under the influence of numerous factors. Dominant influences are the soil, precipitation level and management *(Lazarević et al., 2003; Ćupina et al. 2005)*.

However, hilly-mountainous region is characterized by intensive depopulation process over the last 40 years, so many areas are abandoned or holdings only with old family members. Consequence of this situation is decline in the intensity of anthropogenic influence, primarily in exploitation of grasslands. Considering that the most of the meadow associations are located in forest zones, the absence of utilization or reduced intensity of grazing lead to their transformation towards primary forest associations. This is one of the reasons why land area under grasslands has decreased in hilly-mountainous region. Main characteristic of livestock feed production in these regions is extensive production and dominant way of conservation is preparation of hay.

Process of property extension and differentiation of market producers is more distinct in lowland regions, so far, whereas in the mountainous regions it is almost negligible.

Average productivity of grasslands is very low (meadows 1,8 t ha^{-1}, pastures 0,6 t ha^{-1}), although in years with sufficient precipitation, more productive grasslands without fertilization can give over 4 t ha^{-1} of hay *(Vučković et al. 2005)*. However, numerous researches indicate that by application of mineral fertilizers, especially nitrogen, yields can be increased several times in very short time.

It can be concluded that only the application of NPK fertilizer is the most rational one where nutrient ratio is 2-3:1:1. Which quantities of fertilizer will be applied depends on many factors but the most important are grassland production potential and weather conditions. If production potential and precipitation amount are higher, quantities of mineral fertilizer should be higher *(Stošić and Lazarević, 2007)*. Norms for basic fertilizing for our natural grasslands, depending on the said factors, are following: N 40-100 kg ha^{-1}, and P and K each 30-50 kg ha^{-1}. In regard to period of the application of mineral fertilizers, research results show that all quantities should be applied before the start of vegetation *(Lazarević, 1994, Stošić et al.,1996)*.

Application of only P and K will result in moderate increase of yield after several years of application. Therefore, it is neccesary to use initial higher doses of these nutrients (P and K fertilizers), due to poor content of these nutrients in soils and partial immobilization which may occur after several years of utilization *(Stošić, 1972)*. Combination of P and K can have larger influence on the share of legumes than increase of yield if there are over 10% of legumes in the grassland *(Stošić, 1974)*. Since grasslands of Serbia have low share of legumes, nitrogen fertilizers have decisive importance, but they need to be applied always in combination with P and K fertilizers *(Stošić et al. 2004)*.

Mineral fertilizers, beside productive, also have impact on floristic changes. Nitrogen fertilizers induce development of species from the family *Poaceae*, whereas PK fertilizers induce development of legumes. This leads to dynamic changes in plant associations and their transformation towrds more productive, such as: *Agrostietum vulgaris, Festucetum rubreae* or their transitional forms *(Lazarević et al. 2003)*.

Significant role in production of livestock feed in hilly-mountainous region have sown grasslands, where production of 7-10 t ha^{-1} of hay can be realized *(Lazarević et al., 2005)*, even though production of 11,7-13,0 t ha^{-1} was also recorded *(Lazarević et al., 2004)*. Sown grasslands have faster growth in spring period, and with specific crop choice with various maturation periods, it is possible to project the period of their utilization *(Tomić et al., 1989)*.

Contrary to natrual grasslands where it is very difficult to maintain and increase the share of legumes, in case of sown grasslands this is possible. It is not easy to accomplish this in hilly-mountainous region due to the reduced choice of legumes which are used in mixtures, because of poor soil conditions, but it is feasible. Increased aciditiy narrows the choice of legumes for composing of mixtures *(Lazarević et al., 2005)*.

Considering the importance of legumes, primarily due to their quality, it is necessary to adapt and adjust the fertilization system during exploitation. In the first 2 to 3 years, NPK ratio should be equal, and later, with the decrease of legumes, it is necessary to increase the quantity of N fertilizer. In this way the exploitation period of grasslands is prolonged and better economic results are achieved.

Scientific potential for forage crops investigation

The serious scientific forage crops investigations in Serbia started in the 50s and the 60s of the 20th century and the first cultivars of forage crops are created in 1964. By now it is created a great number of economically important cultivars of forage crops *(Đukic et al. 1996)*. Active breeding programs on forage crops have been developing in State’s scientific institutions:

- Institute of field and vegetable crops Novi Sad, Department of forage crops: alfalfa, red clover, annual forage legumes, sorghum and Sudan grass.
- Institute for forage crops Kruševac: alfalfa, red clover, bird's foot trefoil, white clover and a number of perennial grasses.
- Centre for agricultural and technological researches, Zajecar: alfalfa, bird's foot trefoil, red clover and sainfoin.

Only two institutes are researching forage crops at the moment: Institute of field and vegetable crops Novi Sad and Institute for forage crops Kruševac. There are 27 researchers working in them on seed and plant improvement programme and technologies of production and preservation of livestock feed. Besides aforementioned, about ten researches are now active. They are from:

Faculty of Agriculture Zemun, University of Belgrade.

Faculty of Agriculture, University of Novi Sad.

Faculty of Agronomy Čačak, University of Kragujevac.

Faculty of Agriculture Kosovska Mitrovica, University of Priština.

There are 25 regional Agricultural extension services working with practical application of scientific results and education of farmers. In 2009, 15 Centres of rural development are also founded in order to support the development of local communities and rural areas.

Attained results of forage crops investigations

The total number of created cultivars up to recently is: 25 cultivars of alfalfa, 10 cultivars of red clover, and 5 cultivars of bird's foot trefoil, 1 cultivars of sainfoin and 1 cultivar of white clover. These cultivars have a lot of positive agronomical traits which give high contribution to livestock feed production and animal husbandry development *(Đukic et al.2007)*.

In addition to there are 18 created cultivars of perennial grasses: orchard grass (4), timothy (2), Italian ryegrass (1), meadow fescue (1), tall fescue (3), red fescue (2), tall oat grass (2), and perennial ryegrass (1) which is appropriate for growing in grass-legumes mixtures *(Tomić and Sokolović, 2009)*.

Refer to the annual crops breeding, at University Department of Agriculture Novi Sad, are created 5 cultivars of winter (feeding) pea and 10 cultivars of spring sorts for different (ways of) production (grain and feed), 11 cultivars of different species and varieties of legumes, 8 cultivars and hybrids of sainfoin and 3 cultivars of Sudan grass *(Mihailović et al., 2007)*.

Beside these domestic cultivars, there are a great number of foreign cultivars of almost all important plant species. The domestic cultivars, however, dominate in the large-scale production, and are proved to be better or equal to foreign cultivars. General opinion, confirmed by the results, is that the breeding program and germplasma of forage crops available at Scientific Institutes in Serbia are on high level and are not behind programmes and investigations in developed countries. There are more problems in a sufficient grain production due to either ecological condition for some cultivars or bad organization and lower level of availability of mechanization to farmers. In this paper, it is pointed out that all the domestic cultivars are characterized by high potential for quality biomass yield and good adaptability to climatic condition, that enable stability of yield during the period of their exploitation.

However, lower average annual yield is obtained, which is not over 40% of real genetic potential. Despite of good soil and climatic condition for forage crops growing, unfavourable economic position of agriculture and insufficient application of agricultural measures affect very low average annual yield (Figure7).

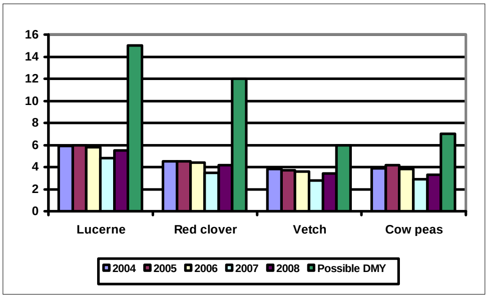

Figure 7. Realised DM yield (2004-2008) in compare to possible DM yield (t ha^{-1})

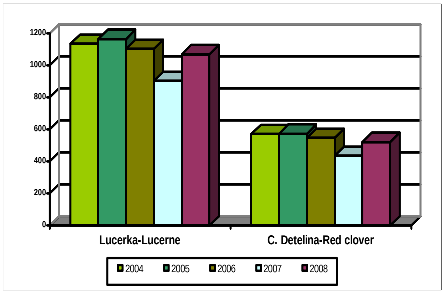

Figure 8. Total hey production (000t) of alfalfa and red clover (2004-2008).

The most frequent causes of unsatisfactory results are: choice of inadequate terrain or crops, period and sowing methods, insufficient protection against weed and pests, insufficient and inadequate fertilizing and wrong time of harvest and high losses during preservation and conservation *(Erić et al.1996)*. Extreme dry years are not rare. In those years, there are much lower quantities of livestock feed in comparison with average yield (Figure8).

There are great disparities between the science achievements and their practical application in this area of crop production.

Conclusion

Natural conditions (soil and climate) in Serbia are favourable for forage crops production and high quality, safe livestock food. Different edaphic and climatic conditions indicate the need for choice of areas for forage crop production, appropriate choice of crops and consistent application of modern technologies.

Scientific results, which reflect in numerous cultivars and hybrids of important forage crops and production and conservation technologies, provide great potentials for development of livestock production. However, the economic position of agriculture, especially animal husbandry and farming, and insufficient implementation of scientific results leads to unsatisfactory results in practice.

It is necessary to overcome this great gap between scientific achievements and their implementation in practice as soon as possible. This includes institutional transfer of knowledge, improvement of economic situation in the country, increase of government support of agricultural production and enlargement of land properties.

Also, it is necessary to introduce European standards and Halal standards as soon as possible, which will enable marketing of livestock products on markets of Western Europe and Middle East.

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Stanje u proizvodnji krmnog bilja u Srbiji

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Rezime

U radu su prikazane karakteristike klime i zemljišta kao osnovnih preduslova za proizvodnju krmnog bilja. Iako se radi o maloj teritoriji, zbog reljefnih karakteristika, klimatski uslovi u Srbiji značajno variraju. Prosečan godišnji nivo padavina se kreće od 540 mm na severu zemlje, do oko 1000 mm u nekim planinskim predelima. Zbog velikih razlika u prosečnim temperaturama izazvanih nadmorskom visinom, planinske rejone karakteriše značajno skraćenje vegetacionog perioda. U nizijama u kojima su, pored povoljnijih klimatskih uslova rasprostranjena zemljišta većeg proizvodnog potencijala, gaji se najveći deo oraničnog krmnog bilja. Od ukupno 466.000 ha na kojima se gaji oranično krmno bilje, dve trećine zauzimaju lucerka i crvena detelina, dok se na ostalim površinama gaje žuti zvezdan, jednogodišnje leguminoze (stočni grašak i stočna grahorica), krmni sirak, sudanska trava i ostale vrste. Značajan izvor stočne hrane predstavljaju i sejani travnjaci koji se gaje na 155.000 ha. Sa povećanjem nadmorske visine učešće oraničnog krmnog bilja u obezbeđenju stočne hrane se smanjuje, da bi u planinskim rejonima skoro jedini izvor predstavljali prirodni travnjaci koji se

prostiru na 1.454.000 ha. U radu su prikazani istraživački potencijali i osnovni rezultati naučnog rada na krmnim biljkama. Tokom višedecenijskog rada stvoren je veliki broj visokoprinosnih sorti ekonomski značajnih krmnih vrsta. Takođe, kreiran je niz tehnologija proizvodnje koje omogućavaju dobijanje visokih prinosa krme u različitim agroekološkim uslovima. Uprkos tome, zbog nepovoljnog stanja u poljoprivredi i nemogućnosti primene savremenih agrotehničkih mera, prosečni prinosi i ekonomski efekti koji se u praksi ostvaruju su nedopustivo niski.

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ALTERNATIVE BREEDING STRATEGIES TO EXPLOIT HETEROSIS IN FORAGE CROPS

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Invited review paper

Abstract: The exploitation of heterosis in hybrids or hybrid like varieties is being discussed since long time. In many forage crop species heterosis for biomass yield could be detected. However, under sward conditions heterosis is much less pronounced than under spaced plant conditions and can be expected in a range of 10 to 20%. Due to the heterozygous state of the parents only heterotic increase (i.e. panmictic mid-parent heterosis PMPH) can be estimated. This review presents alternative breeding strategies with respect to the creation of variation, selection and variety construction in diploid selfincompatible species. Additionally, special cases like self-fertile inbred lines and doubled haploids (DH) will be considered. The concept of heterosis, the identification of heterotic patterns and the development of divergent gene pools are outlined. Heterosis can be exploited to different degrees in several types of varieties like CMS-hybrids, SI-hybrids, semi-hybrids or synthetics. The different approaches will be presented and discussed on theoretical grounds and practical application based on experimental data from perennial ryegrass.

Key words: gene pools, heterosis, hybrids, breeding strategies

Introduction

Forage crop breeders in Europe are located in the centre of diversity of their crops. For many decades they took profit from this diversity and collected ecotypes which after simple phenotyic selection were directly used as new varieties. Since the 70th, forage crop breeding was intensified and breeders intermated whatever materials they had at their disposition. This lead to a large number of new varieties listed, mostly synthetics. On the other hand, many newer varieties are somehow interrelated and are part of a mixed gene pool *(Bolaric et al. 2005)*.

With artificial hybridization the breeder creates genetic variation by allowing recombination to occur between chromosomes from different genotypes. *(Baenziger et al 2006)* stated, '*A key issue in using hybridization to create new* *variation is selection of the parents. Despite the obvious importance of this issue much more research has been done on methods of selection in breeding populations than on selection of parents to create these populations'*. This is specially true in forage crops where mostly whole populations were mated, knowing nothing about the origin or the ancestry of these parents.

Although genetic variation is collected, created and identified, the dilemma faced by the plant breeders is to separate the desirable variation from the undesirable *(Baenziger et al. 2006)*. Simply, genetic variation must exist to achieve genetic improvement. Regardless of the breeding objective, a critical step is identifying sufficient genetic variation to meet that objective. In forage crops, a long list of objectives could be compiled. However, many objectives are only poorly defined. This is partly due to the complexity of the traits and the fact that forage is not an end product. There are numerous publications describing variation in forage crops, however, mostly on the phenotypic level. In the last decade diversity has also been analysed on the molecular level *(Bolaric et al., 2005; Kölliker et al., 2005; Posselt, 2005)*.

Creating the base populations

When choosing the sources of germplasm the breeder should have already clearly defined his breeding objectives. Instead of directly intermating unknown materials, it is worthwhile to evaluate the source materials according to the traits of interest. Furthermore, a preliminary grouping of the materials according to time of flowering and relatedness facilitates the establishment of the base population. Referring to maize breeding *Hallauer and Miranda (1981)* stated:'*Choice of germplasm is a critical decision that requires considerable thought. Hasty decisions either to eliminate or to decrease number of growing seasons required may in the long run increase the number of growing seasons required to develop usable materials. In many instances the selected germplasm will be the basis of the breeding program for the lifetime of the breeder. Choice of germplasm will determine maximum potential improvement that can be attained via breeding; the breeding system used will determine how much of that maximum potential can be realized'*.

The number of genotypes selected for the creation of the base population, and the mode of intermating varies according to the objectives and technical facilities available (Table 1).

Table 1. Examples of base population construction *(Posselt 2010)*

Origin	Single-source	Multi-source	
genetic base	broad	narrow	very broad
genetic material	regional ecotypes	distinct genotypes	25 varieties
no. of parents	100	8	100
population size	1,440	3,500	13,000
reference	*Charmet and Debote 1995*	*Wilkins 1985*	*Johnston and McAneney 1994*

Furthermore, the breeder has to decide whether to establish one or several base populations, and if these will be used in short-term or long-term selection programs. Especially in research studies, mostly 'closed' populations are used for the purpose of comparing response to selection. In practical breeding programs, the option of "open" populations is more promising. The upgrading of the breeding population can be done for the following reasons: i) increase of the already reduced genetic variance, ii) avoidance of inbreeding through the introduction of unrelated material, and iii) introgression of new genes affecting newly defined traits of interest. Depending on the purpose of upgrading, a back-up of the original base population which underwent some type of relaxed selection could already fulfil most of the requirements made. Otherwise, narrow breeding or backcross populations, superior families or other so called elite breeding material could be useful in broadening the genetic base of the respective base population. After creation of the base population it may be worthwhile estimating the overall performance of the population. In general, populations with a large phenotypic variation have lower means than those with a narrow base.

Knowledge of performance *per se* enables a comparison with the best current varieties. If one assumes that a base population yields only 10% less than the best variety, and that the gain from selection (per year) is 1.5% in the base population compared to a general gain in advanced breeding populations of 0.5%, then it will take at least ten years until the new population becomes agronomically competitive.

Besides mean performance *per se,* breeders have also to consider the genetic variability created by hybridization of the selected parents. *Schnell (1983)* combined genetic variance and mean performance in a single criterion called the usefulness criterion U., which can be predicted as: $U_{ij} = C_{ij} + i_a\ h_{ij}\ \sigma_{gij}$, where c denotes the mean of population when crossed with tester j. The parameters i_a, h_{ij} and σ_{gij} refer to selection intensity, square root of the heritability and genotypic standard deviation of the population$_i$ when crossed with tester j. *(Fischer et al. 2010)*. To my best knowledge this formula has not yet been applied in forage crops.

Developing gene pools

Unrelated materials are considered to be more diverse judged from gene frequencies than related ones. The magnitude of heterosis in the hybrid from a cross between two populations is a direct indication of the level of divergence among populations, and is used for the establishment of divergent gene pools in hybrid breeding programs. With respect to open-pollinated varieties (OPVs) and synthetics, quantitative genetic theory indicates that in the population cross obtained by random mating of the two parent populations we can exploit one half of ΔH(PMPH). With large values of ΔH, performance of the population cross often out-yields the higher performing parent population. Thus, in the case of OPVs or synthetics too, it can be rewarding to have separate heterotic groups that are inter-crossed to establish composites released to the farmer, *(Melchinger, 1999)*. This approach is especially attractive in combination with hybrid breeding and/or breeding of synthetics *(Gallais 1991)*.

During the last decade molecular markers were applied in several crop species to investigate diversity patterns of the respective gene pool *(Bolaric et al., 2005, Posselt, 2005)*. The geographic origin of ecotypes is mostly known to the breeder. For released varieties, the situation is rather complex, since breeders have intermated whatever materials they had at their disposition, no simple grouping of varieties is anymore possible and complicates the establishment of divergent gene pools. In a molecular marker study with German ecotypes and varieties of perennial ryegrass it was shown that only 2% of the total genetic variation could be attributed to variation between these two groups of material *(Bolaric et al. 2005)*. Similar results were reported by *Peter-Schmid et al. (2008)* for Italian ryegrass, but somewhat greater proportions for meadow fescue.

In cocksfoot, crosses among European and American varieties showed hybrid effects directly proportional to the putative genetic distance between the parents *(Christie 1970; Christie and Krakar 1980)*. In perennial ryegrass a close relationship between geographic distance and hybrid performance (r = 0.64) was found (Figure 1). In maize, *Moll et al. (1965)* demonstrated the relationship between geographic distance and genetic diversity. However, these authors emphasized the importance of adaptation of geographic distant materials.

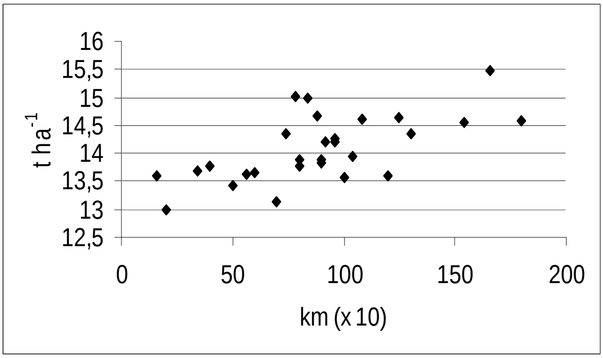

Figure 1. Geographic distance and hybrid performance of diallel crosses among perennial ryegrass populations *(Posselt 2010b)*

The concept of heterosis

The terms hybrids and heterosis are sometimes used synonymously. This is misleading since there are hybrids that do not exhibit heterosis, but there cannot be heterosis without hybrids *(Lamkey and Edwards 1999)*. Following Falconer and *Mackay (1996)*, we will define heterosis or hybrid vigor as the difference in performance between the hybrid and the mean of the two parents and call it midparent heterosis.

$$\text{Midparent-Heterosis (MPH)} = F_1 - \overline{P} \quad \text{with} \quad \overline{P} = \frac{P_i + P_j}{2}$$

This general form can be specified according to the genetic state of the parents *(Lamkey and Edwards 1999)*. IMPH: inbred midparent heterosis if the parents are homozygous inbred lines. PMPH: panmictic-midparent heterosis if two random mating populations are crossed to form an F_1-hybrid, i.e. the difference between the F_1 and the mean of the parent populations. F_2-heterosis is defined as the difference between the mean of the F_2-generation and the midparent value. Random mating in F_1 reduces the F_2-heterosis to 50 % of the MPH. The amount of heterosis in a hybrid requires two conditions: (i) directional partial, complete or overdominance at loci controlling the trait of interest and (ii) differing allele frequencies at those loci in the populations or lines to be crossed *(Falconer and Mackay 1996)*. A detailed review of "Quantitative Genetics of Heterosis" was given by *Lamkey and Edwards (1999)*.

In many species *(Dactylis glomerata, Festuca arundinacea* and *F. pratensis, Lolium perenne* and *L. multiflorum* as well as Alfalfa*)* heterosis for yield could be detected *(Kobabe 1983; Brummer 1999, Posselt 2003)*. All earlier studies were made under spaced plant conditions. Under these non-competitive circumstances both heterosis and inbreeding depression are much more pronounced (in the order of 50 %) than under sward conditions *(Forster 1971, 1973; Posselt 1984b, 1989a,b)*. *Breese (1969)*, who crossed very divergent populations of cocksfoot, stressed the point of adaptation to different environments and showed that the relative amount of heterosis (in this case PMPH) was higher in poorer environments than in high yielding locations. *Gaue et al. (2003)* also found higher PMPH under low-N as compared to high-N conditions in perennial ryegrass. Compared to other outbreeders, heterosis under sward conditions is rather small (5 – 20%). This was observed in population hybrids, SI-hybrids as well as CMS-hybrids. However, it should be kept in mind that in all three cases non-inbred or partial inbred parents were crossed and thus not the full amount of heterosis could be observed.

Identifying heterotic patterns

The importance of heterotic groups and patterns has been discussed in detail by *Melchinger and Gumber (1998)* as well as the interrelationship between genetic diversity and heterosis *(Melchinger 1999)*. If heterotic patterns are not yet known, it is suggested to preselect parents for genetic distance based on molecular or geographic data, and to produce and test diallel crosses among them. The highest performing cross combinations are used to define heterotic patterns. There is evidence suggesting that adapted populations isolated by time and space are the most promising candidates for heterotic patterns *(Melchinger and Gumber1998)*.

In a study based on molecular genetic distance (GD) among German ecotypes of perennial ryegrass distinct gene pools (Northern *vs*. Southern) were identified *(Bolaric et al. 2005)*, although the association between GD and hybrid performance was rather low (r=0.3). In a further study at Hohenheim, pre-grouping of the genetic materials by geographic distances (Figure 1), lead to a rather high association (r=0.64) between GD and performance of the diallel crosses (Table 2).

Average ADMY of the crosses (14.1 Mg ha^{-1}) was only slightly higher than the mean of the parents (13.6 Mg ha^{-1}). The highest performing cross with 15.5 Mg ha^{-1} was P1 x P2. This combination also shows the highest PMPH (12.9%). These parents originate from Wales and Austria and the geographic distance is therefore rather high. P1 shows positive heterotic effects in all crosses and could be the nucleus of a heterotic group.

Table 2. Annual dry matter yield (ADMY) in Mg ha^{-1} of 28 population crosses (above diagonal), their 8 parent populations (diagonal, in bold); and panmictic midparent heterosis (PMPH) in % (below diagonal, in italics) of perennial ryegrass averaged across 2 years and 2 locations *(Posselt 2010b)*

P*	1	2	3	4	5	6	7	8	Mean	GCA
1	13.5	15.5	14.6	14.4	14.6	14.6	14.2	15.0	14.8	7.2
2	*12.9*	14.0	15.0	13.8	14.2	14.7	14.3	14.0	14.6	4.6
3	*10.5*	*11.7*	12.9	14.4	13.7	13.2	13.9	14.6	14.2	1.1
4	*4.2*	*-1.8*	*6.2*	14.1	13.9	13.4	13.6	13.8	13.9	-2.4
5	*8.5*	*3.5*	*3.2*	*0.6*	13.5	13.0	13.7	13.6	13.8	-3.2
6	*11.4*	*9.7*	*2.2*	*0.0*	*-1.2*	12.8	13.6	13.6	13.7	-4.3
7	*2.5*	*0.9*	*2.0*	*-3.8*	*-1.5*	*0.4*	14.3	13.8	13.9	-2.4
8	*9.9*	*0.7*	*8.9*	*-1.3*	*-0.3*	*2.2*	*-1.4*	13.8	14.0	-0.6

P*: 1-‘Aberavon’, 2-‘Fennema’, 3-ecotype PL, 4-‘Weigra’, 5 to 7-ecotypes D, 8-ecotype F

A second heterotic group, i.e. the “opposite” pool to P1 would be P2. P3 shows high PMPH with both P1 and P2, and the question will be whether a third separate group should be selected. If this is not desirable, then P3 should be combined with P1 because of lower heterotic effects than with P2. P 8 has a high yield and shows PMPH in crosses with P1 and P3, but not with P2. Therefore P8 is well suited to be merged with P2. The 4 parents (1, 2, 3 and 8) showing highest PMPH are the also the most distant ones among the whole set of parents tested. All other parents show negative GCA and should not be further considered.

After having identified the two heterotic groups (pool A with P1&3 and pool B with P2&8) the breeder could either start a hybrid breeding program as outlined in Table 3, or broaden the respective gene pools. To assign materials to one of the heterotic groups, two series of testcrosses with the two pools (A and B) as testers need to be carried out. Populations displaying high PMPH with tester A are assigned to pool B and *vice versa*. All materials assigned to a particular pool have to be intermated thoroughly to establish the respective base populations.

Using gene pools in practical breeding

After having identified the two heterotic groups the breeder could either start a hybrid breeding program or create a diverse base population for synthetic breeding. In the procedures outlined in Table 3 it is suggested to create and test full-sib families (FSF). The main reasons are: They are easy to produce in seed islands of a field of rye or triticale and depending on the number of clonal parts enough seed for replicated multilocation trials can be obtained, FSFs can be multiplied individually, remnant seed can easily be maintained in cold storage systems, effective selection can be practiced among FSFs, and FSFs can be used for genetic improvement by recurrent selection within the respective gene pools (for details of FSF selection see *Posselt 2010a*). In *hybrid breeding* the two divergent pools are maintained seperately. A series of testcross hybrids (interpool hybrids) are produced and tested for their yield performance. In *population breeding* the heterotic groups are intermated to create a base population with large genetic diversity. Several cycles of random mating are needed to reach Hardy-Weinberg equilibrium. From this population high performing varietal parents are to be selected. The parents could be FSFs since this type of selection unit is easy to handle. To exploit more heterosis in synthetics, genepools could be kept separate until final synthesis. This is also preferential if ploidization is intended to create a new tetraploid variety.

Table 3 Generalized scheme for alternative breeding strategies using divergent gene pools

Pool A	Pool B		Pool A	Pool B
create POP (AB)			make pair crosses	make pair crosses
Random mating (2 – 3 times)			test performance per se of the FSFs	
make pair crosses			factorial crosses FSF(A) x FSF(B)	
test the full sib families (FSF)			test all interpool hybrids	
select the best FSFs for synthesis			select the best as	
synthetic			hybrid	synthetic

Dimensioning of a breeding program

Population improvement by inta pool recurrent selection enables the breeder to repeatedly capture heterosis.

With closed gene pools, the degree of inbreeding (expressed as F) which is allowed should be taken into account. Assuming inbreeding depression of 1% per cycle (i.e. F=0.01), then the population size should be 50 non-inbred plants or 25 FSFs. If a selection intensity of 10% shall be applied, then 250 pair-crosses are to be made and the best 25 of them will be recombined to produce the improved population *(Posselt 2010a)*. The 10 best from each pool are intermated in a factorial manner and the best inter-pool hybrid will be a potential new variety. So far, no experimental data is available. Compared to other crops, the number of testcrosses is rather small.

Pre-selected parents are available

Demarly (1977) presented breeding schemes (Figure 2) to exploit heterosis on the bases of four non-inbred parents. The first example refers to the classical scheme of synthetic construction. In the second case pair-crosses are carried out to produce full-sib families. If two FSFs are mated and further multiplied after synthesis, this will be also a synthetic. In the first hybrid scheme, the two FSFs are multiplied *inter se* (this results in a partly inbred family coded FSF[2]) before synthesis or hybridization. In those crops where inbreeding is possible, partly or fully inbred lines are mated to create FS-families before hybridization of the new hybrid.

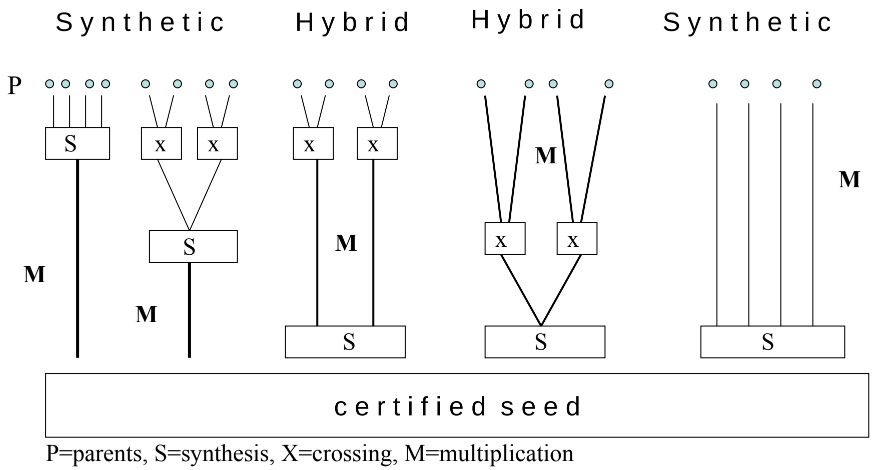

Figure 2. Exploiting heterosis in 4-parent cultivars *(Demarly, 1977)*

This scheme is applied in the breeding of self-incompatible hybrids (SI hybrids) since the FSF is a single cross hybrid and depending on the relatedness of the parents will show heterosis. The hybridization of two FSFs will result a double cross hybrid. However, in most cases hybridization in the sense of controlled crosses cannot be achieved. In the last case it is assumed that fully inbred lines or doubled haploids (DH) can be produced before synthesis.

Self fertility: In many fodders crops and amenity grasses the production of inbred lines is hampered by self-incompatibility. However, as was shown by *Posselt (1982*) genes for self fertility exist in many populations and self fertile inbred lines were developed at Hohenheim in perennial ryegrass (*Utz and Oettler 1978*). In spring rye it was observed that synthetics from self fertile materials yielded 10 to 15% less than comparable synthetics of self-incompatible material (Singh *et al*. 1984). The average amount of self-pollination in open-pollinated self fertile rye populations was estimated to range between 35 and 40%. For the progenies of crosses among the above mentioned self fertile inbred lines of perennial ryegrass, self-pollination rates of 0 to 63% were observed *(Posselt, unpublished data)*. In conclusion, *the use of self fertile material should strictly be avoided in breeding synthetics because complete out-crossing cannot be guarantied*. However, such self-fertile inbred lines could be useful in cms-based hybrid breeding.

Doubled haploids (DH): In several grass species it was possible to develop DH lines *via* antherculture at Hohenheim *(Posselt, unpublished data*). However, the rate of success was highly genotype dependent. Moreover in a large number of perennial ryegrass DHs no seed set after selfing could be obtained. These DH plants were therefore strictly self-incompatible. However, DHs can be easily maintained vegetatively. From a set of five unrelated donor plants two DH-lines were obtained from each of them. Two types of synthetics were synthesized, i) a 5-clone synthetic and ii) a 10 DH-line synthetic. In comparative field experiments of the Syn-2 over two years at two locations, a slight but not significant superiority of the DH-synthetic was observed at a yield level of about 12 Mg ha^{-1} of annual dry matter yield (ADMY). A possible explanation for this *(Röber et al. 2005)* is that DH-lines carry hardly any genetic load due to a strong selection pressure against deleterious recessive genes in the haplophase. Further research on the use of DHs in forage plant breeding is needed.

Hybrid breeding

Hybrids derive from controlled matings of two parent components i and j and allow the breeder i) to fully exploit the panmictic-midparent heterosis (PMPH; *Lamkey and Edwards 1999*) of crosses between genetically distant populations (heterotic groups) and ii) to capitalize not only on GCA but also on SCA effects *(Geiger and Miedaner 2009)*. The genotypic value of a hybrid can be described as $Y_{i \times j} = m + GCA_i + GCA_j + SCA_{ij.}$, were $Y_{i \times j}$ is the hybrid performance, m is the mean of all crosses, GCA_i and GCA_j are the GCA effects of the parents i and j, and $SCA_{ij.}$ is the specific combining ability of the two respective parents.

Hybrid breeding demands i) identical reproduction and multiplication of the parental components on a large scale, ii) controlled crossing of parents on a large scale and iii) fertility of hybrids if seeds are to be harvested. Biological prerequisites are i) a sufficiently high degree of heterosis for economically important traits and ii) a suitable hybridization mechanism. Because of the flowering biology of forage crops, at present only CMS (cytoplasmic genic male sterility) or SI (self-incompatibility) are suitable hybridization systems.

In self incompatible species like fodder crops and amenity grasses, a single cross from two non-inbred parents [(AB) x (CD)] corresponds with a DC based on four inbred lines and is sometimes referred to as ‘cryptic double’. Due to its genetic constitution it is a segregating F_1 .

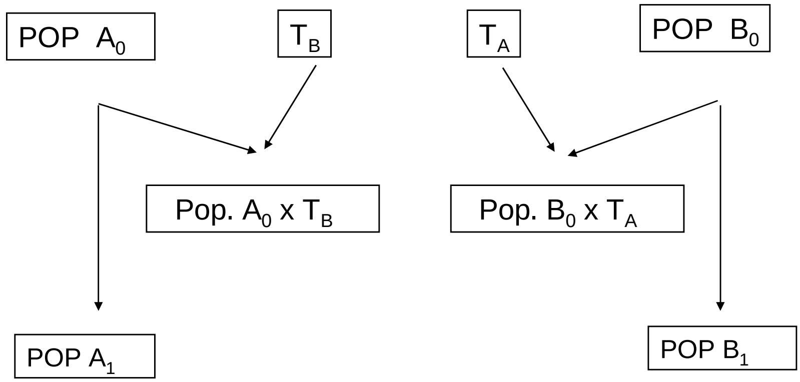

Figure 3. Generalized scheme of reciprocal recurrent selection for improving two parent populations in hybrid breeding *(Posselt 2010a)*

The hybrid breeding scheme in Figure 3 implies that reciprocal testcrosses are made to identify the best genotypes of the candidate hybrids. The best genotypes from each pool are recombined for intra-populational improvement. In perennial crops the selection units for recombination could be clones or families as previously described. After several cycles of reciprocal recurrent selection when a sufficient yield performance in the testcrosses is reached the best selection units from each pool are the potential hybrid parents. In crops where inbreeding or the production of DH-lines is easy, such as maize, intra-population improvement of the respective pools is accompanied by inbred or DH-line development. The final SC is identified by factorial crosses among inbred or DH-lines of the two heterotic groups

CMS-Hybrids

As an example of cms-hybrid breeding, hybrid production in rye (*Secale cereale* L.) will be briefly described. Current hybrid varieties are crosses between a cms-SC as seed parent and a restorer synthetic as pollinator: (A_{cms} x B) x SYN_{Rf} . The SC_{cms} and the SYN derive from different heterotic groups. The hybrid can be classified as a DC, since the pollinator synthetic is based on two inbred lines. Parent B is a self fertile non-restorer inbred line. In practice, rye hybrids are produced as a mixture of 90% SC_{cms} and 10% of SYN_{Rf} as pollinator. Thus, the final hybrid consists of about 90% hybrid plants *(Geiger and Miedaner 2009).*

In grasses with a similar flowering biology hybrid breeding can follow the rye breeding procedure, if inbred lines can be developed. This was done in the former Hohenheim hybrid program with perennial ryegrass *(Posselt 1984b):* The cms-source was maintained by crossing to one of the inbred lines available. The BC_2 was used as cms-tester and the candidates were non inbred plants from the opposite pool *(Posselt 1989b)*. The forementioned BC_2 was crossed to a non-related inbred line to produce the SC_{cms} as seed parent, while a two-parent synthetic was developed as pollen parent. Hybrid production was like in hybrid rye. The program had to be abandoned, because the cms materials were not stable in pollen sterility.

Ruge et al. (2002) reported about a new and stable cms source (named MSL-cms) in perennial ryegrass. A practical hybrid breeding program was initiated and the first hybrids are in VCU trials in Germany (Luesink, NPZ Hohenlieth, Germany; pers. communication).

SI-Hybrids

The production of SI-hybrids in grasses based on the gametophytic two-locus incompatibility system was proposed by *England (1974)*. The procedure depends on the ability to self an individual genotype to produce the expected portion of genotypes which are homozygous at zero (hom 0), one (hom 1) or both (hom 2) of the loci. Two further generations of intermating the S1-line by open pollination (R) in isolation are necessary to obtain equilibrium (0.5 hom 0 and 0.5 hom 1) in the S1R2. If two different lines with appropriate SI-genotypes are mixed together, a seed crop is produced which consists maximal (83%) of F1-hybrids. The remaining 17% are inbreds from intermating within the parental S1R2- lines. Under the intense competitive conditions of a sward it is assumed that only the more vigorous hybrid plants will survive *(Posselt 1993)*.

Table 3. Heterosis in SI-hybrids of L. perenne *(Posselt 1993)*

Set	S_1*-lines (n=10)	SI-hybrids (n=25)	
	midparent value	mean	max.
1	100	104	120
2	100	108	114
3	100	109	115

In an experiment 30 S1R2 lines were grouped arbitrarily into three sets with 10 lines each. The 10 lines were crossed in a 5 x 5 factorial manner. The 25 SI-hybrids and the 10 parental lines of each set were field plot tested in comparison to a standard variety at two locations for three successive years. On average, hybrids out-yielded the partial inbreds only slightly (7%). The best SI-hybrid yielded 10.3 Mg ha^{-1} ADMY and out-yielded the standard variety by about 5%. Highest PMPH was 20%. It has to be mentioned though that in this study all inbred lines had been derived from a single gene pool. Additional heterotic effects and thus higher hybrid performance can be expected if the parental lines originate from different heterotic groups.

From the original clones which were used for line development, synthetics were constructed. Twelve SI-hybrids were tested in comparison with their respective Syn-2(2) synthetics. On average the SI-hybrids out-yielded the synthetics by 10%.

Eickmeyer (1994) investigated the SI-hybrid system in Italian ryegrass under spaced plant conditions. He found much higher MPH (up to 80%). By means of electrophoreses he was able to estimate hybridisation rates, which often were less than the theoretical expected 83%. Critical points are: the S1R2-lines have to be different in their S and Z-alleles, time of flowering has to match and equal amounts of pollen should be released by the two lines. A further critical point is the maintenance of the S and Z composition during S1 multiplication. Migrating S or Z-alleles are assumed to have selective advantage.

If SI-hybrid production were to be carried out on 100 ha, 2,000 kg of seed would be needed. The 1,000 kg of each line would be produced on 2 ha being isolated as best as possible. The 40 kg for sowing the 2 ha should be produced under pollen proof conditions in isolation cabinets.

Semi-hybrids

The general idea of creating population hybrids in grasses has been described by *Burton (1948)*, who suggested the term 'chance hybrids'. Other authors *(Kobabe 1983, Brummer 1999*) favour the expression 'semi-hybrids'. Inter-populational hybridization results in 50% inter-populational and 50% intrapopulational crosses. Theoretically, one half of the potential heterosis can be exploited in such 'semi-hybrids'. If the populations derive from distinct heterotic groups, the 'semi-hybrid' can display a large proportion of PMPH and also out-yield the better parent *(Melchinger 1999)*. An example of the variation in population hybrid performance in perennial ryegrass is given in Table 2.

In Italian ryegrass, *Bertling (1993)* analysed parent populations and their offspring populations by means of electrophoresis. In several crosses, the rate of hybridization was much less (35%) than the expected 50%. Flowering synchronization between the two populations is one bottle neck of this system. Occasionally, in diallel population crosses of unrelated materials, significant SCA variance is observed, which is often an indication of non-matching pollination. In many cases, divergent populations will also be different in their phenotypic appearance which may lead to great heterogeneity in the resultant 'semi-hybrid' and it is doubtful that DUS requirements can be fulfilled. However, phenotypic similarity can be controlled much easier in narrow populations like full-sib families. Thus, referring to Figure 3, FSF selection is suggested, and the 'semi-hybrid' derives from crossing phenotypically similar FSFs.

Combining hybrid and synthetic breeding

In terms of synthetic breeding, a FSF equals a synthetic of two parents [Syn-1(2)] and, reaches equilibrium after further multiplication [Syn-e(2)]. The crossing product of two pre-selected synthetics [Syn-e(2)A x Syn-e(2)B] is a Syn-1(4)AB. or could be called a hybrid. This Syn-1(4)AB should be superior to a four-parent equilibrium synthetic [Syn-e(4)], because in the latter only part of the PMPH is retained.

GCA vs. SCA

Theoretical results *(Reif et al. 2007)* demonstrated that a decrease in genetic distance will lead to a predominance of variance due to specific (SCA) versus general combining ability (SCA) effects, and thus to a larger σ^2_{SCA} σ^2_{GCA} ratio. Larger variation due to SCA reduces accuracy in predicting hybrid performance on the basis of GCA effects. Therefore, success of hybrid breeding programs depends on the genetic distance among heterotic pools *(Melchinger and Gumber, 1998)*.

The ratio of $\sigma^2_{SCA} : \sigma^2_{GCA}$ is a criterion of the importance of SCA for a particular trait. In maize *(Melchinger 1999)* it was shown that SCA was much more important for grain yield than for forage yield (12.9 *vs*. 3.9). No such comparisons are known in the forages. However, it has been assumed that in vegetative traits like biomass production additive gene action is much more important than non-additive effects (*Breese and Hayward 1972*), while in generative traits like seed yield non-additive effects are more pronounced.

Application of molecular markers

DNA markers have been widely used to describe genetic variation in the species of interest, however, so far only few attempts have been made to use the diversity information in practical breeding. The relationship between genetic distance (GD) and yield of diallel crosses was investigated in perennial ryegrass *(Posselt 2005)*. Since so far mostly anonymous markers were applied, the association between genetic distance and yield performance of population hybrids was not very high. However, because only a limited number of populations could be tested as diallel crosses, pre-selection of promising populations by marker application could be worthwhile. The assumption that genetically diverse parents out-yield populations from genetically similar parents was confirmed in perennial ryegrass *(Kölliker et al. 2005)*. The latter group selected individual genotypes from a set of elite clones according to their genetic diversity based on AFLP marker data and developed synthetics with contrasting levels of diversity (narrow vs. wide). The more diverse synthetics out-yielded the narrow ones. In the context of the breeding procedures outlined above, marker application could be useful in i) pre-selection of populations, ii) selection of diverse individuals before establishing the respective gene pools, and iii) selection of promising parents to reduce the number of testcrosses. Marker assisted selection is beyond the scope of this review, however, the interested reader is referred to a recent review *(Roldan-Ruiz and Kölliker 2010).*

Further considerations

In general, hybrids of arable crops are cultivated under monoculture conditions. With the exception of alfalfa, this is not the case in forage crops. They are sown in mixtures of several species The greater uniformity of hybrids might be of a disadvantage to explore niches in the mixed swards, or they might dominate the sward in an unwanted fashion. More probably even, there will be a dilution effect, since only part of the heterosis can be exploited due to the reduced amount of hybrid plants in the mixture. Thus, it is an open question, how perennial ryegrass hybrid varieties will perform in association with white clover as a companion crop.
In the amenity grasses, no advantages can be seen for hybrid varieties, since yield performance is not a breeding objective and uniformity is not superior than in synthetics. Moreover, most amenity species are used in mixtures anyway.

Iskorišćavanje heterozisa kod krmnih biljaka korišćenjem alternativnih strategija oplemenjivanja

U. K. Posselt

Rezime

Dobijanje heterozisa prilikom kreiranja hibrida i sorti nastalih hibridizacijom predmet je diskusija duže vreme. Kod mnogih vrsta krmnih biljaka pojava heterozis za prinos biomase je detektovana. Ipak, u proizvodnim uslovima travnjaka, heterozis je mnogo manje izražen u odnosu na oplemenjivačke uslove u matičnjacima pojedinačnih biljaka i može iznositi od 10 do 20%. Na osnovu heterozigotnog stanja samih roditelja može se proceniti doprinos heterozisa PMPA (tj. panmiktički heterozis u odnosu na prosek oba roditelja). Ovaj rad opisuje neke alternativne strategije oplemenjivanja, mogućnost dobijanja nove varijabilnosti, selekciju genotipova i kreiranje sorti kod diploidnih stranooplodnih biljaka. Dodatno će biti razmatrane self-fertile inbred linije i dvostruki haploidi (DH).

Istaknuti su koncept heterozisa, identifikacije heterotičnih obrazaca i formiranje divergentnih genskih pulova. Heterozis se može dobiti u nekoliko tipova sorti kao što su CMS-hibridi, SI-hibridi, poluhibridi ili sintetici. Biće prikazani i diskutovani različiti pristupi sa teorijskog aspekta i praktične primene, na osnovu eksperimentalnih podataka dobijenih na engleskom ljulju.

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BREEDING OF PERENNIAL FORAGE LEGUMES IN SERBIA, RESULTS AND FUTURE DIRECTIONS

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Abstract: Perennial forage legumes have important role in providing efficient, viable and sustainable systems of animal production. Alfalfa and red clover is base of forages in arable land, while on low quality soil and hilly mountain region, beside red clover, birds foot trefoil, white clover and in same causes sainfoin have important role. Due to their importance, breeding of alfalfa and red clover started in Serbia in the middle of twenty century, while other forage legume breeding started later. The aim of this paper is present achievement of forage legumes breeding in Serbia and their plan for further period. Divergent selection materials (local and wild populations, introduced material) and different breeding methods were used to create high productive domestic cultivars with acceptable persistence, which is important factor for high and stable yield during entire exploitation period. Until now, breeders in Serbia, were created over 30 varieties of perennial forage legumes. Domestic varieties are well adapted on local environment and provides high and stable forage yield during all period of exploitation.

Key words: perennial forage legumes, breeding, variability, breeding methods

Introduction

Perennial forage legumes play an important part in grassland farming in many regions of the world. The well documented advantages of forage legumes are their high forage production and superior feeding value, as their contributions to the nitrogen economy of sward through nitrogen fixation *(Tomić et al., 2007)*. Due to rich and variable genetic base they have good adaptability on different environmental conditions and wide area of growing. They are cultivated in dry regions with 200 mm annual precipitation without irrigation as well as in humid regions with 2500 mm precipitation. Grown on the arable land, alfalfa and red clover can provide cheap forage of high nutritive value and digestibility. Also, some perennial legume species could provide the protein component in forage when grown on the low quality soils not suitable for alfalfa.

White clover and birds foot trefoil, are especially highly capable of extracting soil nutrition and have minimal demands in relation to growing conditions. They are also adapted to shallow and poor soil where no other plant would be able to grow and achieve the same forage yield.

Suitable choice of forage species, which depend on soil quality, climatic condition, altitude and farmer requirements, could provide protein rich fodder feed, which is usually insufficient in animal husbandry. Depending on the environment condition, perennial forage legume species have a different importance in various regions.

The most important forage legumes in Serbia are alfalfa (200 000 ha) and red clover (120 000 ha). In mountainous and hilly areas, red and white clover and bird's foot trefoil have important place in animal husbandry development as a components of legume-grasses mixtures, Breeding of forage legumes in Serbia started in the middle of the twentieth century in the Institute for forage crops (alfalfa, bird's foot trefoil, red and white clover), Institute for field and vegetable crops (alfalfa and red clover) and Center in Zaječar (alfalfa, bird's foot trefoil, red clover and sainfoin). Until now breeders in Serbia created near 40 forage perennial legumes cultivars (Table 1).

Table 1. Perennial forage legumes cultivars breeding in Serbia

Species / Organization	Alfalfa	Red clover	White clover	Birds foot trefoil	Sainfoin
Institute for forage crops, Kruševac	6	5 (2n) 2 (4n)	1	2	-
Institute for field and vegetable crops, Novi Sad	11	3	-	-	-
Center, Zaječar	4	1	-	2	1
Total	21	11	1	4	1

Numerous results confirm that domestic cultivars provide high and stable forage yield during the entire utilization period and they are proved to be much better than the introduced alfalfa cultivars *(Radović et al., 2004a; Katić et al., 2005)*. Similar results were obtained in comparative testing of foreign and domestic cultivars and populations of red clover *(Vasiljević et al., 2005a; Lugić et al., 2002; Vasiljević et al., 2010)* and birds foot trefoil *(Radović et al., 2003)*. It is confirmed that the domestic cultivars in relation to production, quality, morphological and biological characteristics were superior to introduced varieties. All of this affect that domestic cultivars are widely spread in agricultural production, and introduced cultivars could find place in the market just in years which are not favourable for seed production that have affect the lack of seeds.

This paper will present approach of forage legumes breeding in Serbia, focus on results and further work.

Problems in breeding

Regarding their importance as forage crops, great attention in breeding program was paid on this species all over the world. Considering that, scientific efforts have been and are being devoted annually to improvement of both yield and quality of alfalfa. As a result, numerous forage legumes cultivars, especially alfalfa, have been created in last fifty years.

In spite of that, genetic increases in alfalfa yield have been small, compared with those realized in most grain crops. However, increasing the forage, despite the well-developed breeding programs of its kind, was slightly. According to research *Holland and Bingham (1994)* dry matter yield of alfalfa was increased for about 20% for the last 100 years. It is believed that about 10% increase in yield, is a result of improved technology in agricultural production, which is only 10% contribution to the breeding of this species. This yield increase was a result of a large number of populations of different geographic and genetic backgrounds as well as work with a variety of plants in the population. Compared with other agricultural species, the results achieved in increasing the yield of alfalfa biomass are very small. Similar results are achieved in other forage legumes breeding.

One of the most obvious is the perennial growth habit, so one experimental strain must be evaluated for several years before decisions can be made in selection program. Large portion of the yield increase in many crops had been achieved by altering regulatory processes and increased the proportion of plant assimilates going into the desired plant organs without increasing total plant growth. But, it isn't possible in forage legumes, because the total plant is used for forage.

Another reason is the genetic and reproductive characteristic of those species. Perennial forage legumes are mostly cross pollinated species, tolerates comparatively little inbreeding. Therefore, the getting a homogenous material is very difficult *(Osborn et al., 1998*). Natural autotetraploidy is common occurrence in alfalfa, white clover, bird's foot trefoil, while sainfoin population could be diploid and tetraploid. For that reason inheritance of most agronomical important traits in perennial forage species is therefore quite complex and could make difficulties in breeding.

Source of variability

Results of breeding primarily depend on the extent and type of variability in breeding materials, and choosing of the appropriate selection methods. As previously stated, perennial legumes are mostly autotetraploid and cross pollinated species, which causes a high variability among and within populations. High variability for numerous morphological and productive traits has been obtained for alfalfa *(Radović et al., 1996; Katić et al., 2004),* red clover *(Lugić. 1999; Vasiljević et al., 2001a)*, white clover *(Lugić et al., 1998; Lugić et al., 2001)* and birds foot trefoil *(Radović et al., 2004b)*. Variability for forage yield and forage yield components are frequently used in breeding program for developing cultivars with high forage production and quality. Although the populations of forage legumes had a wide genetic base, for most important traits, increase the existing variability by introducing new material is always desirable. Various sources of variability were used in forage legume breeding in Serbia.

Local populations - One of the particularly important sources of variability for perennial species is local populations, which are highly adapted to the agro-ecological condition in which are grown *(Miladinović, 1972)*. The selection of breeding material which is generally adapted to the ecological condition of the region is a major requirement for achieving high and stable yield in perennial species. This is one of the reasons why many local varieties showed better results than introduced species (*Rumbaugh et al., 1988*). First domestic cultivars of alfalfa (K-1, M-2, Banat, Bačka) and red clover (K-3, K-9, Kolubara and Avala) are created from local population. Collecting and evaluating a huge number of populations from different part of Serbia are still good source for yield stability and field persistence. Results obtained by many researches proved the value of local population as a starting selection material for breeding cultivars with broad genetic base.

Wild flora - Serbia is due to high variability of geographical, climatic, pedological and land configuration condition, in floristic way, on of the most complex area of East Europe. Strong migration and influence from other of the other European regions, particularly from East Europe, were very important for great wild population variability of forage crops in flora of Serbia. Natural meadows and pastures are rich sources of variability for many traits. There is a need to determine the diversity of forage crops and their wild relatives grown in Serbia and use them in breeding program.

Wild populations of birds foot trefoil, red and white clover are common component of natural grasses in all part of Serbia. They are differing in yield, quality, but their tolerance to stress condition, tolerance to growing and persistence in mixtures with other species is most important. Wild population of birds foot trefoil, collected from different part of Serbia, besides local population, was initial material for obtaining high productive and persistent genotypes *(Mijatović et al., 1986; Radović et al., 2003)*. Initial selection material for creating new varieties of bird's foot trefoil (Zora, Bokor, Sumadija and K-37) and white clover cultivar K-33 *(Lugić et al., 1996)* were wild population.

Wild relatives. The introduction of wild relatives of alfalfa, *Medicago sativa,* primarily in alfalfa breeding process, an attempt was made further increase genetic variability and adaptability of breeding material *(Rumbaughet al, 1988)*. The need for diversifying and broadening the existing genetic base of alfalfa led to their introduction of related species into breeding program. First interspecies crossing (*M. sativa X M. falcata*) were made in Institute for field and vegetable crops in Novi Sad. As a result of this inter species crossing, two alfalfa cultivars were created, NS Mediana and Novosadjanka H-11. Those cultivars were characterized by increased winter hardiness, tolerance to lodging and major disease, and they are suitable for the cultivation of the soil slightly lower quality. Wild flora is rich source of other relatives from genus Trifolium, which is characterized by high genetic variability of species, with different level of ploidy *(Mrfat-Vukelić et al., 2003)*. Wild species from *Trifolium* genus could be used as forage crops, especially in hilly mountain regions of Serbia where the providing of protein components in animal food still are problem. Wild populations of *Trifolium pannoicum* collected from different part of Serbia showed high location effect on morphological traits as on quality of dry matter *(Lugic et al., 2005)*.

In the last few years Institute for forage crops in Kruševac organized few expeditions every year for collecting new variability from wild flora of Serbia. As a result, rich collections of *T. repens, T. pretense*, *T. montanum, T. medium, T. alpestre*, *T. campestre, T. pannonicum, Lotus corniculatus* and *Medicago falcata* were collected and a part of collection are in the nursery at experimental field of Institute for forage crops in Kruševac.

Introduced selection material. Rich collection of autochthonous breeding material isn't enough for successful breeding. Increasing variability in selection material could be achieved by introducing divergent genetic material, which could be new source of diversity. Considered that, yield increase in alfalfa was a result of a large number of populations of different geographic and genetic backgrounds. Nowadays, intensive international cooperation of scientific workers has allowed the exchange of selection materials, therefore research institutes have a rich collection and cultivars usually have, more or less, genes from divergent germplasm. Introduced cultivars usually showed lower forage yield and field persistence, especially in longer period of investigation, mainly as a response of poor suitability on new environment. Anyway, they could be new source of variability, especially for same traits which is missing to domestic cultivars *(Radovic et al., 2009b)*.

Breeding criteria

Main goal of forage legumes breeding is forage yield increasing, with maintenance of good forage quality.

Previous efforts to obtain the plant breeding material with high content of raw protein, while maintaining high yields, did not give adequate results. Therefore, more attention was paid to forage digestibility parameters (NDF, ADF, lignin) for which greater variability was find in the alfalfa cultivars *(Marković et al., 2008; Vasiljević et al., 2009)*. One of the indirect ways of improving the quality is breeding of genotypes tolerant to the frequent cutting, and tolerance to lodging *(Katić et al., 2008)*.

Beside that, special attention was paid on field persistency and stable forage yield, which are complex traits, especially important for perennial forage species. Field persistence depends mostly of resistance to abioticu (soil, drought, low temperature...) and biotic factors (various pathogenic organisms) under field conditions, which affect duration of exploitation period. As a result of long term breeding on field persistence in Institute for forage crops were created two red clover cultivars K-38 and K-39, which achieved better forage yield in third year of utilization, which was main complain for previos cultivars *(Lugic et al., 2006)*.

Special attention, particularly in North America, is dedicated to alfalfa resistance to diseases and pests, which greatly affects the yield and quality obtained from biomass. As a result of the large number of researchers today, most alfalfa cultivars are characterized by medium or high resistance to important diseases and pests, but have not reached full strength and still not enough to protect alfalfa from the strong attack of pathogens. Forage crops disease wasn't object of investigation in Serbia a long time. In the last twenty years more detailed examination of the crown and alfalfa root diseases caused by *Fusarium sp.* and *Colletotrichum sp.* were started. For now rich collection of pathogen isolates was formed, as a good base for further investigation. Variability among and within alfalfa cultivars for tolerance to *Fusarium sp. (Krnjaja et al., 2004)* and *Coletotrichum sp. (Vasić et al., 2009)* was found.

For species which usually grow in mixture with high competitive grasses, as birds foot trefoil, red and white clover, the most important traits, besides yield and forage quality, is improving compatibility and slow re-growth after cutting which is crucial for long term persistence legume component in mixtures.

The problem of increasing soil acidity is, recently, more and more pronounced in Serbia. Alfalfa is one of the sensitive plant species to increased soil acidity and, thereby, its growing area is reducing. These problems put another task to alfalfa breeding - creating alfalfa genotypes with increased tolerance to slightly acidic soil.

Breeding methods

The success of a long-term breeding program, as any perennial species breeding, largely depends on the choice of appropriate selection methods and the initial values of properties that we want to improve. Choosing the most appropriate breeding methods and its application are conditioned by the extent and type of genetic variability, genetic correlation between traits and heritability for most important agronomic traits.

Mass selection method takes important place in the forage legumes breeding, primarily because of its simplicity and efficiency. It is especially suitable for breeding of wild and local populations and for those features to that already made natural selection. The first alfalfa and red clover cultivars, created in the middle of 20 century in Serbia, were created by mass selection from local populations *(Djukić et al., 1996)*. Today this method is used mainly in pre-selection and maintenance of cultivars.

Recurrent phenotypic selection is often used in alfalfa breeding. It consists of several cycles and one cycle of selection involves evaluating a number of plants, selecting individuals with desirable traits and to obtain progenies under conditions of controlled pollination. The advantage of this method is that the father was also selected, while the mass selections of plants fathers are not controlled. Recurrent phenotypic selection gives good results for properties with a medium or high heritability. The application of recurrent phenotypic selection to achieve significantly better results than using mass selection. It is used to choose parental line for breeding.

Polycross is most widely used forage legume breeding method (Progeny Test Selection), which arises from a wide variety of synthetic gene basis, obtained by varying the number of parents. In order to achieve heterosis in synthetic cultivar, it is necessary that parents have good combining ability. For low heritability traits, such as yield, for the selection of parents, methods which include the use of progeny tests are recommended.

Synthetic cultivars are developed in order to increase yield stability and cultivar adaptability, as well as to avoid inbreeding depression in the generations of maintenance *(Holland and Bingham 1994)*. In order to avoid inbreed depression it is necessary to use at least 5 parents. Therefore, it is suggested for the development of synthetic cultivars to use more than 4 and up to 100 parents (components) *(Rumbaugh et al., 1988)*. In practice, much larger number of parents are used, usually 20 to 100, although there are examples that the number of parents moving up to 1000 *(Chloupek, 1994)*. Synthetics with the broader genetic base (greater number of parents) have a higher level heterozygote and they are less sensitive to the interaction of genotype x environment which enables them to better adaptability and high and stable yields trough the whole exploitation period *(Hill et al., 1988)*.

The most forage crops cultivars created in Serbia are synthetics. Numbers of parental line in cultivar is different and depend of breeder, but don't excise 30 parental lines.

Induced polyploidy Unlike alfalfa, white clover and bird's foot trefoil, which are natural tetraploids, red clover populations are diploid. This fact opens the possibility for using induced polyploidy for getting tetraploid red clover forms. By duplication of chromosomes, induced by colhicine *(Krstic et al., 1983)*, in Institute for forage crops in Kruševac two tetraploid cultivars of red clover, K-27 and K-32 were created. Tetraploid red clover cultivars are characterized by larger leaves, higher yield and better forage quality *(Lugic et al., 2002)*. Unfortunately, domestic tetraploid cultivars showed low tolerance to drought, which can be overcome with irrigation. In Serbia red clover is growing mostly without irrigation and sensitivity to drought could affect significant decreasing of forage yield. That fact and high coast of seed were the reason why tetraploid red clover cultivars, besides their good performance, didn't find their place in market in Serbia.

Hybrids Perennial forage legumes are cross pollinated species that allow self fertilization in a small percentage and they have more or less severe inbreeding depression, which is affected on decreasing forage yield, especially in alfalfa. Negative self-fertilization effect is manifested by a drastic reduction in yield (up to 30%) and vigour of alfalfa, but after the first cycle of self fertilization. Inbreeding depression is more strongly expressed than it shows inbreeding coefficient *(Gallais, 2003)*, therefore it is difficult to obtain inbreed lines, and the creation of hybrid is rather difficult and very expensive.

Inheritance and investigation of heterosis of forage legumes was object of investigation at the same beginning of breeding in Serbia *(Krstić, 1972)*. Using inbreed line as parental line for synthetic cultivars, as getting single and double hybrids of perennial legume was on of the common investigation *(Radović et al., 2001; Vasiljević et al., 2005a; Milić et al., 2009)*.

Hybridization method or creation of simple and double hybrid increases the genetic gain, due to the possibility of exploiting both additive and dominant genetic variance components.

New tools

Development of classical quantitative genetics to solve this problem is very important, as well as using new methods such as the application of molecular markers *(Gallais, 2003)*. The use of methods of molecular markers in breeding alfalfa may help in determining the genetic diversity between and within the studied population, selection of potential parents of remote populations and in the synthetic study of inbreeding and heterosis in this species. The first attempt of applying molecular marker procedures in forage crop breeding has been done.

There were same attempts to use new methods for solving breeding problems in forage legumes. One of them was tissue and cell culture. *In vitro* regeneration of alfalfa, red clover *(Radović, 1998)* and birds foot trefoil were obtained, as genetic transformation of *L. corniculatus* by *A. tumefaciens*. Also screening of red clover regenerant *in vitro* on fusaric acid was done. There was same positive correlation between tolerance to fusaric acid *in vitro* and tolerance of plant regenerated from culture on *Fusarium sp. (Radović et al., 2003)* However, that research didn't get application in breeding program.

Conclusion

Despite a variety of research in alfalfa, problems remain to be solved, such as increasing the productivity, improve the quality of dry matter while maintaining high yields, improving the persistence, and to develop germplasm for special purposes. Those are the new challenges for the forage crops breeders.

It is necessary to continue to search for the optimal selection methods, which enable the achievement of maximum heterosis. Development of classical quantitative genetics to solve this problem is very important, as well as using new methods such as the application of molecular markers.

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Oplemenjivanje višegodišnjih krmnih leguminoza u Srbiji, rezultati i budući pravci

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Rezime

Višegodišnje krmne leguminoze imaju važnu ulogu u organizovanju efikasnog i održivog sistema stočarske proizvodnje. Lucerka i crvena detelina su najvažnije krmne vrste na oraničnim površinama, dok na zemljištima lošijeg kvaliteta i u brdsko-planinskim reonima, pored crvene deteline, žuti zvezdan, bela detelina i u nekim slučajevima esparzeta imaju veoma važnu ulogu u obezbeđenju krme. Shodno njihovom značaju, oplemenjivanje lucerke i crvene deteline je u Srbiji započelo sredinom dvadesetog veka, dok je oplemenjivanje ostalih višegodišnjih krmnih leguminoza počelo kasnije. Cilj rada je da predstavi rezultate u oplemenjivanju krmnih leguminoza, kao i da ukaže na dalje pravce oplemenjivanja ovih vrsta. Korišćenjem divergentnog selekcionog materijala (lokalne i divlje populacije, introdukovani materijal) i primenom različitih selekcionih metoda stvorene su visoko produktivne domaće sorte lucerke, žutog zvezdana, esparzete, kao i crvene i bele deteline koje se odlikuju odličnom poljskom perzistencijom, što je jedan od uslova za postizanje visokih i stabilnih prinosa u toku celog perioda eksploatacije. Do sada su selekcioneri krmnih višegodišnjih leguminoza u Srbiji kreirali 38 sorti. Domaće sorte se odlikuju širokom genetskom osnovom, koja im omogućava odličnu prilagođenost agroekološkim uslovima.

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NOVEL DIRECTIONS OF BREEDING ANNUAL FEED LEGUMES IN SERBIA

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Review paper

Abstract: The total of 36 cultivars of annual feed legumes has been developed in Serbia. One of the main directions of breeding annual feed legumes is further improvement of the most widespread crops such as feed pea and common vetch cultivated for traditional purposes. Another goal of the annual feed legume breeding is to find novel uses of the most widely distributed crops. One of the best winter protein pea, L-574, has a potential for grain yields of more than 5 t ha^{-1}. One of the strategic goals of the breeding programme of annual feed legumes in the Institute of Field and Vegetable Crops is to restore the neglected and underutilized crops into the Serbian agriculture. The selection of the best progenies resulted in the development and registration of the first Serbian feed faba bean cultivars, Gema and Šarac. The introduction of novel annual legume crops in the Serbian agriculture comprises both those that are already regarded as crops and are widely cultivated in other countries and those that are still regarded as a part of the wild flora. The first Serbian white lupin cultivars, Vesna and Panorama, are highly tolerant to the presence of calcium in soil. The most promising of the wild annual legume species is large-flowered vetch that is characterized by rather prominent tolerance to low temperatures, enhanced earliness in comparison to the other vetch species and a potential for more than 30 t ha^{-1} of green forage and 8 t ha^{-1} of forage dry matter.

Key words: annual feed legumes, breeding, quality, stress, yield.

Introduction

Breeding annual legumes in Serbia has its beginnings in the early decades of the last century and has always progressed together with the development of agronomy and other related fields. The most important annual legume crop in Serbia today is soybean (*Glycine max* (L.) Merr.), while the most important food legume is *Phaseolus* beans *(Mihailović et al., 2009b)*. Apart from them, among the most widely cultivated annual legumes are pea (*Pisum sativum* L.) and vetches (*Vicia* spp.). In Serbia today, breeding soybean in carried out in the Institute of

Field and Vegetable Crops in Novi Sad and the Maize Research Institute Zemun Polje in Belgrade, while the breeding *Phaseolus* beans and other vegetable legumes is active in both Institute of Field and Vegetable Crops and the Institute of Vegetable Crops in Smederevska Palanka. The Novi Sad institute is also the only one in Serbia that has breeding programmes on various annual feed legumes, with an exception of the Institute for Forage Crops in Kruševac with hairy vetch (*Vicia villosa* Roth) breeding programme.

Table 1. Annual feed legume cultivars developed and registered in Serbia

Species, season and cultivation purpose	Name	Year of registration
Winter forage pea	NS-Dunav	1977
	NS-Pionir	1977
	Pionir	2006
	Kosmaj	2006
	Pešter	2007
Winter dual-purpose pea	Cer	2006
Spring forage pea	NS-Lim	1992
	Trezor	2008
Spring dial-purpose pea	NS-Junior	1992
	Jantar	2009
Spring protein pea	Moravac	1994
	Jezero	1995
	Javor	2002
	Partner	2007
	Kristal	2007
	Dukat	2007
Winter common vetch	Novosadska 624	1967
	NS Sirmium	1979
	Neoplanta	2005
	Tara	2006
	Morava	2006
Spring common vetch	Novosadska 5590	1967
	Beograd	1970
	Novi Beograd	1997
	Perla	2009
Winter hairy vetch	Kruševačka 10	1979 (2007)
	NS Violeta	1979
	NS-Viloza	2007
Winter Hungarian vetch	NS Panonika	1979
	Panonka	2007
Winter bitter vetch	Perper	2008
Spring feed faba bean	Gema	2007
	Šarac	2007
Spring grass pea	Studenica	2009
	Sitnica	2009
Spring white lupin	Vesna	2008
	Panorama	2008

So far, 36 cultivars of annual feed legumes in Serbia have been developed in total (Table 1), namely 17 cultivars of feed pea, 9 of common vetch (*V. sativa* L.), 3 cultivars of hairy vetch, two cultivars of Hungarian vetch (*V. pannonica* Crantz), one of bitter vetch (*V. ervilia* (L.) Willd.), two cultivars of faba bean (*V. faba* L.), two cultivars of grass pea (*Lathyrus sativus* L.) and two cultivars of white lupin (*Lupinus albus* L.).

Breeding annual feed legumes in Serbia is aimed at further increase of forage, grain and biomass yields and enhancement of forage, grain and biomass quality, as well as to the improvement of the tolerance to various biotic and abiotic stresses *(Mihailović et al., 2008c)*. The usual methods used are mass selection from wild populations and local landraces, as well as pedigree and bulk methods of selection from hybrid populations. The application of maker-assisted selection (MAS) is in its beginnings.

Improvement of the most widespread annual feed legumes

One of the main directions of breeding annual feed legumes in Serbia is further improvement of the most widespread crops such as feed pea and common vetch cultivated for traditional purposes.

The emphasis in breeding winter forage pea is upon the development of the lines with satisfying tolerance to low temperatures and more prominent earliness, with non-decreased forage yields and quality. Such line would be able to be cut earlier and leave more room for sowing the succeeding crops.

Answering to the demands of one part of the market for one cultivar that could produce both forage and grain yields, making is suitable in both ruminant and non-ruminant feeding, new lines belonging to the type of the cultivar NS-Junior are developed.

Breeding spring protein pea is aimed mainly at the selection of the lines with increased earliness and with afila leaf type, since it proved to be at least equal in grain yields in comparison to the cultivars with normal leaf type, while with significantly increased standing ability *(Mihailović and Mikić, 2004)*. Apart from this morphological feature, there are others changes in plant architecture, such short internodes and determinate stem growth that are also incorporated in new lines *(Mikić et al., 2006)*.

Regarding quality, apart from retaining or increasing the crude protein content in grain dry matter in pea, a goal of all modern protein pea breeding programmes is to decrease the content of various anti-nutritional factors, most notably trypsin inhibitors *(Mikić et al., 2009d)*.

Breeding common vetch is aimed at increasing tolerance to low temperatures in winter genotypes and drought in spring genotypes, with enhanced earliness and improved forage yield and quality in both. At the same time, one of the constant goals remains a significant increase in seed yield, as one of the strategic issues for a reliable cultivation of this crop *(Mihailović et al., 2007c)*.

New purposes for the "old" annual feed legumes

Another goal of the annual feed legume breeding is to find novel uses of the most widely distributed crops. Following this strategy, pre-breeding of the winter dual-purpose pea has begun, resulting in the development of the lines with great potential for both forage and grain yields, such as the cultivar Cer, with about 30 t ha^{-1} of green forage, 7 t ha^{-1} of forage dry matter and more than 3100 kg ha^{-1} of grain *(Mihailović et al., 2007a)*.

The cultivation of winter cultivars may significantly increase the area under a crop, especially in the regions with temperate climates. By this reason, a programme of the development of the winter protein pea was established in 2004. The first results of the evaluation of the tolerance to low temperatures in several genotypes of diverse geographic origin were encouraging *(Mikić et al., 2007)*. One of the best lines selected from hybrid populations, L-574, has a potential for grain yields of more than 5 t ha^{-1} *(Mihailović et al., 2008a)* and is currently in the process of registration in Serbia.

In southern Europe and the Mediterranean, as well as in Australia, common vetch is cultivated not only for forage, but for grain as well *(Matić et al., 2005)*. The modern common vetch dual-purpose cultivars, such as the Australian cultivar Morava, have potential for grain yields of up to 3100 kg ha^{-1} *(Mihailović et al., 2007b)*.

Re-introduction of neglected annual feed legumes

In a similar way to the other Balkan and South East European countries, Serbia has begun to lose many of its traditional annual legume crops in favour of the most profitable ones. One of the strategic goals of breeding programme of annual feed legumes in the Institute of Field and Vegetable Crops is to restore the neglected and underutilized crops into the Serbian agriculture.

Widely present in the wild flora throughout Serbia, hairy vetch has gain more interest recently, as a both forage and green manure crop. During several past years, the expeditions carried out in various regions of Serbia resulted in the collecting and *ex situ* conservation of a considerable number of hairy vetch accessions. Certain hairy vetch genotypes may produce up to 50 t ha^{-1} of green forage and nearly 11 t ha^{-1} of forage dry matter *(Mihailović et al., 2008d)*.

Hungarian vetch plays much more regional role in comparison to the other vetch crops. However, its wild populations may represent a valuable source of new genetic variability and the desirable traits such as the tolerance to low temperatures, earliness and satisfactory forage and seed yields *(Mihailović et al., 2009a)*.

Faba bean is one of the most important crops on its way back in the feed production in the agriculture of Serbia. Collected local landraces have served as the quality basis for the establishment of the first Serbian feed faba bean programme *(Mihailović et al., 2006c)*. The accessions that proved best in the evaluation of their potential for both grain, forage and biomass production were included in the hybridization. The selection of the best progenies resulted in the development and registration of the first Serbian feed faba bean cultivars, Gema and Šarac, with average yields of more than 4500 kg ha^{-1} of grain and more than 45 t ha^{-1} of green forage *(Mihailović et al., 2010)*.

Once well-known in central and southern parts of Serbia, bitter vetch today is completely forgotten. The evaluation of both forage and grain yields in this crop confirmed its great potential for dual-purpose cultivation, with a good tolerance to low temperatures and a prominent earliness in comparison to the other vetch species, as well as with yields of about 31 t ha^{-1} of green forage and 7 t ha^{-1} of forage dry matter *(Mihailović et al., 2006b)*.

The youngest among the re-introduced annual legume crops is grass pea, once also widely grown for both human consumption and animal feeding. Today, grass pea may be found extremely sporadically in certain regions of Serbia and Srpska, especially lower Herzegovina, where it serves as a complementary pulse in human diets *(Mikić et al., 2009b)*. A small breeding programme on grass pea in Novi Sad is based upon the evaluation of mainly introduced material, such as Polish cultivars and French landraces, resulting in the development and the registration of the first Serbian grass pea cultivars, Studenica and Sitnica, suitable for both forage and grain production.

Introduction of novel annual feed legumes

The introduction of novel annual legume crops in the Serbian agriculture comprises both those that are already regarded as crops and are widely cultivated in other countries and those that are still regarded as a part of the wild flora.

Despite the fact that it originated in the southern regions of the Balkans, white lupin is still completely unknown in Serbia. The main obstacle to its cultivation on rich soils such as chernozem dominating northern parts of Serbia is its susceptibility to high content of calcium in soil. The preliminary trials with white lupin were aimed at selecting the tolerant genotypes to high alkaline soil reaction *(Mihailović et al., 2006a)*. Further steps led to the development of the first Serbian white lupin cultivars, Vesna and Panorama, highly tolerant to the presence of calcium in soil *(Mikić et al., 2009a)* and with a potential for grain yields of more than 5000 kg ha^{-1} and more than 350 g kg^{-1} of crude protein content in grain dry matter *(Mikić et al., 2010)*.

A preliminary testing of narrow-leafed lupin (*L. angustifolius* L.) accessions on chernozem soil confirmed their inability to tolerate its alkaline reaction, resulting in a necrosis at early stages of plant development. However, its cultivation on an acid pseudogley soil was successful and showed that this crop may produce more than 2000 kg ha^{-1} *(Mihailović et al., 2008b)*.

The evaluation of certain sub-tropical species, such as cowpea (*Vigna unguiculata* (L.) Walp.), pigeon pea (*Cajanus cajan* (L.) Millsp.) and hyacinth bean (*Lablab purpureus* (L.) Sweet) proved successful. Forage yields in some genotypes of pigeon pea are higher tha 50 t ha^{-1} of green forage and 14 t ha^{-1} of forage dry matter (*Mihailović et al., 2006d*), while grain yields in certain cowpea accessions may surpass 3000 kg ha^{-1} *(Mihailović et al., 2005)*. The selection of the lines with satisfactory forage and grain yields and an appropriate photoperiodic reaction is carried out and may result in the cultivars suitable for dual-purpose cultivation in the agro-ecological conditions of Serbia.

In order to conserve the wealth of the wild legume flora of Serbia, many expeditions have been carried out in various regions of Serbia, resulting in hundreds of new accessions of mainly wild species of vetches and vetchlings (*Lathyrus* spp.) maintained in the Institute of Field and Vegetable Crops. Many of these have been included in the evaluation of the potential for primarily forage production *(Ćupina et al., 2007)*. The most promising species is large-flowered vetch (*V. grandiflora* Scop.) that is characterized by rather prominent tolerance to low temperatures, enhanced earliness in comparison to the other vetch species and a potential for more than 30 t ha^{-1} of green forage and 8 t ha^{-1} of forage dry matter *(Mikić et al., 2009c)*. At the same time, narrow-leafed vetch () may also represent a potential forage crop, with up to 20 t ha^{-1} of green forage and 5 t ha^{-1} of forage dry matter *(Mikić et al., 2008)*.

Conclusion

Breeding annual feed legumes in Serbia is aimed at both improving the existing and widely cultivated crops and introducing novel ones. A wide variability of morphological traits and agronomic characteristics in all these crops represents a mighty tool in their further enhancement and the development of cultivars suitable for growing in diverse agro-ecological conditions.

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Nova usmerenja oplemenjivanja jednogodišnjih stočnih mahunarki u Srbiji

V. Mihailović, A. Mikić

Rezime

U Srbiji, ukupno, stvoreno je i priznato 36 sorti jednogodišnjih stočnih mahunarki. Jedno od osnovnih usmerenja oplemenjivanja jednogodišnjih stočnih mahunarki je dalje unapređenje najviše gajenih useva, poput stočnog graška i obične grahorice. Drugi cilj oplemenjivanja jednogodišnjih stočnih mahunarki jeste iznalaženje novih namena najviše gajenih useva. Jedna od najboljih linija ozimog proteinskog graška, L-574, poseduje potencijal za prinose zrna veće od 5 t ha^{-1}. Jedan od strateških ciljeva oplemenjivanja jednogodišnjih stočnih mahunarki u Institutu za ratarstvo i povrtarstvo je i vraćanje zanemarenih i neiskorišćenih useva u poljoprivredu Srbije. Izdvajanje najboljih potomstava proishodilo je stvaranjem prvih srpskih stočnog boba, Geme i Šarca. Uvođenje novih useva u poljoprivredu Srbije obuhvata one koji se već gaje u drugim oblastima i zemljama i one koji se još uvek smatraju delom samonikle flore. Prve srpske sorte bele lupine, Vesna i Panorama, poseduju visoku tolerantnost na prisustvo zemljišnog kalcijuma. Najperspektivnija među samoniklim jednogodišnjim mahunarkama je krupnocvetna grahorica, sa veoma izraženom tolerantnošću na dejstvo niskih temperatura, poboljšanom ranostasnošću i potencijalom za prinose veće od 30 t ha^{-1} zelene krme i 8 t ha^{-1} suve materije krme.

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PERENNIAL FORAGE GRASSES BREEDING IN SERBIA: ACHIEVEMENTS, LIMITS AND PROSPECTS

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Abstract: Perennial grasses are basic components of natural and sown pastures and meadows and therefore represent important ingredient of voluminous ruminant feed. Most of perennial grasses can be used for livestock feed, but most important species for forage production are cocksfoot, perennial ryegrass, meadow fescue, tall fescue, Italian ryegrass, tall oat grass, red fescue and smooth brome.

Grass genotype (cultivar) has great effect on total yield and successful utilization of grasslands; therefore, their improvement is very important issue. Grass breeding has strong, 50 year long tradition in Serbia. As a result, in domestic scientific institutions, 19 forage cultivars of perennial grasses have been created and included into cultivar list. The most important prerequisite for successful breeding is large breeding germplasm with enough genetic variability. Incorporation of local autochthonous Serbian population is approach to breeding for adaptability and persistency. Main breeding criteria are yield and quality of dry matter, maturation, persistency and tolerance to drought. The most successful method of breeding of perennial grasses is modified phenotypic recurrent selection, followed with synthetic breeding. In future, breeding of grasses in Serbia should be directed to introduction of new modern breeding methods, hybrid breeding, detection and improvement of traits associated with drought resistance and involving of new species of perennial grasses into breeding programs.

Key words: perennial grasses, breeding, cultivars, methods, criteria, prospects

Introduction

Perennial grasses represent very complex and heterogeneous group of cosmopolitan plants. On Balkan peninsula and Serbia live cool season perennial grasses which are acclimatized to change of seasons and different habitats, from lowlands, with fertile and deep soil, to hilly-mountainous habitats over 1200 m a.s.l., with shallow soils with lack of minerals and humus.

Exactly this broad adaptability to different agro-ecological and climatic conditions considerably increases agronomical importance of perennial grasses on the multiple bases. First, most of them are cenobionts and edificators of whole series of plant associations on natural meadows which covers large acreage in Serbia, over 1 455 000 hectare or almost 29% of total agriculture land in Serbia *(Stošić and Lazarević, 2009)*.

Presence of grasses in agriculture is dating from period of nomadic animal husbandry. First written traces about this group of plants (Italian ryegrass) in agriculture originated from 12th century *(Beddows, 1953)* and from 17th century (perennial ryegrass). Today, representing base of the sustainable animal husbandry and animal feed production, they are essential components of ruminant diet as components of natural and sown pastures and meadows *(Stošić et al., 2005),*utilized by hay making or as haylage and silage *(Dinić et al., 2003)*.

Only small number of perennial grass species is adapted to intensive forage production and they are most important for this purpose in Serbia: cocksfoot (*Dactylis glomerata* L.), perennial ryegrass (*Lolium perenne* L.), meadow fescue (*Festuca pratensis* Huds.), tall fescue (*Festuca arundinacea* Schreb.), timothy (*Phleum pratense* L.), Italian ryegrass (*Lolium multiflorum* Lam.), tall oat grass (*Arrhenatherum elatius* (L., P. Beauv. ex J. Presl&C.Presl.), red fescue (*Festuca rubra* L.) and smooth brome (*Bromus inermis* Leyss).

Nevertheless, perennial grasses have extremely important function in soil, fresh water and natural habitats conservation. In addition, some of them (perennial ryegrass, red fescue, tall fescue, Kentucky bluegrass and bent grasses) are main components of amenity grasslands in northern and temperate climate.

Grass genotype (cultivar) has great effect on total yield and successful utilization of grasslands and therefore, their improvement has been very important issue. However, all distinct forage grasses characteristics (especially pereniality and anemophily) make breeding of this group of plants extraordinary complex and species specific. Therefore, there is no universal breeding method and criterion for all grass species. Also, breeding process of perennial grasses *per se* is very long, resulting in releasing of cultivar after more than 10 years of demanding scientific work.

If we look on history scale, in addition to natural selection, which was dominant very long, first selection and breeding of grasses was carried out totally unconsciously, by cropping. Using of new technologies, farmers were unconsciously improving and adapting of grown grass genotypes to new contemporary management systems and use. Formal and methodical breeding of perennial grasses, established on scientific knowledge, started almost simultaneously last decade of 19th Century in Great Britain and USA. From that time grass breeding has been developed constantly and rapidly. Especially important event for development of grass breeding was establishment Welsh Plant Breeding Station in Aberystwyth in 1919, where first long-time breeding programs of perennial ryegrass and other grasses were designed.

First steps in this field of research in Serbia happened considerable later than abroad, but still long time ago. In the middle of last Century, precisely 1961. Institute for forage crops was established in Kruševac. It is aimed as specialized scientific institution for breeding and research of production technologies and utilization of forage crops. In this research field breeding of perennial forage grasses are important topic. Cultivars bred in Kruševac are main components of mixtures and animal feed, especially in hilly-mountainous regions of Serbia.

Current achievements in perennial grass breeding in Serbia

Perennial grasses breeding has strong, 50 years long tradition in Serbia especially in Institute for forage crops in Kruševac. Because of scientific and breeding work, 19 cultivars were created and released on Serbian market *(Tomić and Soković, 2007)* where they found large acceptance in local agro-ecological conditions. Those are forage cultivars of cocksfoot, Italian ryegrass, perennial ryegrass, timothy, meadow fescue, tall fescue, red fescue and tall oat grass (tab.1).

All domestic registered forage cultivars, according demanding testing and releasing process in network of micro field trials, have high genetic potential for production traits. They are characterized with high biomass production with good chemical composition in Serbian agro-ecological conditions *(Soković et al., 2004a; Tomić et al., 2007)*, and improved filed persistency, different maturity and drought tolerance.

Table 1. Created forage cultivars and DMY of perennial grasses bred in Serbia (adapted from *Tomić and Soković, 2007*)

Grass species	Cultivar	Year of releasing (re-registration)	Dry matter yield (t ha^{-1})
Cocksfoot	K-6	1976	7.67 – 11.6
	K-7	1976	6.46 – 10.1
	K-rana	1992	9.12-10.44
	K-40	2001	10.59
Timothy	K-15	1979	7.5-15.01
	K-41	2002	6.56
Italian ryegrass	K-13	1979 (2006)	10.36 -14.07
	K-29t	1994	15.5
Meadow fescue	K-21	1986	11- 11.5
Tall fescue	K-19	1982	13.3-17.72
	K-20	1982	13.15-15.66
	NS-10	1979	12.11
Red fescue	K-14	1979 (2006)	7.44-10.56
Tall oat grass	K-12	1979 (2006)	8.23-12.46
	K-16	2007	8.5-12.3
Perennial ryegrass	K-11	2006	6.45

Creating the base breeding germplasm is first prerequisite for successful breeding. This germplasm and its variability will determine maximum potential achievements through breeding. First steps in grass breeding in Serbia were made on introduced plant material mainly of Eastern European origin and subsequent incorporation of local autochthonous populations in germplasm for increasing genetic variability and introgression of genes for drought tolerance.

Because of this pioneer work using mass selection cultivars of cocksfoot (K-6 and K-7), timoty cv. K-15, red fescue cv. K-14, Italian ryegrass cv. K-13 and tall oat grass cv. K-12 were created. Some of those firstly bred cultivars are reregistered in 2006. and still are components of sown meadows in Serbia. First perennial grass cultivars, in general, are created by population breeding and improvement. Therefore they have large phenotypic and especially genetic variability *(Hayward, 1970)* and represent very important source for further breeding.

Further breeding efforts were made on tall fescue landraces introduced from USA and acclimatized on local climate conditions over 30 years *(Krstić et al., 1982)* Goal was to create cultivars with improved forage quality. Final steps of breeding were consisted of poly-cross hybridization of vegetatively propagated clones of chosen genotypes in repeat mass selection. Result was registration of two intermediate cultivars K-19 and K-20 with DMY over 13 tha-1 and improved DM quality. They are still in agricultural practice.

First cultivar breed using Serbian autochthonous germplasm is meadow fescue K-21, released 1986. After that time all cultivars created have local wild populations as main part of breeding germplasm. Also some cultivars with special characteristics were created, such as early maturing cocksfoot K-early *(Tomić et al., 1995)* and tetraploid cultivar of Italian ryegrass K-29t *(Tomić and Popović, 1996)* made by seedlings colchicine treatment.

Novel breeding programs and strategies included local wild populations improved with multiple recurrent selections *(Sokolović, 2006; Tomić and Sokolović, 2007)*. First step in breeding is determination of germplasm and base population variability and characteristics. Many breeding efforts were made on this field by introducing and characterizing of autochthonous populations *(Sokolović, 2001; Sokolović et al., 2002; Sokolović et al., 2004c)* or introduced cultivars *(Sokolović et al., 2010)*. Large pool of genetic variability and desirable traits has been discovered.

More the two decades long breeding has resulted in creating of some new cultivars of most important grass species. Among them first realized was cocksfoot K-40, early-intermediate cultivar, morphologically similar to K-6, which riches annually 10.5 tha^{-1} *(Sokolović et al., 2004a)*. Also, timothy cultivar K-41 was tested and included in state approved cultivar list in 2002. Two latest cultivars are perennial ryegrass K-11*(Sokolović et al., 2007)* and tall oat grass K-16 *(Sokolović et al., 2008)* released in 2006 and 2007, respectively.

Nowadays two potential cultivars of cocksfoot are in trail system of Commission for cultivar registration. Medium and late maturity cultivars are in third and first investigation year, respectively, and both were completely created using autochthonous collected populations. Some efforts have been done in breeding of cocksfoot genotypes with special traits for orchards landscaping *(Babić et al., 2009)*. Covering the soil during winter periods or during rain spells is the only guaranty against soil erosion and perennial grass covers is in fact optimal to protect vineyards and orchards soils on slopes against erosion. Parent components for potential new cultivar for orchard land planting, was characterized and poly-cross is formed.

During last decade, large efforts was performed in broadening of genetic base for breeding of perennial grasses in Serbia, either with introduced cultivars from abroad or collecting promising populations from different hilly mountainous habitats across Serbia *(Tomić, 1997; Sokolović et al., 2003b; Tomić et al., 2009).* Serbian meadow flora is very rich with populations of all cool-season perennial grasses, due to fact that Serbia is part of the area (Mediterranean and Near east) which represent centre of their origin. Those populations are acclimatized on local agro-ecological and climate conditions and therefore have comparative advantage with introduced genotypes *(Sokolović et al., 2003b; Sokolović et al, 2004a)*. Local ecotypes have natural variation and very often have satisfactory yielding performance in comparison with selected cultivars, which referred them for direct phenotypic selection for cultivars release (*Posselt and Willner, 2007*).

Many collecting projects have been carried out locally, but also on international level (SEEDnet). Most of the accessions are already included in pre-breeding process *(Sokolović et al., 2004c; Sokolović et al., 2006; Babić et al., 2010).* Therefore, breeding germplasm of majority species which are included in Serbian breeding programs is almost totally composed of those population.

Used some methods and criteria and their limitations

Due to large diversity of perennial grass species and their distinct characteristics (perenniality and anemophily), there is no universal breeding method or criterion. This makes breeding of this group of plants extraordinary complex and species specific, or it can de said that genetic architecture of species determine used breeding method.

Basically, forage grasses breeding in Serbia is aimed to increase of dry matter yield, its quality in sense of chemical composition and digestibility, as well as to the improvement of the field tolerance to drought and *Puccinia* sp *(Tomić et al., 1999)*. Dry matter yield (DMY) is still most important traits of forage grasses in

Serbia and represent “bottom line” of all breeding programs. This criterion is always used in breeding scheme, bat small number of papers considered DMY breeding *per se (Burton, 1982, Ceccarellii et al., 1980).* Lot of breeding programs is aimed to improve different forage grasses traits (DMY components) shoving height correlation coefficients with yield *(Carlson, 1990; Sokolović et al., 2003a; Sokolović, 2006).*

However, improvement of DMY *per se* is possible by breeding *(Carlson, 1990)*, and progress in this trait breeding has been made in Serbia recent years *(Sokolović et al., 2007; 2008).* This is due to large genetic variability in breeding germplasm mostly formed from local populations *(Sokolović et al., 2004c, 2006; Babić et al., 2010)* and dominantly used breeding methods (phenotypic mass and recurrent selection). Phenotypic recurrent selection (PRS) method is powerful selection tool, designed for rapid increasing of desirable genes frequencies with protection of genetic variability and minimizing of inbreeding depression. This method uses most of additive variance within population and provides noticeable progress even for traits with extremely low heritability. If selection response is not adequate (usually for traits with heritability below 10%), progeny testing should be included or breeding cycles repeated.

For forage grasses breeding in Serbia this method has been modified after *Burton, (1982)* by introducing clone replication of chosen plant, eliminating fertilization with undesirable pollen and therefore enabling control of hybridization. This makes concentrations of “good” genes faster, shortening breeding cycle and recurrent selection in total. It is not knows is phenotypic recurrent selection best for forage grasses, since there are no papers comparing the results of different breeding methods on forages *(Brummer, 2005).* Same author quoted that long-lasting PRS is basis of breeding for yield in forages and that is possible to upgrade it with all modern breeding methods. However, PRS method on extremely broad germplasm will lead to very slow respond and accumulation of desirable characteristics controlled by additive genetic effect *(Brummer, 1999).*

Even there were continuous improvement of DMY in forage perennial grasses in Serbia *(Sokolović et al., 2007; Sokolović et al., 2008)* and existing genetic gain in breeding was proven *(Sokolovic, 2006)*, some limitations appear in breeding process. First of all, as long as breeding last, the effects and results of breeding decreasing. Best solution is germplasm renewing by introducing of new populations and variability. Also, it is possible after selection within population, to improve germplasm variability by hybridization of chosen genotypes. This will lead to renewing of breeding germplasm, increase of genetic variability with reduction of unpleasant genotypes in the same time. Some steps in that direction were made in Serbia on perennial ryegrass *(Sokolović, 2006)* and meadow fescue *(Babić, unpublish data).*

Furthermore, plant selection for improved DMY is usually performed in spaced plant nurseries *(Sokolović et al., 2002, 2004c, 2005),* where this trait shows excellent heritability *(Sokolović et al., 2004b)* and genetic gain or heterosis *(Sokolović, 2006).* It is published that spaced plant selection could be successful, but achievement significantly differs between perennial grass populations *(Hayward, 1983).* Genetic gain in breeding is decreasing with reduction of plant distance in nursery *(Hayward and Vivero, 1984).* In general, DMY breeding results on spaced plants (most used design in forage breeding in Serbia), are not totally reliable in prediction of yield in dense stand of perennial ryegrass *(Hayward and Vivero, 1984)*, tall fescue *(Rotilli et al., 1976)*, reed canarygrass *(Casler and Hovin, 1985)* and smooth brome *(Carpenter and Casler, 1990)*. On the other hand, there are published results, which confirm improvement DMY by spaced plant breeding *(Fujimoto and Suzuki, 1975; Sokolović, 2006; Sokolović et al, 2010)*

Besides DMY improvement, some efforts have been made to breeding of phenological traits. Time of maturity is extremely important trait and genotype synchronization in this trait has multiple weight (in hybridization and adequate applied management on time). According to *Casler (1988)* early genotypes of cocksfoot showed better persistency in mixture with alfalfa. Also, late perennial ryegrass cultivars showed better frost tolerance *(Humphreys and Eagles, 1988).* However, most important is forming palette of cultivars with different maturity time, which allows composition of mixtures for various ways, time and duration of utilization. In Serbia, breeding of this trait complex is especially developed on cocksfoot *(Tomić et al., 1995; Sokolović, unpublish data)*.

Forage chemical composition also represent important breeding criterion and it is usually included in all forage grasses breeding programs. Improvement of crude protein content, fiber (ADF; NDF) and lignin (ADL) content *(Claessens et al., 2005; Casler, 1999)*, DM digestibility or palatability *(Casler and Carpenter, 1989)* and WSC *(Wilkins et al., 2003)* by breeding is also possible and promising. In perennial grasses breeding in Serbia those traits were investigated and improved in all programs *(Tomić et al., 2002; Sokolović et al., 2002; Ignjatović et al., 2004).*

Improvement of resistance to rust, *Puccinia coronata* Corda f.sp. *lolii* Brown and *Puccinia graminis* f. sp. *graminicola*, common disease of Italian and perennial ryegrass in Europe, is among major goals in ryegrass breeding programs. Certain breeding efforts were made and some resistance was found *(Tomić et al., 1999)*, but new sources of resistance are still needed.

Since that forage production in Serbia have been organizing mostly without irrigation and that recent years droughty periods during spring and summer have been appearing, improved drought tolerance rises as most important criterion. Natural selection has strong impact on this trait, because acclimatized populations always have better drought tolerance. Therefore, in Serbian breeding programs local populations and landraces are incorporated in germplasm for all perennial grasses. Breeding programs for drought resistance are consisted of improving of field persistency and plant life duration. As a result, cultivar K-11 was released 2006 *(Sokolović et al., 2007).*

Seed yield of forage grasses is important trait for breeding, also. This trait is very often in negative correlation with other important traits, breeding subjects. Breeding of seed yield is possible *per se (Ceccarelli et al., 1981)* or via numerous seed yield components *(Sokolović et al., 2006).*

Concerning final steps in applied breeding method, almost all grass cultivates in Serbia are synthetic or improved populations *(Krstić et al., 1982; Tomić et al., 1995; Sokolović et al., 2007, 2008)*. Synthetics (S_1 generation) are obtaining by intercrossing of chosen genotypes (S_0) (parents) in all combinations (polycross). S_1 seed is multiplied in few subsequent generations for production of enough amount of certified seed (S_3 or S_4) for market *(Brummer, 1999).* According to *Reheul et al. (2003),* the yield performance of syn1 and syn2 generations did not differ significantly although there was a tendency of syn1 over yielding syn2. In addition, for two different synthetics (based on 3 and 4 different clones respectively) syn1 generation tended to yield more herbage than the syn2 but not significantly. There are some hybrids made by clones dialel pair-crosses that yielded in F2 generation more than syn2, or even more than syn1 *(Ghesquiere and Baert, 2007).* Parents for synthetics have been choosing for potential high combining ability for important agronomic traits, what can be proven by polycross or topcross test *(Wilkins, 1991; Sokolović, 2006)* and superior pair-crosses could be combined to synthetic cultivar.

Inbreeding of parents is possible and increases genetic variability among individuals in a population. Greater genetic variability among inbred progeny can increase the effectiveness of selection and the amount of genetic improvement in a breeding program *(Fehr, 1987).* Nevertheless, inbreeding of open pollinated forage grasses very often reduces many important traits through inbreeding depression. However, crossing in breeds to produce synthetic strains can restore vigour sufficiently to guarantee the use of that procedure in a breeding program. In most of the breeding programs, the number of parents for the synthetic varies between 3 and 15, and in that range influence of the number of parents is limited. According to *Posselt (2000),* the optimum number of parents varies from seven to nine, depending on the inbreeding depression. The less clones are used the greater the differences between the generations are expected *(Ghesquiere and Baert, 2007).* Using fewer components also reduces the risk of heterogeneity and enhances the selection intensity, but increase probability for inbreeding depression. In Serbia synthetics are made from poly-cross of 4-10 parents *(Sokolović et al., 2007; Sokolović et al., 2008).* After *Foster et al. (2001),* synthetic breeding starts with basic population of 2000 to 5000 genotypes, continuing with 200 chosen genotypes inter crossed in few different poly-cross according maturity, time and finishing in poly-cross, and formed synthetic with 7 to 10 parents chosen after half sib analysis.

Hybrid breeding is most important method in major crops, but concerning forage grasses specificity it is impossible to perform classical hybrid breeding like in maize. However, some hybrid breeding modification in forage grasses is promising and valuable. All that work is aimed to rich the phenomena of heterosis, most preferred characteristics of hybrid progeny. It represents their superiority in F1 generation in one or more traits of interest compared with parents. In forage grasses hybrid breeding heterosis effect for DMY is looking more than 60 years and it is detected in many grass species *(Brummer, 1999; Posselt, 2003; Sokolovic, 2006),* but limitations caused by grass fertilization system and the amount of hybrid seed obtained are crucial for insufficient progress. In addition, one cycle of hybrid seed multiplication can reduce heterosis effect for 50% *(Posselt, 2003).* Those reasons determine lack of hybrid cultivars of perennial forage grasses.

However, there are some experimental procedures, which can be potentially useful for grass hybrid breeding. Intercrosses of partially selfed parents (multiplied in isolation) suggested by *England (1974)* give 83% of hybrid progeny. These so called SI Hybrids (Self-incompatibility) are able to show heterosis effect of 20% *(Posselt, 1993).*

Brummer (1999) put forward discussion about “semi-hybrids” and their use in forage breeding, but general idea about this type of hybrids (“chance hybrids”) was created by Burton. Main idea is to inter-cross prosperous selected population pre-multiply in isolation to produce enough F1 seed for commercial use. Why “chance hybrids”? Because, theoretically by probability principle, 50% of progeny will be inter-populational and rest of them intra-populational crosses. In practice, this percent is rather smaller, about 35% (*Posselt, 2003*). Although, time of parents flowering and its synchronization represent “bottle neck” of this method, heterosis is achieved in several perennial grasses (*Foster, 1971; Posselt, 2003*).

Identification and development of heterosis groups and patterns for semi-hybrid breeding or any other crosses with expected heterosis is essential *(Posselt, 2010)*. If heterosis groups are not known, they should be looked for in genetically distanced genotypes and populations or with genetic diverse origin *(Christie and Krakar, 1980)*. Some effort was made in genetic gain prediction in breeding process perennial ryegrass *(Soković, 2006)*. This parameter is valuable for choosing of most appropriate applied method (*Casler, 1999*), but genetic gain depends of breeding material per se, its variability and traits of interest heritability.

Though, commonly used method for genetic distance determination in forage grasses in Serbia is “phenotyping”, molecular markers methods are chipper and less time-consuming methods, like isozyme systems markers *(Humphreys, 1992; Soković, 2006)* or DNA molekular markers *(Foster et al., 2001)*. The application of DNA makers in grass breeding in Serbia is in its beginnings.

One of the largely used breeding tools is production of polyploid cultivars which differs from diploids in improved traits (DMY, its quality and persistence).

This was achieved on *Lolium multiflorum* by colchicines induction on vegetative tillers. Obtained tetraploid genotypes were selected and incorporated by polycross in cultivar K-29T *(Tomić and Popović, 1996)*.

What we need to do in future – forage grasses breeding prospects

Recent years breeders have been faced with many challenges, problems to be solved and limitations inn breeding programs. Some of those challenges, thought almost decade old are *(Stuber, 2001)*: identification of useful genetic parameters in diverse populations and lines, introgression of genes of interest in breeding germplasm, improvement of recurrent selection program based on phenotypic respond, investigation of heterosis and hybrid combining ability prediction, understanding and modification of interaction between genotype - environment, genotype - genotype and genotype - pathogen.

On the other hand, well known is that prediction of heritability *(Sokolović et al., 2004b)*, genetic correlations *(Sokolović et al., 2005)*, genetic gain *(Sokolović, 2006)* and interaction between genotype and environment are priceless in formulation of efficient strategy and breeding method. Therefore, future breeding of perennial forage grasses in Serbia should be directed to several issues and goals:

- Breeding for which real production conditions exist. This means breeding to competitiveness with grasses and compatibility with legumes. Therefore, breeding should be performed in shorter plant distances or with legumes (white clover) and grasses (short) intercropped, simulating real production condition.
- Increasing resistance and tolerance to drought by improvement of physiological and morphological traits (efficiency of plants to survive summer drought by dormancy *(Norton et al., 2007)*, water use and nutrients absorption efficiency, size and opening of stoma, root development and dept *(Crush et al., 2007; Bonos et al., 2004)*.
- Breeding for different maturity cultivars (for mixtures with different purposes and period of utilization
- Developing polyploid cultivars of natural diploid forage grass species
- Studying of interspecies hybridization, (*Lolium-Festuca* complex), for combining best traits of both species.
- Developing and identification for heterosis groups, (populations), of most important grass species, for hybrid breeding, especially semi-hybrids and combination with synthetic breeding.
- DNA molecular markers establishing, for determination of genetic variability in breeding germplasm *(Bolarić et al., 2005)* or genetic distances for production of superior hybrids *(Kolliker et al., 2005)*.

- Introduction of novel species of forage perennial grasses in breeding which are characterized by broad genetic base and adaptability to different agro-ecological conditions

Conclusion

In Serbia perennial grasses breeding has 50 year long tradition with 19 forage cultivars created. All cultivars are characterized by high genetic potential for yield and quality of dry matter. Most used breeding method in grass breeding in Serbia was phenotypic, recurrent selection, modify to make breeding process shorter and more effective, followed with polycross and synthetic breeding. Most important criteria are yield and quality of dry matter, maturity, drought tolerance, persistency and seed yield. In future grass breeding special attention of breeders should be paid to breeding in real production conditions, breeding for improved drought resistance (especially root development and dept) and different cultivars maturity, developing heterosis groups (populations) for hybrid breeding, polyploid cultivars, interspecies hybridization and introducing of new methods (DNA molecular markers) and novel species of forage perennial grasses in breeding process.

Oplemenjivanje višegodišnjih krmnih trava u Srbiji: dostignuća, ograničenja i perspective

D. Soković, Z. Tomić, S. Babić

Rezime

Višegodišnje trave su vrlo bitna komponenta kabaste stočne hrane preživara, s obzirom da su i osnovne komponente prirodnih i sejanih pašnjaka i livada. Većina vrsta višegodišnjih trava se može koristiti za ishranu životinja, dok su najbitnije za proizvodnju krme ježevica, engleski ljulj, livadski vijuk, visoki vijuk, mačiji rep, italijanski ljulj, francuski ljulj, crveni vijuk i bezosni vlasen. Pošto sorta ima veliki uticaj za uspešnu proizvodnju krme, oplemenjivanju se posvećuje velika pažnja. U Srbiji oplemenjivanje trava ima dugo 50-ogodišnju tradiciju i kao reultat u domaćim Institutima je do sada kreirano 19 krmnih sorti. Uspešno oplemenjivanje sorti krmih kultura je raznovrsna oplemenjivačka germplazma, visoke genetičke varijabilnosti. Oplemenjivanje sorti adaptabilnih i perzistentnih na pojedina svojstva podrazumeva uključivanje lokalnih populacija u taj proces. Glavni oplemenjivački kriterijumi su prinos i kvalitet suve materije, vreme stasavanja, poljska perzistencija i otpornost na uslove suše. Najčešće korišćena metoda, u stvaranju novih sorti višegodišnjih krmnih trava, je modifikovana fenotipska

rekurentna selekcija, praćena polikros ukrštanjem i stvaranjem sintetičkih sorti. U budućnosti oplemenjivanje trava u Srbiji treba ići u pravcu uvođenja novih savremenih methoda oplemenjivanja, formiranje različitih vrsta hibrida, detekcija u unapređenje osobina povezanih sa otpornošću na sušu i uključivanje novih travnih vrsta u proces oplemenjivanja.

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APPLYING MOLECULAR MARKERS TO FORAGE BREEDING

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Invited review paper

Abstract: Genetic gain in forage crops is generally low for most complex traits like yield. In this paper, I suggest that we can improve gain by using alternative selection methods. Additionally, applying genetic markers to breeding programs can provide further gains, particularly if genome wide markers can be developed to inexpensively enable off-season, marker-only selection. Some alternative methods are discussed.

Key words: genetic markers, recurrent selection, genome wide selection

Introduction

The goal of forage breeders is to develop cultivars improved for one or more characteristics. The ability of breeders to improve a given trait depends on the presence of heritable genetic variation in the crop's germplasm resources for the trait and an ability to select for the trait in a cost-effective and timely manner *(Brummer et al., 2009)*. Because many traits of importance cannot be assessed quickly (consider long-term persistence, which may require nurseries lasting five or more years) or cheaply (chemical composition, for example), alternative selection methods need to be used.

Conventionally, a correlated trait that is easy to assay has been used to select indirectly for the primary trait of interest, and as long as the correlation is reasonably strong, indirect selection can be effective. In the absence of a correlated trait, indirect selection can be done using molecular genetic markers, provided a linkage between marker and trait can be identified (or even better, if the exact allele of a gene causing a phenotype is found). Marker-assisted selection would therefore help breeders to select difficult phenotypes in a timely and cost-effective manner.

We have two avenues forward. On the one hand, we can attempt to reduce complex traits to their component constituents. This can be accomplished by mapping (and eventually cloning) quantitative trait loci (QTL) and then using linked markers to manipulate the QTL in breeding populations. When cloned, the gene(s) represented by a QTL can be transformed into breeding lines (in theory) and manipulated with markers without attendant linkage drag. This approach is certainly appealing, and success at mapping QTL for complex traits has been successful in many species, including alfalfa yield *(Robins et al., 2007a,b)*, persistence *(Robins et al., 2008)*, and selfed seed yield *(Robins and Brummer, 2010)*. (See *Brummer (2004b)* for a more comprehensive review of alfalfa mapping.) Alternatively, gene expression analyses using microarrays or high throughput sequencing can help identify genes associated with particular phenotypes. For example, we have recently identified a number of genes that may be involved with heterosis for biomass yield in tetraploid alfalfa *(Li et al., 2009)*. These candidate genes can be used either through marker selection or transformation to modify the trait of interest.

Unfortunately, most important agronomic traits, like yield or persistence, are complex. The underlying genetic structure of complex traits, even in mammalian systems, is poorly understood:

> All these (recent) observations suggest that the extent of regulatory information in the mammalian genomes is far greater than previously thought…. This creates challenges for resolving the complex bases for common human diseases, where gene networks rather than master genes drive specific phenotypes.…" *(Mattick et al., 2009)*.

Although this quotation refers to mammalian genetics, we can presume that agronomic traits are under similarly complex control. Thus, while we can identify QTL for these traits, full understanding of their underlying genetics is unlikely in the near future, particularly in forage crops.

The second avenue is to ignore QTL and simply consider overall genetic merit of individuals. In reality, despite the hype about genomics (and associated transcriptomics, proteomics, and metabolomics), all that really matters to the plant breeder – and to the forage farmer - is the phenome. The phenome can be defined as all the phenotypes a plant can express across developmental time under a range of environmental conditions. What we as forage breeders want is a cultivar that expresses a desirable phenotype under the conditions it will experience when grown in real-world situations. Therefore, we assess phenomes under a range of conditions – multiple locations and years – that hopefully represent the climatic conditions in which the resulting cultivar will be grown.

Conventionally, our field evaluations give us an assessment of genetic merit – i.e., the breeding value – of individual plants (genotypes) if we use progeny testing. The beauty of conventional plant breeding is that the complexity underlying the trait is essentially immaterial; if our phenotypic evaluation is accurate, then we can improve the trait regardless of the number of structural genes or regulatory factors involved in its expression. The shortcoming of the gene-by-gene reductionist approach is that any single gene within the population will be affected by all the others affecting the trait, and the end result may not be predictable. (Of course, the effect may be highly predictable, in which case using that particular gene or QTL in the breeding program would be very sensible.)

We return now to the problem of selecting indirectly using markers but without focusing on particular genes or QTL. Recently developed high-throughput markers systems based on single nucleotide polymorphisms (SNPs) provide breeders the capability of assessing the entire genome – or at least significant parts of it – at once. Genome-wide markers enable breeders to conduct genomewide selection, in which a breeding value is computed for every individual marker *(Meuwissen et al., 2001; Bernardo and Yu, 2007)*. If these markers sample the entire genome, then collectively they will explain a large portion of the additive genetic variance for a given trait. It does not matter which genes involved in the trait are associated with which marker; all that matters is the overall genetic merit of an individual based on the summation of the breeding values of the marker alleles that it contains. Selection is then made based on breeding values computed from the markers, rather than breeding values derived from phenotypic evaluation, for example.

Designing a Breeding Program

Consider first how we might better design a breeding program to achieve improved genetic gain. Before markers are introduced into a program, carefully consider how well the current breeding methods result in the desired outcome. In my experience, simple changes to breeding methodologies may enable greater genetic gain, and in fact, may result in higher gain than could ever be obtained by incorporating markers into the program.

We know that simple phenotypic recurrent selection based on individual plants is inferior to progeny testing if trait heritability is low. Among progeny tests, the half-sib family evaluation is the most common, due to the simplicity of producing seed from open pollination of a set of genotypes. The limitation of half-sib evaluation, of course, is that (in diploids) only ¼ of the additive genetic variance is present among families, while the remaining ¾ is within families *(Fehr, 1987)*. Other types of progeny testing are better, particularly if the relative magnitude of dominance variance is small compared to additive variance. Of particular value is the use of selfed families (if they can be produced), in which all additive variance is among families *(Brummer, 2008; Fehr, 1987)*. For a given selection intensity, gain per cycle can easily be double under selfed family selection compared to half-sib family selection.

Considering only half-sib evaluations, several options are possible *(Brummer and Casler, 2008; Casler and Brummer, 2008)*. The method begins with intercrossing a number of individuals from a base population to produce half-sib families that can be planted into replicated, multilocation trials (Figure 1). Based on the evaluation, the breeder could recombine either the parents of the best families (A) or the best individuals from the best families (B). The conditions under which each is favored are discussed in the paper, but generally, the second method is favored, in part due to the need for a second generation of recombination in A in order to develop families for the next cycle of evaluation.

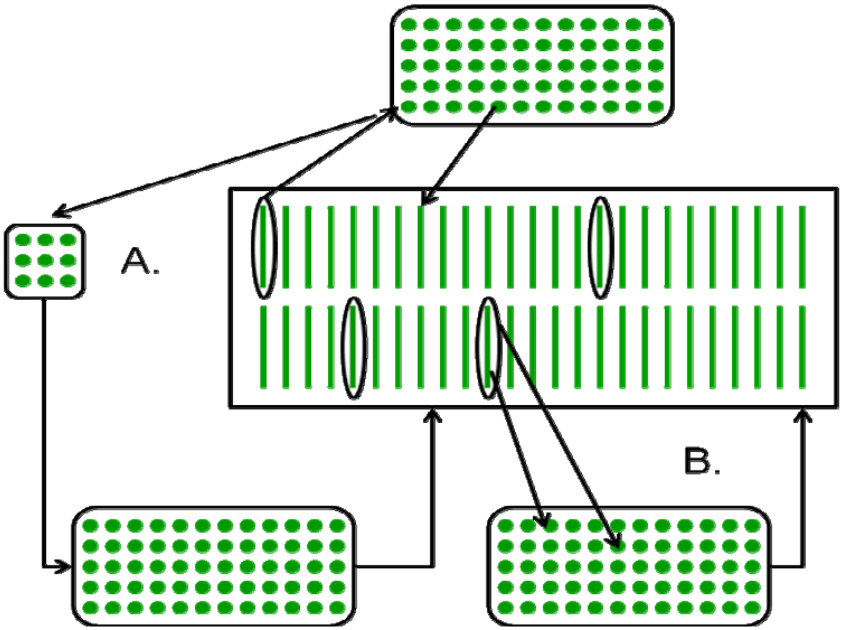

Figure 1. Half-sib family evaluation. Progenies are initially developed by open-pollination of members from a base population. The best families are identified. (A) The parents of the best families are removed to an isolated area and recombined. A subsequent recombination is needed to regenerate families for the next cycle. (B) The best individuals from the best families are recombined, with sufficient individuals selected to produce the desired number of families in the next cycle.

This platform is generally suitable for many breeding applications, and it can serve as a model to show how genetic markers can be incorporated into a program. In the case of marker-assisted selection of single genes or QTL, the method is relatively well developed in many crops, and applying it to forage species is rather trivial – that is, plants are evaluated with the marker(s) and those with the marker (and any other traits of interest) are selected, recombined, and so on. Of more complexity is the use of markers to improve traits when marker-trait associations are not known, such as in the genome wide method described above.

Markers can be used in several ways. First, using selection method B in Fig. 1, markers can be used to assist selection of individuals within families (Figure 2, A). Most simply, a select set of markers can be used to assess paternity, to ensure that only parents of the best families contribute to the next cycle *(Riday, 2010)*. This method can be both inexpensive and rapid, and would result in significant genetic gain, even in the absence of either whole-genome marker scans or specific marker-QTL associations. Paternity testing also provides information on relationships among individuals (and families) and can also contribute to improved gain by capturing genetic variance among paternal families if evaluations are done on individual genotypes rather than on a family-wide basis.

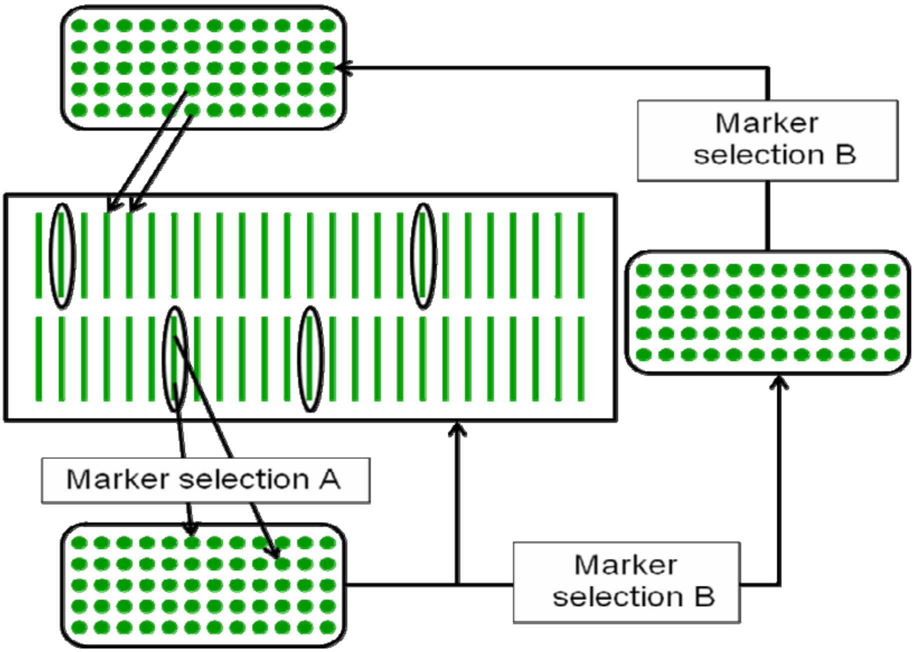

Figure 2. Points of marker incorporation into a half-sib family evaluation program. Marker selection A could be based only on a few markers to assess paternity. Alternatively, the parents of the families can be evaluated with genome wide markers, for which breeding values can be computed based on the phenotypes evaluated on their progeny families. Based on marker breeding values, individuals can be selected from within the best families (A). Following recombination, seedlings can be grown and selected with marker-based breeding values in the greenhouse (B).

Paternity testing, called “a poor man’s marker assisted breeding strategy” by Riday, does not enable continued selection beyond one cycle, because the markers themselves have no predictive value. Genomewide marker evaluation would enable one or more cycles of marker-only selection (Figure 2, B), which could be conducted in the greenhouse off-season. The resulting gain could be significant if the markers explain a substantial fraction of the variation in the trait. Eventually, the predictive power of the markers will diminish as desirable allele frequencies increase. Therefore, after several cycles of marker-only selection, breeding values need to be reevaluated as shown for the first cycle.

The biggest limitation of genome wide methods at the current time is the lack of sufficient markers. Current sequencing methods produce large amounts of sequence, although a given “read” is relatively short *(Varshney et al., 2009)*. Nevertheless these sequences are relatively cheap and can be used to identify large numbers of SNPs that can be developed into markers.

We are currently using high throughput sequencing of alfalfa transcriptomes to identify SNPs in genes related to cell wall synthesis and other important traits. Nevertheless, even with many SNP markers, the cost of genome-wide marker analysis on many individuals in a breeding population is high, and probably cost-prohibitive for most applications. The most promising alternative is to simply use high-throughput sequencing directly as markers; strategies for doing this, particularly in polyploidy species without inbred lines, is still being developed. Genetic gain can also be improved in other ways. For instance, capturing heterosis in the cultivar development process can lead directly to yield gain *(Brummer, 1999)*. Examples such as this show that improving yield does not require the application of genomics, and in fact, applying genomics could be counterproductive to improving cultivars if it diverts limited funds from breeding methods that are known to work *(Brummer, 2004)*.

Conclusion

Our goal is to efficiently develop improved forage cultivars. Perhaps the most important gains can be made by simply re-evaluating our breeding methods and changing how we breed to better estimate breeding values. Evaluating phenotypes is critical to all breeding, and to the extent that we can get a better evaluation of phenotypes by changing methods, we will improve gain. Markers can be used in many ways, but most directly, their use to assess paternity may be the most sensible for most forage breeding applications. In depth QTL discovery and marker-assisted selection can help, but for complex traits, developing genome-wide marker methods to enable marker-only selection will have the largest impact over time.

Upotreba molekularnih markera u oplemenjivanju krmnih biljaka

E. C. Brummer

Rezime

Genetička dobit u oplemenjivanju je generalno niska za sve kompleksne osobine, kao sto je prinos krme. U ovom radu je predložen način kako unaprediti genetičku dobit korišćenjem alternativnih selekcionih metoda. Takođe, uključivanje genetičkih markera u oplemenjivačke programe može dodatno unaprediti dobit, posebno ako se razviju raznovrsni i pouzdani molekularni markeri za sprovođenje vansezonske selekcije bazirane isključivo na markerima. Neke alternativne methode su obrađene u radu.

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GENETIC RESOURCES IN SERBIA, MAIN ASPECT ON FORAGES

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Abstract: Presented results refer to genetic diversity in general, and main emphasis is on forage species, major and minor crops, as well as number of cultivars of major species selected so far. Also work on conservation of PGR, *in situ* protection and *ex-situ* management is presented. The state of forage crops ex-situ collection, national PGB, storing and regenerating of forage genetic resources, as well as activities relating to gathering of accessions and expanding of the national collection, and national collecting program are presented. State of forage crops *ex situ* collections stored in breeding Institutes in Serbia and state of regional and international collaboration are presented and forage PGR accessions evaluated.

Key words: genetic resources, forage crops, gene bank, Serbia

Introduction

Serbia is located in Southeast Europe, in the central part of the Balkan Peninsula, between 41°51' and 45°54' latitude and between 18°48' and 23°00' longitude. Serbia is referred to as the cross-roads of Europe. Its river valleys make up the shortest link between Western and Central Europe, on the one side, and the Middle East, Asia and Africa, on the other. Longest river is Danube (flows 588 of its 2.783 kilometer long course through Serbia). The Republic of Serbia covers an area of 88,361 sq. km. Northern part in the Pannonia Plain is mainly flat (Vojvodina), while its central and southern areas consist of highlands and mountains. The flatlands are mainly in river valleys: Mačva on west, Morava Valley in central part, Stig and the Negotin flatland in eastern part of Serbia. The Morava-Vardar valley is the most important connection between the north and the south of the Balkan Peninsula, and passage for breakthrough of Mediterranean climate. Fifteen mountains are over 2,000 meters height, with highest top Djeravica in the Prokletija mountain range (2,656 m). Climate in Serbia is temperate continental, with a gradual transition between the four seasons of the year and average annual precipitation from 600 mm to 800 mm in the plains and from 800 mm to 1,200 mm in the mountains. An average annual temperature is 11-12°C. The temperatures in January and June average -1 - +1°C and 22 - 23°C respectively.

According geographic position Serbia is part of Mediterranean area one of the largest center of origin and divergence of many plant cultivated species important for agriculture, especially forage plants. According to the most recent estimates the flora of Serbia contains 3662 taxa, i.e. 3272 species and 390 subspecies, *(Kišgeci and Cvetković, 1998)* making Serbia a country with very high floristic diversity and density per unit area compared to other European countries. All plant species are included in 141 families and 766 genera. Considering the vegetation diversity of Serbia, about 600 to 1200 plant associations have been identified *(Lakušić, 2005)*, including forests (deciduous and coniferous), steppes, meadows, pastures, vegetation of sand dunes and saline soils, swamps, alpine tundra, etc. Among them, after detail analysis, 273 meadow and pasture associations were determined in Serbia and included in 46 vegetation alliances from 24 rows and 10 classes of grasslands *(Kojić et al., 2004)*. The most diverse vegetation class are *Festuco-Brometea* (Br.-Bl. et R. TX. 1943) with 1194 plant species (41.8% of all plant species in Serbia) and *Molinio-Arrhenateretea* (R. Tx. 1937) with 895 species included *(Lakušić, 2005)*.

Farmland covers 70% of the total surface area of Serbia, while 30% is woodland. Total agricultural land is on 5 093 000ha with 1454000ha of permanent grasslands *(Statistical yearbook of Serbia, 2009)* especially in hyli-mountinous area. Natural features, diverse relief and climatic diversity result in great wealth of grassland plant species (including many endemic plants). Serbia is therefore rich source of autochthonous plant genetic resources (PGR) of forage plants *(Ćupina et al., 2006; Soković et al., 2007a; 2009)*, since most of the forage cultures have wild relatives in the nature which show high degree of genetic variability and specificity *(Lugić et al., 2007; Soković et al., 2007b; Babić et al., 2009; Radović et al., 2009)*.

Fabacea family, which includes very important forage leguminous plants, in Serbian flora is presented with 34 genera among which the most important and interesting are the following genus: *Trifolium* with 50 species, *Vicia* with 27 species, *Medicago* with 11 and *Lotus* with 4 species *(Josifović, 1976)*. In the second important family *Poaceae* there are 70 genera of which, from the aspect of production, the most interesting are *Poa* with 17 species, *Phleum* with 8 species, *Festuca* with 21 species, *Lolium* with 5 species, *Agrostis* with 6 species, *Dactylis* with 3 species and *Bromus* with 14 species. Many of them contain numerous sub-species, varieties and forms. Natural ecosystems of meadows and pastures whose main components are above mentioned species are present in Serbia on large areas mainly on higher altitudes, on hilly-mountainous terrains *(Stošić and Lazarević, 2009)*. All associations are well developed, of stable floristic composition and wide diversity. This great diversity of forage plants has enormous importance, not only as source for breeding, but also as source of natural plant genetic diversity. They represent basic prerequisites for sustainable forage production, but very low potential of these eco-systems is utilized today. The possibility of their enhanced utilization requires serious work on previous genetic resources protection in Serbia. Due to large species diversity and their complex relationships, those eco-systems are extremely vulnerable and should be protected.

State of diversity of forage crops in Serbia

General plant diversity. Serbia is characterized by a huge geographic and biological diversity reflected in the richness of its indigenous flora which exists as various vegetation formations of terrestrial and aquatic ecosystems. Dominant ecosystems in Serbia include deciduous forests, steppes, coniferous forests, meadows and alpine "tundra".

Serbia is considered as one of the 158 world biodiversity centers, based upon the total number of plant species (including mosses) and territory size (biodiversity index of 0.718). Also many individual plant communities have been identified, including steppes, meadows, pastures, vegetation of sand dunes and saline soils, swamps, alpine tundra, etc make Serbia a country with very high floristic diversity and density per unit area compared to other European countries. The main factors of such floral diversity are: historical background of vegetation development, geographical position, climate, relief, presence of streams.

Forage crops diversity. Total number of plants species used in Serbian commercial agricultural production is 233 (without flowers). There are 185 commercial agricultural species of field crops, with 205 different kinds of crops because some species contain several forms (*Brassica oleracea* ssp. *capitata* and *sabauda*; *Beta vulgaris* ssp. *vulgaris* and *conditiva*; etc). Maize and wheat are the most widespread crops in Serbia. Other important cereals in country are barley, oat and rye. Main industrial crops grown in Serbia are: sunflower, soybean, sugar beet, oil turnip. All those major crops is partly or totally used as animal food.

From fodder crops, the highest importance in Serbia (**major fodder crops**) has: alfalfa (*Medicago sativa* L.), red clover (*Trifolium pretense* L.), forage (silage) maize (*Zea mays* L.), forage pea (*Pisum sativum* L.), common vetch (*Vicia sativa* L.), grasses (orchardgrass (*Dactylis glomerata* L.), meadow fescue (*Festuca pratensis* Huds.), Italian ryegrass (*Lolium multiflorum* Lam.), perennial ryegrass (*Lolium perenne* L.), timothy (*Phleum pratense* L.)) and fodder beet (*Beta vulgaris* L.).

Minor fodder crops in Serbia, with rare utilization, are legumes: white clover (*Trifolium repens* L.), birdsfoot trefoil (*Lotus corniculatus* L.), sainfoin (*Onobrychis viciifolia* Scop.), Crimson clover (*Trifolium incarnatum* L.), subterranean clover (*Trifolium subterraneum* L.), Hungarian vetch (*Vicia pannonica* Crantz.), hairy vetch (*Vicia villosa* Roth.), field bean (*Vicia faba* L.), grass pea (*Lathyrus spp*.), and grasses: red fescue (*Festuca rubra* L.), tall oatgrass (*Arrhenatherum elatius* (L.) P. Beauv. ex J. Presl & C. Presl.), *Agrostis spp*., tall fescue (*Festuca arundinacea* Schreb.), Kentucky bluegrass (*Poa pratensis* L.*)*, smooth brome (*Bromus inermis* Leyss.), Sudan grass (*Sorgum sudanence* (Piper.) Stapf), sorghum (*Sorghum bicolor* (L.) Moench).

Some of mentioned species are characterized by high number of intra-species taxa, such as subspecies, varieties and forms. All those plant species have been using as forage very long and they have been improved by breeding many time for better utilization (Table 1).

Table 1. Number of created varieties of fooder crops in Serbia ***(Djukić et al., 2007; Tomić and Sokolović, 2007; Mihajlović et al., 2007c)***

Perennial legumes		Perrenial grasses		Other forage species	
Alfalfa	24	Tall oatgrass	3	Field pea	10
Red clover	10	Cocksfoot	4	Field bean	2
Birdsfoot trefoil	5	Tall fescue	3	Common vetch	8
Sainfoin	2	Meadow fescue	1	Hairy vetch	2
White clover	1	Red fescue	2	Hungarian vetch	1
		Italian ryegrass	2	Sorghum	7
		Perennial ryegrass	2	Sudan grass	3
		Timothy	2	Fodder beet	3
Total number	42		19		36

Most of created cultivars contain autochthonous wild populations and genotypes from old adapted cultivars from abroad and represent great and important resource of genetic variability. It is noticeable that number of registered cultivars of minor crops is significantly lower then in major crops, but great value of genetic resources in this group of plants lies in a numerous old, primal varieties and breeding populations, landraces, indigenous material, etc. However, there is a danger of genetic erosion of these resources caused by gradual introduction of modern high-yielding cultivars.

State of diversity of wild plants. In marginal area, which is not used for agriculture, and on natural grasslands on uplands there are many wild relatives of crop plants. Serbian grasslands are very rich with populations of almost all forage plants, due to fact that Serbia is center of their origin and distribution (Mediterranean and Near east). Local ecotypes and populations have natural variation and very often have satisfactory yielding performance in comparison with introduced cultivars *(Sokolović et al., 2003; Sokolović et al, 2004; Sokolović et al,* *2010)*, which referred them for direct phenotypic selection for cultivars release *(Posselt and Willner, 2007)*.

The most valuable native plant genetic resources, components of Serbian natural grasslands, are legumes (genera *Medicago, Lathyrus*, *Trifolium* and *Vicia*) and grasses, especially *Festuca, Agrostis* and *Poa*. In the natural plant communities there are also many wild populations of all most important fodder species which show a high degree of genetic diversity.

Protected and endangered species. It is important to emphasize that 215 plant species is protected by *Direction of natural rarities protection* in Serbian flora, or 17.7% *(Kišgeci and Cvetković, 1998)*. The *Direction* includes 12 fern species, 7 angiosperms and 196 vascular plants. In aim to protect the biodiversity in Serbia, scientific publication "Red data book of Serbian flora and fauna" was written in 1999. This book contains the preliminary list of the most threatened plants, according to IUCN, the International Union for Conservation of Nature. Some of threatened plants are included in the worlds and European Red List.

Some of them can be found on meadows and grasslands, and therefore represent part of association with possible forage utilization. They should be protected as very rare genetic resources with their natural habitats. Some of them are listed here: Paeonia tenuifolia (sand Deliblatska peščara), Adonis vernalis – yellow pheasant's eye (mountain Fruška Gora, sand Subotička peščara), Drosera rotundifolia (mountain Stara Planina, Vlasina river), Pulsatilla grandis (Fruška Gora, Deliblatska peščara), Leontopodium alpinum (mountains Kopaonik, Mučanj, Prokletije), Gentiana lutea (mountain Golija, Kopaonik), Cypripedium calceolus (mountain Suva Planina - protected by UNESCO as world's natural rarity), Lilium martagon, Stipea pennata, etc. Although significant efforts in protection of natural resources (especially in meadows and pastures) has been made, a great number of plant species has already disappeared.

Forage crops PGR conservation in Serbia

Two models of conservation are possible for forage crop genetic resources: First is dominant and represent maintaining of seed samples *ex situ* in gene banks. Also, autochthonous populations, ecotypes, landraces or cultivars can also be maintained *in situ* (for landraces or cultivars it can be referred to as "on farm").

The state of in-situ forage crops PGR protection. The main objective of *in situ* conservation is to maintain the environment which has led to development of the distinctive properties of the genetic resources. *In situ* conservation allows that genetic evolution and specific environment, which developed such unique genetic resource, continue influence on further development of forage plants.

Humphreys (2003) quoted that “although it is diminishing year by year through genetic erosion, a wide range of valuable genetic resources are still available in the natural or semi-natural grasslands of Europe”. During the past few decades, the protection of fodder crops *in situ* has received increasing interest over Europe *(Boller and Greene, 2010)*, especial in grassland dominated regions which are centres of diversity.

This type of PGR protection is not developed in Serbia, although it is necessary and quite possible in uplands of Serbia, to protect and conserve various types of natural grassland from their degradation or destruction by inadequate grassland management or uncontrolled production of other cultures (potato or raspberry). This is achievable by contracting between state and grassland owners (farmers) on longer time scale, for preserve traditional grassland management and production.

Within fodder crop accessions grown *in situ* in Serbia dominate domestic varieties or old landraces. First created landraces (cultivars) mostly originated from native populations or cultivars form Eastern Europe *(Djukić et al., 2007; Tomić and Sokolović, 2007)*. Nowadays, after more than 50 years, all these landraces don’t have any commercial significance, most of them are already excluded from the cultivar list, but have enormous importance as valuable source of genes. Among them the most famous and most distributed were K-1 and M-2 landraces of alfalfa (*Medicago sativa* L), K-17 of red clover (*Trifolium pratense* L.) and K-6 and K-7 of cocksfoot *Dactylis glomerata* L. Very rarely some old cultivars introduced long time ago from abroad could be found in fodder production on farms (*Trifolium pretense* cv. Reichersberger*, Trifolium repens* cv. Angeliter Milka*, Arrhenatherum elatius* cv. Wena, *Beta vulgaris var. crassa* cv. Rossa mammouth, *Brassica napus var.napobrassica* cv. Bangholm wilby otofte and Hoffman's Gelbe, *Festuca pratensis* cv. Jabelska, *Lolium perenne* cv. Naki, *Lotus corniculatus* cv. Bosnalotus, *Phleum pretense* cv. Foka, *Vicia villosa* cv. Poppelsdorfer). After long period of growing in Serbia, these foreign old cultivars changed their genetic structure and became adapted to local conditions.

Domestic population of forage crops could be still found *in situ* in the mountain regions, growing on farm on mountains, such as Mt. Rtanj, Kopaonik or Goč. On those localities, the seeds of *Medicago falcata, Trifolim pratense, Trifolim repens, Trifolium medium, Lotus corniculatus, Dactylis glomerata, Festuca pratensis, Fesuca arundinacea* and *Lolium perenne* have been collecting, but there are no agreements on *in situ* conservation of those meadows and pastures with owners.

Forage crops PGR *ex-situ* management. By *ex situ* conservation, genetic structure of the original seed sample is preserving. The crucial is that all actions concerning ex-situ management like collecting, storage, regeneration or distribution is aimed keep presence and frequency of alleles within the population as constant as possible. As well as this type of PGR preservation is an important tool for conserving genetic diversity, it is also powerful tool to provide genetic resources for a broad range of users *(Greene and Morris, 2001)*.

The decision on the development of the National Plant Gene bank was brought in 1988. The construction of plant gene bank building started in 1990. Unfortunately, due to the breakdown of former Yugoslavia and enormous financial difficulties, it has not been completed. The aim of the formation of national plant gene bank was to ensure equal treatment, conservation and utilization to all the genetic resources in the state, as well as to ensure the adequate national policy in this field. The project of establishment of plant gene bank was realized through two parts: "Development of plant gene bank", "Collecting of genetic resources for the needs of plant gene bank". In the realization of the second part of project, 26 scientific research institutions of agriculture participated. In *ex-situ* national collection, the greatest number of accessions represents old varieties and populations. For the national collection it can be generally reported that it does not reflect the state in natural ecosystems. The most represented species are the economically most significant cultivated species (maize, wheat, sunflower, barley). However, from the aspect of genetic variability for our region, many species were not paid sufficient attention to (medicinal and aromatic plants, textile plants, some vegetable species, and a series of wild relatives of the species).

The state of forage crops *ex-situ collection* National PGB. In national collection there are 283 accessions of perennial forage species in total. Among them 159 accessions are perennial legumes and 124 accessions perennial grasses (Table 1). The majority of accessions belong to lucerne (71), but in whole, collection is mostly made of local populations.

Table 1. Existing collection of forage crops in Gene Bank of Serbia

Perennial legumes		Perennial grasses	
Species	No of samples	*Species*	No of samples
Medicago sativa	71	*Agrostis stolonifera*	34
Trifolium pratense	24	*Agrostis gigantea*	15
Trifolium repens	53	*Agrostis capilaris*	35
Trifolium hibridum	6	*Lolium perenne*	10
Trifolium montanum	4	*Lolium multiflorum*	4
Lotus corniculatus	1	*Dactylis glomerata*	12
		Festuca arundinacea	5
		Festuca pratensis	3
		Festuca rubra	2
		Phleum pratense	4

State of accessions in National PGB for forage crops has not differed recent years *(Tomić 1997; Tomić et al., 1999)*, only collections which have been keeping in state breeding Institutes have developed *(Tomić et al., 2009)*.

Storing and Regenerating of Forage Genetic Resources. *Ex situ* conservation of forages usually involves the storage of seed in gene banks in standard temperatures of 0–4◦ C for active collections and −18◦C for base collections, both at low seed moisture (3–7%). Durability of seed storage is dependant on seed condition and initial germination in the storage beginning. If seed plants are grown and harvested under optimal conditions and initial germination was >95%, duration of storage for many forage species is predicted to be 100 years or longer when stored at −18◦C (*Sackville-Hamilton et al. 1998*). Germplasm storage conditions should be optimized to maximize longevity, since seed regeneration is the most expensive activity of germplasm *ex situ* management.

Collected forage crops material of Serbian PGB is today stored at the Maize Research Institute "Zemun Polje" in Belgrade. Seed samples are kept in a cold chamber with controlled conditions at +4°C and approximately 50% relative air humidity. A part of accessions are documented on the electronic data base including passport, characterization and evaluation data. Internationally recommended descriptor lists (IBPRG/IPGRI) were followed wherever it was possible. Nevertheless, National Plant Gene bank does not have enough financial sources to carry on with the characterization, evaluation and documentation of samples.

Certainly, seed accessions have to be regenerated when seed quantity or viability drops below threshold limits and newly collected seeds need to be increased for distribution. Careful consideration needs to be given to regeneration to minimize any genetic change during the process, especially in forages, since they are largely out crossers and genetically heterogeneous.

From 1996, most of forage crops accessions in Serbia PGB were regenerated only ones, in 1999 *(Tomić and Sokolović, 1999).* A part of accessions has critical germination ability and must be regenerated. Therefore project named "Determination of seed quality of forage crops national collection" financed by Ministry of Agriculture, Forestry and Water Management of the Republic of Serbia was conducted. All 283 accessions were analyzed for germination in Institute for forage crops in Krusevac and it was determined that 145 accessions (57 legumes and 88 grasses) should be regenerated because their germination dropped below 50 %. Germination of accessions was between 30 and 98%, and considering long period of storage, fortunately, only few accessions had to low germination for regeneration.

As a result of such low seed accessions germination project proposal concerning seed regeneration was applied to Ministry of Agriculture, Forestry and

Water Management, and three-year project titled "Regeneration of seed accessions of forage crops National collection" was started 2007.

Figure 1. Pollination isolation for grass seed regeneration in filed of barley on experimental field of Institute for forage crops in Kruševac

Figure 2. Pollination cages with honey bees for isolated seed regeneration of alfalfa accessions on experimental field of Institute for forage crops in Kruševac

Plants were sown in isolation (cereals for grasses, Figure 1. and chambers for legumes Figure 2) on experimental field of Institute for forage crops, Kruševac. First regenerated seed accessions were collected last year. Project is continuing in 2010.

Broadening of *ex situ* PGR forage crops collection

Activities relating to gathering of accessions and expanding of the National collection. Depending on objectives, there are two basic criteria in making a strategy for collecting forage crops *ex situ.* If the primary objective is to enlarge genetic diversity of the breeding germplasm, the relative degree of genetic variation within and among sampling sites will affect decisions about the number of sites to be visited and the number of individuals to collect per site. When the objective is to find new genes affecting particular characters, the choice of collecting sites will be influenced by the presence of environmental or management factors that are selective forces for traits of interest.

First method of expanding forage crops PGR collection, especially for breeding, is introducing seed accessions with desirable traits from abroad (plant introductions). There are some specialized fodder crops gene banks with enormous accessions number, open for exchange and sending of chosen seed samples (Table 2).

Table 2. Major *ex situ* collections of forage legumes and grasses *(Boller and Greene, 2010)*

Country/international organization	Access. number	Major genera in collection
Australian Medicago Genetic Resource Centre, Adelaide, South Australia	45,640	*Medicago* (mainly annual species), *Trifolium*, *Lotus*, *Astragalus*, *Hedysarum*, *Trigonella*, *Atriplex*, *Onobrychis*, *Melilotus*; >1500 grasses
Contact	www.sardi.sa.gov.au/pastures/genetic_resources	
CGIAR International Center for Agricultural Research in the Dry Areas (ICARDA), Aleppo, Syria	20,031	*Medicago* (annual species), *Trifolium, Astragalus, Onobrychis, Trigonella, Scorpiurus*
Contact	singer.cgiar.org/index.jsp	
European Cooperative Programme for Plant Genetic Resources (ECPGR)	56,444	*Trifolium*, *Festuca*, *Lolium*, *Dactylis*, *Medicago*. Landraces and varieties developed in European countries, as well as local ecotypes.
Contact	www.ecpgr.cgiar.org/; http://eurisco.ecpgr.org	
USDA National Plant Germplasm System	33,142	*Medicago*, *Trifolium*, *Festuca*, *Elymus*, *Dactylis*, *Lolium*, *Eragrostis*, *Poa*, *Bromus*
Contact	www.ars-grin.gov/npgs/	
Genetic Resource Centre for Temperate Pasture Legumes, South Perth, Western Australia	9,184	*Trifolium* (mainly annual species), *Ornithopus* and *Biserrula*
Contact	www.agric.wa.gov.au/content/past/pl/genetic_resource_index.htm	

Other method for accession number increase is collecting from wild flora for preserving natural biodiversity or rising genetic variability of breeding germplasm.

National collecting program. On the relevant proposal of the Ministry of Agriculture, Forestry and Water Management of the Republic of Serbia, at the session held on August 18, 2005, adopted “Strategy of the agricultural development of Serbia”.

"National Program for conservation and sustainable use of plant genetic resources for food and agriculture" is in progress, and planned to be an integral part of the "National Agricultural program 2009-2011". This program refers to:

1. Bringing legislation in line with EU Directives;
2. Adaptation of the existing national database of international standards (FAO, IPGRI);
3. Establishing the National Gene bank;
4. Supporting the work of the study of agro biodiversity (collection, identification, characterization, documentation, conservation of old varieties, wild relatives, endemic species, etc.);
5. Supporting the exchange of genetic materials, access and benefit sharing, training of staff, public awareness raising, promoting international cooperation;
6. Development of combined traditional farm systems and agricultural production based on autochthonous varieties of plants;
7. Protection of traditional knowledge and cultural heritage, development of rural tourism, production of local products with protected geographic origin, etc;

Several projects relating collecting of perennial forage grasses and legumes was performed recent years financed by Ministry of Agriculture, Forestry and Water Management through "Program for conservation and sustainable use of plant genetic resources for food and agriculture" or within breeding projects in organization of Institute for forage crops, Krusevac and Institute of field and vegetable crops, Novi Sad.

State of forage crops ex situ collections hold in breeding Institutes. As a result of great number of expeditions across Serbia, mostly to higher hilly-mountainous terrains, researchers from Institute for forage crops, Kruševac, (Table 3) collected 201 accessions of most important perennial forage grasses (meadow, tall and red fescue, perennial and Italian ryegrass, cocksfoot, tall oatgrass, timothy, meadow foxtail, smooth brome) and legumes (lucerne, yellow lucerne, birds foot trefoil, sainfoin, red, white, Hungarian and mountain clover). Most of accessions are autochthonous populations, whereas 58 represent domestic landraces.

Table 3. Collection of forage plants in the Institute of forage crops, Kruševac ***(Tomić et al. 2009)***

Species	No. of accessions	Species	No. of accessions
Festuca pratensis	26	*Medicago sativa*	17
Arrhenatherum elatius	25	*Trifolium pratensis*	22
Tall fescue	5	*Lotus corniculatus*	17
Dactylis glomerata	31	*Onobrychis sativa*	7
Phleum pratense,	10	*Medicago falcata*	5
Festuca rubra	2	*Trifolium montanum*	6
Bromus inermis	5	*Trifolium repens*	7
Alopecurus pratensis	5	*Trifolium pannonicum*	5
Lolium multiflorum	3		
Lolium perenne	3		
Total	115		86

In the Institute of field and vegetable crops, Novi Sad, in Department of forage crops, activities are carried out on conservation, collection and storing of genetic resources as basis for improvement and creation of new cultivars *(Mihailović et al., 2007a; Vasiljević et al. 2007)*. In this Institute, great effort has been made in collecting of major forage plant species which contain high genetic variability to be used in breeding. Great number of accessions of annual legumes (Table 4), lucerne and red clover (Table 5), and Brassica were introduced from abroad or collected from wild flora.

Table 4. Collections of annual leguminous plants per genera in the Institute of field and vegetable crops, Novi Sad *(FAO State report, 2009)*

Genus	No. of accession	Genus	No. of accessions
Arachis	4	*Macrotyloma*	1
ajanus	7	*Mucuna*	1
Calopogonium	1	*Ornithopus*	4
Cicer	66	*Pisum*	688
Lablab	3	*Stylosanthes*	1
Lathyrus	87	*Trigonella*	1
Lens	51	*Vicia*	1226

Annual forage legumes collection (AFLC) in Institute of field and vegetable crops consists of 67 species from 16 genera *(Vasić et al., 2007)*. The species with most numerous accessions are pea (*Pisum sativum* L.) with about 600 accessions and common vetch (*Vicia sativa* L.) with more than 500 accessions *(Mihailović et al., 2007b)*. Three hundred accessions are from Serbia, while others are from 77 countries. From total number of accessions about 700 are advanced cultivars, about 200 are breeder's lines, about 300 are local landraces, while the rest belongs to wild populations and genetic stock *(Mihailović et al., 2007a)*. Most important accessions donors are ICARDA, Allepo, Syria; IPK, Gatersleben, Germany; INIA, CRF, Spain; IPGR, Sadovo, Bulgaria *(Mihailović et al., 2006)*.

Table 5. Collection of samples of lucerne and red clover in the Institute of filed and vegetable crops, Novi Sad *(FAO State report, 2009)*

Species	Origin	Population	Cultivars	Experimental lines	Landraces
Medicago sativa	Domestic	2	15	500	
	Introduction	4	80	-	
	Total	6	95	500	
Red clover		53	516		54

Forage brassicas collection is in the very beginning, with several accessions of fodder kale (*Brassica oleracea* var. *viridis*) and white mustard

(S*inapis alba* L. subsp. *alba*). Main goal is improving it in the same way and with the same activities as the AFLC *(Mihailović et al., 2007c)*.

State of regional and international collaboration. Regional and international collaboration in forage PGR in Serbia is based on several programs such as SEEDNet and ECPGR.

SEEDNet (South East European Development Network on Plant Genetic Resources) programme was established in 2004. The programme is financially assisted by SIDA (Swedish International Development Cooperation Agency) and CBM (Centrum for biologist manifold) as executing agency. The aim of the SEEDNet programme is to contribute to the establishment and strengthening of national programmers on PGR in order to secure the conservation of PGR in the region, to promote a sustainable utilization of PGR and to strengthen collaboration, networking and linkages among various stakeholders at both national and regional levels through pooling of resources and use of comparative advantages available in the various institutions and countries. Also through this project NGB in Serbia is supplying with necessary equipment for beginning of its official work.

National group of researchers who are engaged in work on genetic resources of forage plants are participants of regional project realized in the last few years named "Regional collecting expedition and *ex situ* conservation of *Trifolium pratense* L., *Medicago falcata* L., *Dactylis glomerata* L. and *Festuca pratensis* Huds". The main goals of this project are: collecting of seed of autochthonous populations, setting up seed trials and conduct regeneration, characterization and further evaluation in several countries-WG members, multiplying the seeds for long term storage, documenting all obtained data of characterization and evaluation and make it available on line and strengthen regional, sub regional and international cooperation and documentation for a broad use and exchange of information and seed.

During two years of project conducting, several expeditions were organized (Rtanj, Fruška gora, Kopaonik, Pešter, Tara, Vlasina) and many seed samples were collected (Table 6).

Table 6. Number of collected accessions through regional SEEDNet project

Mandate species	No. of accessions	Mandate species	No. of accessions
Trifolium pratense	30	*Dactylis glomerata*	20
Medicago falcata	18	*Festuca pratensis*	17

Evaluation of forage PGR accessions. To make *ex situ* collection accessions useful to potential users, especially breeders, seed accessions need to be characterized and evaluated, and this information needs to be completely available to potential users. Generally, characterization focuses on simply inherited traits while evaluation is mainly directed to quantitative traits. International Descriptor Lists have been published for forage grasses and forage legumes (*http://www.bioversityinternational.org/*). This provides a starting point for characterizing accessions in a standard format. Unfortunately the cost of germplasm evaluation is high, particularly for forage crops which generally exhibit large genotype by environment interactions due to their out crossing nature. Another difficulty is appearing if evaluation is made in real production conditions in swards. This rise total cost of evaluation due to trait evaluated (interplant competition, persistence and sward growth pattern, grazing tolerance and yield).

Evaluation of all collected accessions is praxis in breeding Institutes in Serbia, so most of populations are characterized and evaluated already *(Lugić et al., 2007; Soković et al., 2007a,b; Babić et al., 2009; Radović et al., 2009)*. Current activities on AFLC are maintaining the passport database according to the FAO multiple descriptors, *ex situ* characterization, *ex situ* evaluation, short-term storage and regeneration each year in field conditions *(Ćupina et al., 2006)*.

Genetski resursi u Srbiji – krmne biljke

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Rezime

Prikazani rezultati se odnose na genetički diverzitet generalno, a glavni akcent je na diverzitetu krmnih kultura glavnih i manje prisutnih useva, kao i broj do sada selekcionisanih sorti najvažnijih vrsta. Prikazan je rad na konzervaciji biljnih genetskih resursa, *in situ* zaštiti i *ex-situ* upravljanjem. Takođe je prikazana postojeća situacija koja se odnosi na *ex-situ* sakupljanje krmnih kultura, nacionalnu banku biljnih resursa, čuvanje i regenerisanje genetskih resursa krmnih biljaka, kao i aktivnosti koje se odnose na sakupljanje uzoraka i proširenje nacionalne kolekcije i nacionalni program kolekcije. Predstavljeno je stanje *ex situ* kolekcija krmnih kultura koje se čuvaju u institutima koji se bave selekcijom biljaka u Srbiji, kao i regionalna i međunarodna saradnja.

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FORAGE LEGUMES SEED PRODUCTION IN SERBIA

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Abstract: The most important forage legumes in Serbia are alfalfa, red clover, field pea and vetch. It is estimated that forage legumes are cultivated on 290,000 ha, that is, on 8 % of the arable land of Serbia. The prevailing form of utilization of forage legumes in Serbia is the production of voluminous feed (green forage, hay, haylage, silage), with the exception of protein pea that is used in the form of grain. Due to this, there are developed the cultivars with extremely high aboveground biomass, with high proportion of leaf in the total forage yield and slim and slender stems with high digestibility. Such morphology implicates a significant susceptibility to lodging and therefore makes seed production difficult. By this reason, the main characteristic of the seed production of all forage legumes in Serbia with the exception of protein pea is an extremely wide variation of yield, depending on weather conditions in a specific year. The average seed yield in alfalfa and red clover is 250 kg ha^{-1}, in field pea 2500 kg ha^{-1} and in vetch 1200 kg ha^{-1}.

Key words: alfalfa, red clover, forage pea, vetch, seed

Introduction

The most important and the most widely distributed forage legumes in Serbia are alfalfa, red clover, field pea and vetch. According to *Erić et al. (2000)*, in the period of 1984-2000 alfalfa in Serbia was cultivated on about 200,000 ha. We do consider the cultivated area under alfalfa uncertain, since it is often overlapped with red clover in the official data. On the basis of the sold quantities in the market, it is estimated that alfalfa in Serbia is grown on about 180,000 ha, representing 5 % of the arable land. Out of this, alfalfa in Vojvodina in 2003 was grown on about 60,000 ha *(Karagić et al., 2004)*.

According to the official data, red clover in Serbia is grown on 120,000 ha, (SGRS, 2002-2008), but the seed producers regard its area much smaller, that is, from 60,000 ha to 80,000 ha *(Katić, pers. comm.)*. It is cultivated mostly in western parts of Serbia and the acid soils of central Serbia, while in Vojvodina it is used only for seed production. The area under field pea and vetch in Serbia is about 30,000 ha, mostly in northern and central parts of the country *(Mihailović et al., 2003b)*. Vetch in Serbia is grown on about 7000 ha *(Mikić et al., 2006)*.

Regarding the cultivation areas, the annual requirements for the forage legumes seed in Serbia are 800-1,000 t for alfalfa, 300-400 t for red clover, about 3,500 t for field pea and 800-900 t for vetch. The value of the forage legumes seed market in Serbia is estimated on about 1 billion dinars, that is, about 10 million euros.

Alfalfa seed production. The alfalfa seed production in Serbia is located mainly in Vojvodina. According to the official data, the alfalfa seed in Vojvodina was produced on 1,400 ha in 2002. In 2007-2009, the certified seed was produced on 1,688 ha, 2,544 ha and 2,898 *(Boćanski, pers. comm.)*. Apart from this, it is estimated that non-certified seed is produced on an area greater than 1,000 ha *(Karagić et al., 2007)*.

Alfalfa is genetically prepared to render extremely high forage yields, which are often in negative correlation with seed yield *(Huyghe et al., 2001; Bolanos-Aguilar et al., 2002)*. In regions with semi humid climate, weather conditions in the year of growing are the main source of variation in alfalfa seed yield. *Karagić et al. (2003)* determined a highly significant negative correlation between alfalfa seed yield and precipitation sum in Vojvodina during the flowering stage of the seed cut (r = -0.72).

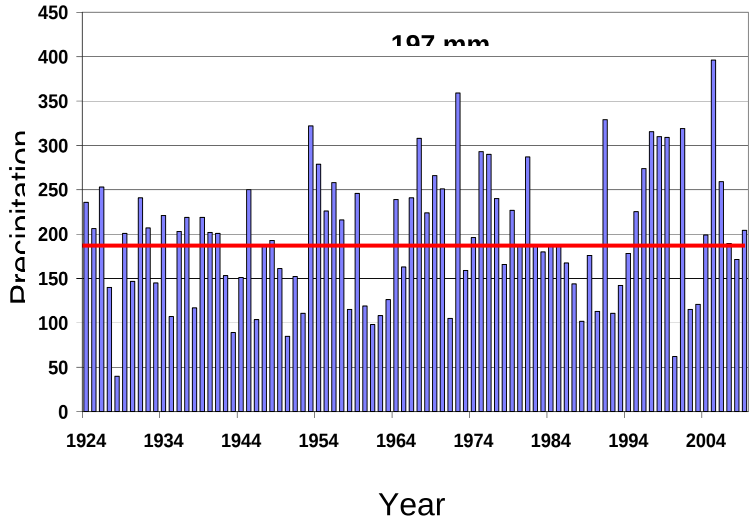

Figure 1. Precipitation sums for June, July and August, at Rimski Šančevi location, for the period 1924-2008

A successful alfalfa seed production is carried out in the regions with clear, sunny and hot summer days combined with low precipitations. The annual sum should not be higher than 450-600 mm, with 90-110 mm in July and August at most, that is, not more than 180 mm in June, July and August *(Žarinov and Kljuj, 1990)*. Such ecological conditions presume good alfalfa flowering and are optimal for the pollinating activities of bees (Apoidea), thus answering two essential factors for the seed production *(Rincker et al., 1988)*.

The average precipitation sum in June, July and August for the range of 86 years (1924-2009) is 197 mm (Figure 1), slightly higher than the value marked by *Žarinov and Kljuj (1990)* as the upper limit for a successful alfalfa seed production. The proportion of the years with the precipitation sums lesser than 180 mm, that is, the years that are very favourable for alfalfa seed production, is 41.5 % according to this criterion. The proportion of the moderately favourable years (a precipitation sum of 180-250 mm) is 36.6 %, while the proportion of the non-favourable years is 21.9 % *(Karagić et al., 2007a)*.

In the years with excessive precipitations, alfalfa plants are lush and lodge easily. Lodged plants are not suitable for pollination and low seed yields are consequently produced. The most adequate measure to solve this problem is a proper selection of the region. Such appropriate region should be characterized by a minimum precipitation sum during the summer. The most favourable conditions for alfalfa seed production in Serbia are in the regions of north Bačka, bordering regions with Romania in Banat, regions of Kikinda, Zrenjanin, Kovačica and the region of Timočka Krajina (Zaječar). In these regions, the annual precipitation sum, as well as the precipitation sum in June, July and August is significantly lower than at the location of Rimski Šančevi. *Mihailović et al. (2004)* determined that the average alfalfa seed yields in 2003 were 583 kg ha^{-1} in Mid-Banat and 621 kg ha^{-1}, in North Bačka, while in South Bačka it was 181 kg ha^{-1} in average. The favourable regions are characterized by higher alfalfa seed yields in comparison to the yields of other filed crops, especially of soybean and maize, thus increasing the competitiveness of alfalfa seed stand *(Karagić et al., 2007)*. However, due to a relatively small area of the favourable regions, the effect of regions on the alfalfa seed production in Serbia is limited.

Apart from the regionalization of the seed production, the growth of the alfalfa seed stand may be regulated by the cutting regime *(Karagić, 2004)*. The climatic conditions of Serbia favour the production of alfalfa seed from the second re-growth. The first re-growth is used for production of hay. With early first and second cuts, seed may be also produced from the third re-growth.

The late cutting in full flowering ensures a reduced stand density and maximum number of productive shoots. Also, plant height is reduced and number of pods per raceme significantly increases in relation to the systems of early and medium cutting. In addition, cutting date is used for timing the beginning and duration of flowering period in seed crops, with the intention of synchronizing flowering period with maximum activity of pollinating insects.

Consequently, plant sensitivity to lodging is considerably reduced while conditions for alfalfa flowering and activity of pollinating insects are improved, all that resulting in increased seed yield (Table 1) *(Karagic et al., 2007)*.

Table 1. Seed yield depending on cutting schedule in 2001-2004 (kg ha^{-1})

Cutting schedule (C)	Year (Y)				Average
	2001	2002	2003	2004	
c_1 - Budding	163	573	372	234	335
c_2 – First flower	167	589	203	297	314
c_3 – Full flower	225	1041	192	415	468
c_4 - III cut*	108	656	57	199	255
Average	166	715	206	286	343

*I and II cuts in budding

LSD	C	Y	C x Y
0.05	19.53	21.48	39.06
0.01	25.72	28.34	53.45

Alfalfa is allogamous, enthomophilous species, that is, the pollination requires a flower to open, its style to grow and the pollen from another flower to reach its stigma. Pollen grains in alfalfa are wet, sticky, heavy, collected in large balls and unable to be transferred by wind. The most efficient pollinators in alfalfa are wild solitary bees and its relatives from the genera *Anderna, Helictus, Megachile, Melitturga, Melitta, Nomia, Rophitoides, Osmia, Eucera* and *Bombus* (*Goloborodko and Bodnarčuk, 1998*). Certain authors *(Erić and Đukić, 1995; Miladinović, 2001; Jevtić et al., 2002; Jevtić et al., 2007)* point out the importance of the European honey bee (*Apis mellifera* **) for the pollination and seed production of alfalfa. According to *Jevtić et al. (2002)* in two out of four years honey bee was dominant on alfalfa flowers. On the other hand, *Katić (1988), Mihailović et al. (2003), Karagić (2004)* regard the impact of the European honey bee upon the pollination in alfalfa as non-significant since it does not open flowers while collecting nectar.

Neither honey bee nor wild bees are used in the alfalfa seed production in Serbia. It is considered that in the nature there are already enough alfalfa pollinators, as confirmed by high seed yields (> 1000 kg/ha) in favourable agro-ecological conditions *(Karagić et al., 2007)*. There were attempts to introduce and domesticate the wild bee *Megachile rotundate* F. to improve the alfalfa seed production. However, due to unfavourable conditions such as high precipitations very modest results were achieved *(Đukić et al., 1987)*.

In order to achieve high alfalfa seed yields in Serbia, a special attention should be pay to the control of both weeds and pests. The greatest problem in the alfalfa establishment is wild mustard (*Sinapis arvensis* L.), white goosefoot (*Chenopodium album* L.) and creeping thistle (*Cirsium arvense* (L.) Scop.). Weed composition is changed with the stand getting older. The most common weeds in an old alfalfa stand are shepherd's-purse (*Capsella bursa-pastoris*), plantain (*Plantago* spp.), dandelion (*Taraxacum officinale* Web.), stitchwort (*Stellaria media* (L.) Vill.), fleabane (*Erigeron canadense* L.) and perennial broad- and narrow-leaved weeds. One of the greatest problems in alfalfa seed production is dodder (*Cuscuta* sp.).

Weeds decrease the number of alfalfa plants and increase stand density, with a negative impact on the activity of pollinating insects and serious decrease in seed yield. Along with this, they make the harvest heavier and slower and increase the seed losses and seed processing expenses *(Erić et al., 1993)*.

The most significant pests in alfalfa seed production are alfalfa leaf beetle (*Phytodecta fornicata* Brüg.), alfalfa weevil (*Phytonomus variabilis* Hrbst.), alfalfa flower midge (*Contarinia medicaginis* Kief.), alfalfa seed weevil (Tychius flavus Beck.), alfalfa seed chalcid (*Bruchophagus rodii* Guss.), alfalfa (*Adelphocoris lineolatus* Goeze) and common plant bug (*Lygus pratensis* L.). In a hot and dry year of 2000, the attack of alfalfa plant bug decreased the seed yields for 20-90 % on about 600 ha in north Bačka and Banat *(Sekulić et al., 2005)*. Apart from insects, significant pests in alfalfa seed stand are common vole (*Microtus arvalis* Pall.) and common hamster (*Cricetus cricetus* L.).

In order to provide an alfalfa seed stand from weeds and pests it is needed to apply chemical measures. However, the applied pesticides must not significantly decrease the number of pollinating insects during the stage of flowering on alfalfa and that is possible by a proper determination of timing and choice of preparation.

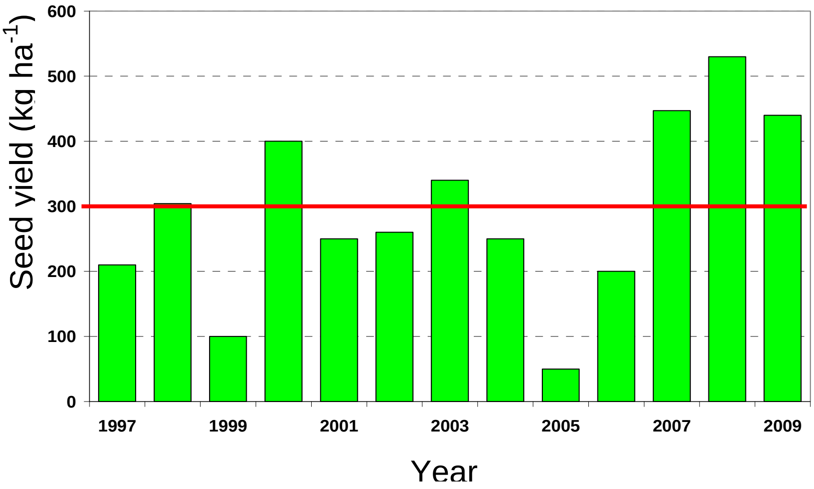

Figure 2. Alfalfa seed yield in the Vojvodina Province in 1997-2009 period

The average alfalfa seed yield in the agro-ecological conditions of Vojvodina is about 250 kg ha^{-1}, with a wide variation depending on the conditions in a year, that is, from 50 to 700 kg ha^{-1} *(Karagić et al., 2003; Mihailović et al., 2004). Mišković (1986)* points that the application of all necessary agronomy measures in average conditions results in seed yields of about 350-400 kg ha^{-1}, while the favourable environmental conditions may lead to the seed yields of 600-800 kg ha^{-1}. The average alfalfa seed yield in Vojvodina for 1997-2009 was 300 kg ha^{-1}, due to a very favourable weather conditions in the last three years (Figure 2).

The main characteristic of the alfalfa seed production in Serbia is a rather wide variation in yield depending on the weather conditions in a year. The seed yields in a very unfavoiurable year may be 10 times lower in comparison to those in a very favourable year *(Karagić et al., 2007)*. Mostly due to a prominent variation in seed yields, in Serbia there are no special alfalfa seed stands with wide row spacing, because the risk of losing profit is very great in unfavourable years. Instead of that, the seed production is carried out in the dual-purpose (forage-seed) stands. In favourable years, the first and the third cuts are used for forage production, while the second cut is used for seed production. In the years with excessive precipitations, farmers give up the seed production and profit only form the alfalfa forage production and thus decrease the risk of production. Specialized, wide-row stands, are used exceptionally for the production of elite seeds.

Red clover seed production. The production of the certified red clover seed in Serbia does not meet the demands for a long time. The widest production of the seed of this forage legume was during the 70s of the last century, when the production in Serbia answered almost all requirements *(Lugić et al., 1999). Bošnjak, (1996)* quotes that, according to the data by Agrozajednica, the group for the improvement of production, processing and market of herbage seeds, the red clover production in Serbia was only 100 t in 1994 while about 250 t in 1993.

According to the official data by the responsible service of the Ministry of Agriculture, Forestry and Water Management of the Republic of Serbia, the red clover seed production was carried out on 141 ha in 2007 and on only 87 ha in 2008 *(Boćanski, pers. comm.)*, showing a constant decrease in the certified red clover seed production in Serbia.

In central Serbia, the non-certified on-farm seed production is dominant. Red clover is established mainly by non-cultivar, non-processed and non-controlled seed. That is one of the main reasons for low seed yields. At the same time, forage yields are also low due to weeds and massive invasions by dodder.

Apart from this, a large portion of red clover seed is still imported, although such actions cannot be justified due to a low adaptability of foreign cultivars in our agro-ecological conditions, as well as lower forage production in comparison to Serbian cultivars *(Vasiljević et al., 2010)*.

The main reason for such conditions is the lack of interest in great seed producers in Vojvodina for the red clover seed production. As in alfalfa, red clover seed production is carried out in the dual-purpose stands. The most important producers of forage legume seeds are large farms with animal husbandry. There the first cut is used for forage production, while a smaller area in the second cut is used for seed production. However, red clover is used in a shorter period of time in comparison to alfalfa, has lower forage yields and is very susceptible to the drought during July and August. By all these reasons, large farms are not interested in red clover cultivation and that makes the red clover certified seed production significantly harder.

On the other side, the red clover seed production in Serbia is much more stable in comparison to alfalfa. In red clover, European honey bee is the most important pollinator. *Jevtić et al. (2002)* determined 76.2 pollinators in red clover in average, with 57.9 honey bees (75.98 %) and 18.3 other pollinators (24.02 %). Also, the research by *Jevtić et al. (2006)* determined 25.4 pollinators in red clover in average, with 21,3 honey bees (83.86 %) and 4.1 other pollinators (16.14 %).

Mainly due to the timely applied agronomic measures, in some locations in Vojvodina, high red clover seed yields were achieved in 2005 (515 kg ha^{-1}), as well as in 2008 (550 kg ha^{-1}) and 2009 (650 kg ha^{-1}) *(Vasiljević et al., 2010)*.

Stjepanović et al. (1990) and *Lugić et al. (1999)* point out that the potential of red clover for seed production is about 1000 kg ha^{-1}, but also that the yield of 500 kg ha^{-1} could be considered very good and realistic in the modern production conditions, with the yields of about 150-300 kg ha^{-1}as the most common in wide production.

Field pea seed production. The production of the certified filed pea seed in Serbia is carried out on an area of about 500 ha each year. The average field pea seed yield is 2500 kg ha^{-1} (Figure 3) (*Karagic et al., 2008*). The seed quantity produced on this area meets about 30 % of the requirements of the Serbian market. The remaining 70 % is sown with non-cultivar and non-certified seed of unknown origin. According to the agronomic classification of field pea by *Mikić et al. (2006)*, the field pea cultivars in Serbia may be divided into three groups. The first one are forage pea cultivars, used exclusively for the production of voluminous feed (green forage, hay, haylage, silage), the second one are dual-purpose cultivars (forage and/or grain production), and the third one are protein pea cultivars exclusively for dry grain production. Due to a rather prominent morphological variability between these three groups, the seed production is faced with diverse problems and requires three different approaches.

The most important morphological differences affecting seed production are related to the type of stem growth, plant height, number of pods per plant and 1000 seeds mass. Very significant are the differences in earliness, as well as in the requirements for mineral nutrition and soil quality, water management and susceptibility to diseases.

Forage pea cultivars have abundant aboveground biomass, indeterminate stem growth and a plant height of 160-180 cm *(Karagić et al., 2007)*. Their crude fibre content is lower in comparison to other types, making their stem slim and very susceptible to lodging *(Mihailović et al., 2003)*. By this reason, all cultivars in this group (in the type of the cultivar NS-Pionir) have poor standing ability, visible even in the beginning of flowering. The conditions for pod forming and filling the already formed seeds in such stands are extremely poor. That makes the moment of the beginning of stand lodging and its intensity the most limiting factors in achieving high seed yields in forage pea. The harvest of a lodged stand is significantly slower and harder, while the seed losses may be higher than 30 %. Along with this, such produced seeds have lower quality *(Karagić et al., 2009)*.

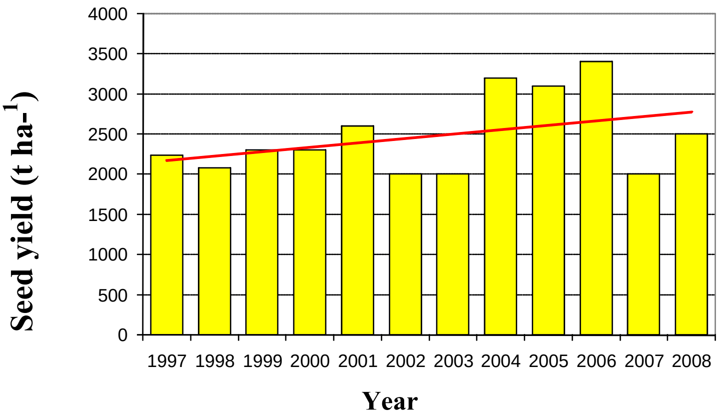

Figure 3. Seed yield of forage pea in Serbia for 1997-2008

In order to decrease lodging, that is, to decrease its negative effects, the seed production is carried out in selected regions, with a special care about the selected field. The highest seed yields are achieved in arid regions of Serbia, where the alfalfa seed production is carried out. Fields of a lower quality and lighter structure are selected. Fertilizers are applied only during the basic tillage and with complex fertilizers rich in phosphorus. Nitrogen fertilizers are usually avoided. Lower seed rates are applied (about 100 kg ha^{-1}) in order to achieve a stand density of 70-80 plants m^{-2}. All this should make a stand less abundant and thus decrease its lodging.

The advantage of such cultivars is their high tolerance to drought and lower susceptibility to diseases than in other cultivars. They can also be cultivated on slightly more acid soils. The damage by pea weevil (*Bruchus pisorum* L.) is the smallest in forage pea cultivars, but the susceptibility to aphids (*Acyrthosiphon pisum* (Harris) is equal to that in other cultivars. They have a very long growing period, with sowing in late September and harvest in mid-July. The average seed yield in forage pea cultivars in Serbia is 1800 kg ha^{-1}, while its price is about 30-40 % higher in comparison to protein pea.

Breeding protein pea has successfully solved the problem of lodging. Regarding the fact that these cultivars are aimed exclusively at grain production, the issue of forage yield and quality has become irrelevant *(Karagić et al., 2008)*. Thanks to this, there were developed the cultivars with determinate stem growth, with a height of 60-80 cm. The internode length is significantly decreased, the proportion of cellulose and lignin in the stem mechanical tissue is increased, the number of nodes in the lower part of stem is increased and in some the afila leaf type is introduced, all of which significantly decreases their susceptibility to lodging *(Mihailović et al, 2004a; Mikić et al, 2009)*.

However, the decrease in plant height led to a decrease in pod number per plant in these cultivars (Figure 4) *(Karagić et al., 2007b)*. By this reason, these cultivars require higher stand densities, that is, about 120 plants m^{-2}, in order to achieve a satisfactory seed yield. The 1000 seeds mass in protein pea cultivars is significantly higher in comparison to the other cultivars and is from 230 g to 300 g, making their seed rates from 260 to 320 kg ha^{-1}.

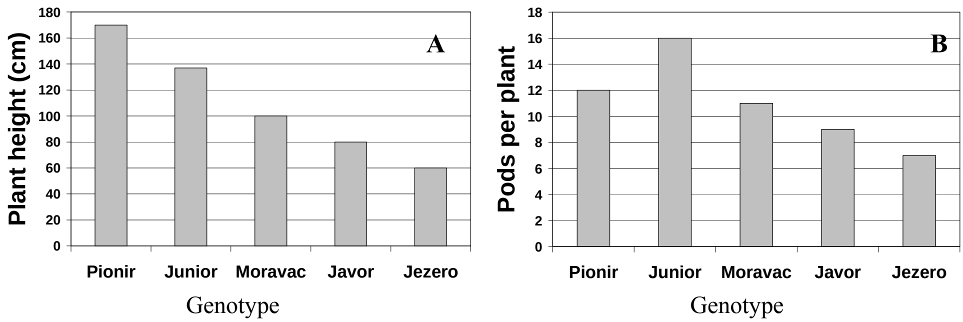

Figure 4. Plant height (A) and number of pods per plant (B) depending on genotype

The highest protein pea seed yields are achieved in humid regiosn of Serbia, such as fertile soils of South Bačka and Mačva. Complex fertilizers, such as 70-100 kg ha^{-1} P_2O_5 and 80-120 kg ha^{-1} K_2O, are applied in the basic tillage, while the nitrogen fertilizers are applied before sowing, with 45-50 kg ha^{-1} *(Karagić et al., 2009)*.

The use of chemical measures against weeds is an obligate agronomy measure in the seed production of all protein pea cultivars. Due to a less abundant aboveground biomass, a timely protection against weeds is of special importance. Since the existence of a large number of herbicides suitable for pea, weeds do not represent a significant problem. The most important problem in protein pea seed production is the protection against pests and diseases. The application of insecticides against pea weevil is carried out in the beginning of flowering, usually overlapping with a massive attack by leaf aphids. Protein pea cultivars are susceptible to diseases, especially powdery mildew (*Erysiphe poligoni* DC.) Due to a uniform flowering and a relatively short growing period of these cultivars, one application of organo-phosphoric insecticides and systemic fungicides is sufficient for the total stand protection.

The average protein pea seed yield in Serbia is 3500 kg ha^{-1}, with amplitude of 2500-5500 kg ha^{-1}.

The dual-purpose field pea cultivars are somewhere between the forage and the protein pea cultivars after their characteristics, their requirements for agronomy measures and their average seed yields.

Vetch seed production. Of three vetch species cultivated in Serbia, namely common (*Vicia sativa* L.), hairy (*Vicia villosa* Roth) and Hungarian (*Vicia pannonica* Crantz) vetches, the greatest commercial significance have both winter- and spring-sown common vetch. Serbian vetch cultivars have a great potential for high seed yield *(Mihailović et al., 2006)*. However, specific morphology causes extremely high losses in the seed production.

The main form of using vetch in Serbia is voluminous feed (green forage, hay, haylage) or green manure in orchards. Accordingly, breeding vetches is aimed at development of the cultivars with extremely high aboveground biomass yield, high proportion of leaf in the total forage yield and slim and slender stems of high digestibility *(Mihailović et al., 2006)*. Such morphology and the chemical composition of cell walls and inter-cellular space of the stem mechanical tissue implicate a significant susceptibility towards lodging *(Karagic et al., 2008)*. Regarding the fact that vetch is used for forage production in the srages from the beginning of flowering to the beginning of pod forming, lodging does not represent a significant problem.

On the contrary, lodging is the most important problem in the vetch seed production in the agro-ecological conditions in Serbia *(Karagić et al., 2003)*. The seed rate for vetch seed production in Serbia is quite high, 100-120 kg ha^{-1}. It has been adopted with no critical approach from the vetch forage production *(Karagić et al., 2004)*.

In forage production, high seed rate may be justified since a dense stand will produce elongated, thin, slender and highly digestible stems. Along with that, a large number of vetch plants quickly covers the ground and decreases the number of weeds in a stand. However, all these stem traits are not desirable in the vetch seed production *(Van de Wouw et al., 2003)*. A stand significantly lodges already in the stage of flowering, with lower parts of plants rotting, a small number of formed pods, slow seed filling and low seed yield and *(Iptas, 2002; Karagic et al., 2008)*.

The vetch seed producers try to delay the beginning of flowering and to reduce the intensity of lodging by growing vetch in mixtures with small grains as a supporting crop. A great number of authors recommend the growing vetch in mixtures with small grains *(Iptas, 2002; Karadag and Buyukburc, 2003; Jong 2006)*, while *Andrzejewska et al. (2006)* think that this is the only way to prevent lodging in vetch.

However, the growing vetch in mixtures with small grains imposes a number of technical and organizational limitations in the vetch seed production. The protection against the narrow-leafed weeds is completely disabled *(Karagic et al., 2008)*, while the maturation of small grains and vetch is not concurrent *(Iptas, 2002)*. Such way of growing demands additional requirements in seed processing, with vetch seed yields often lower in comparison to the seed production in pure stand *(Nikolaev and Kozmin, 1973)*. Along with this, the Serbian legislative does not include the term 'supporting crop', making small grains considered another species and a mixed stand for the vetch seed production discarded.

In Serbia, the lodging is attempted to be solved by regionalization, similarly to alfalfa and field pea. Along with that, by decreasing seed rates and increasing row spacing, stand density is decreased and the susceptibility towards lodging is also decreased, hopefully resulting in higher seed yields *(Karagić et al., 2010)*.

Apart from lodging, a great problem in vetch seed production is the protection from weeds. A very small number of herbicides are useful for common vetch, with insufficient efficiency against weeds (*Malidža, pers. comm.*). An especially great problem are broad-leafed weeds such as poppy (*Papaver rhoeas* L.), white goosefoot (*Chenopodium album* L.), creeping thistle (*Cirsium arvense* (L.) Scop.), hemp (*Cannabis sativa* L.) and weedy sunflower (*Helianthus annuus* L.).

In the agro-ecological conditions of Serbia, vetch seed yields significantly depend on the weather conditions. The average seed yields are 800 kg ha^{-1} in winter- and 1500 kg ha^{-1} in spring-sown common vetch.

Conclusion

The main characteristic of the seed production of all forage legumes, with an exception of protein pea, in Serbia is an extremely wide variation of yield, depending on weather conditions in a specific year.

The agro-ecological conditions of Serbia may be regarded as moderately favourable for the seed production of the majority of forage legumes. The main limiting factor for high seed yields are high sums and non-favourable distribution of precipitations during growing period. Growing technology of alfalfa and field pea seed production is on a relatively high level. By more intensive research it is needed to improve the growing technology of vetch and red clover seed production. The average seed yields of alfalfa and red clover are 250 kg ha^{-1}, of field pea 2500 kg ha^{-1}, and vetch 1200 kg ha^{-1}.

Semenarstvo krmnih leguminoza U SRBIJI

Đ. Karagić, G. Jevtić , D. Terzić

Rezime

Najznačajnije krmne leguminoze u Srbiji su lucerka, crvena detelina, stočni grašak i grahorica. Površine na kojima se gaje krmne leguminoze procenjuju se na oko 290.000 ha, što predstavlja 8% obradivog zemljišta Srbije. Dominantan način iskorišćavanja krmnih leguminoza u Srbiji je proizvodnja kabaste stočne hrane (zelena krma, seno, senaža, silaža), sa izuzetkom proteinskog graška koji se koristi za proizvodnju zrna. Stoga se oplemenjivanjem stvaraju sorte koje se odlikuju izuzetno visokim prinosom nadzemne mase, velikim učešćem lista u ukupnom prinosu, tankom i nežnom stabljikom visoke svarljivosti. Ovakva morfološka građa biljaka implicira značajnu osetljivost prema poleganju, što u velikoj meri otežava proizvodnju semena. Zbog toga je osnovna karakteristika proizvodnje semena krmnih leguminoza u Srbiji, sa izuzetkom proteinskog graška, izuzetno veliko variranje prinosa u zavisnosti od vremenskih uslova godine. Klima Srbije ocenjuje se kao umereno povoljna za proizvodnju semena krmnih leguminoza. Prosečan prinos semena lucerke i crvene deteline iznosi 250 kg ha^{-1}, stočnog graška 2500 kg ha^{-1}, a grahorice 1200 kg ha^{-1}.

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SEED PRODUCTION IN EUROPE WITH SPECIAL FOCUS ON DENMARK

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Communication

Abstract: There are three main production areas for grass and clover seed in the world. These are North America with Villamette Valley in Oregon USA and Peace River Valley in Canada as the biggest areas, EU with Denmark as the most important country and New Zealand. In EU a larger part of the seed production has moved to Denmark over the years due to the favourable mild coastal climate conditions, know-how by growers and the industry and competitive production prices. In most cases the seed production of grass and clovers are in competition with the production of cereals. The need for long term planning in grass and clover seed production makes this more vulnerable to fluctuating prices on cereals. The tendency is that the prices on the competing products and grass and clover seed are not in line at the same time. Seed production in Denmark has been developed over a long period of time and the result is very dedicated seed growers using effective management in the field, combined with large capacity processing lines at the seed companies.

Key words: Seed production areas, climate influence, competition to cereals and prices, Seed production technique in Denmark

Introduction

The intension of the paper is to give an overall view of where and how grass seed is produced. Production has moved and is still moving towards the most effective and competitive production areas. The main criterion here is the competition to cereals. The paper is describing some of the factors behind this movement.

At the end the paper describes more in detail the conditions for seed production in Denmark.

Global seed production

The main global seed production areas of cool season grasses are North America with Villamette Valley in Oregon USA and Peace River Valley in Canada as the biggest areas, EU with Denmark as the biggest country and New Zealand.

In normal years it is estimated that the world seed market consumption is approx. 600,000 tons of clover and grass seed. See illustration 1.

Illustration 1. Main global production areas, cool season grasses

European seed production

In EU 27 the total area of grass and clover seed is around 270,000-305,000 ha per year with an average yield of around 750 kg/ha. This equals 200-230,000 tons. See table 1.

Table 1. EU 27, Production acreage in ha

Species	2007	2008	2009*
Lolium perenne	69.952	67.094	68.208
Festuca rubra	37.353	30.305	33.180
Poa pratensis	9.911	8.721	9.240
Festuca arundinacea	13.569	15.264	17.117
Festuca pratensis	13.516	12.940	10.631
Dactylis glomerata	9.939	11.524	11.930
Lolium multiflorum(Westerwold. + Italicum)	33.897	30.039	35.168
Phleum pratensis	13.962	18.551	17.719
Others	21.789	13.004	11.661
Total Grasses	223.888	207.442	214.854
Medicago sativa L.	42.333	37.287	41.068
Trifolium/clover crops	35.930	31.501	34.382
Total grasses/clover/alfalfa	302.151	276.230	290.304

* Not final figures

In Europe Denmark has gained market shares in the production of grass and clover seed which can be seen in table 2.

Table 2. Production acreage in ha in the EU 27 countries

Country	2007	%	2008	%	2009*	%
Denmark	74.186	33.1	71.285	34.5	79.803	37.1
Germany	27.095	12.1	27.825	13.4	28.869	13.4
France	21.395	9.6	16.958	8.2	17.325	8.1
The Netherlands	19.816	8.9	15.887	7.7	17.661	8.2
Czech Republic	19.619	8.8	16.749	8.1	14.911	6.9
Poland	12.657	5.7	11.312	5.5	11.312	5.3
Sweden	8.938	4.0	9.224	4.4	9.082	4.2
Finland	8.720	3.9	8.854	4.3	8.854	4.1
United Kingdom	5.916	2.6	5.203	2.5	4.329	2.0
Spain	4.581	2.0	4.524	2.2	4.524	2.1
Others	20.966	9.4	19.621	9.5	18.184	8.5
Total Grasses	223.888	100	207.442	100	214.854	100

* Not final figures

The increase has taken place over a long span of years which as it shows in illustration 2.

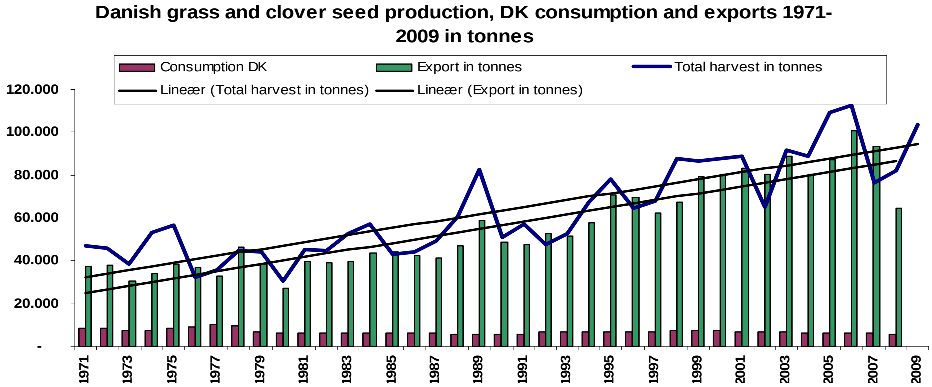

Illustration 2. Danish grass and clover seed production

There are more reasons for this, but the main one is the climatic conditions. Being surrounded by water Denmark is favoured with relatively mild autumns which allow the seed plants to develop a very good basic for the seed production next year. The relatively mild winters makes winterkill a rare thing and the mild spring again favours the development of plants and seed heads. The rather windy weather during spring and early summer gives a good pollination and normally there are sufficient dry weather for a safe harvest with high quality and germination. See illustration 3.

Illustration 3. Map of Europe

The long days combined with the mild climates are also a deciding factor in the optimal development of seed heads and development of the seeds. This combination of climatic conditions is rarely found in other parts of the world.

Moving seed production away for the coast near climate has a heavy negative influence on the seed yield of many of the species. Even in Denmark a difference in distance to the sea of 50 km can mean a drop in seed yield of 20%.

Contrary to grasses winter wheat does not respond to these conditions. If a winter wheat field is treated well and grown on good soil the yield will be high no matter where in Europe it is grown.

This favourable climate in Denmark means that the tradition of growing seed has been developed over many years and there are a high number of very well skilled seed producers. The high quantities produced have also made it possible to develop a very modern industry with needed development in processing and packaging facilities that are spread all over the country.

Main competition is cereals

Grass seed production is a more long term production and is not as flexible as the cereal production. Management is also more demanding compared to growing cereals. Therefore there is a need for a premium in profit to the farmer by growing grass seed.

At the same time fluctuations in yield within the grass seed production can be quite high which makes the long term planning more difficult and courses more price fluctuations.

The grass seed market is a very global market and no production sites are isolated from global trends. The global market makes the fluctuations in currencies very important and especially here the rate of the USD is important. In the last two years the rate of the USD compared to EURO has changed quite dramatically which can be seen in illustration 4.

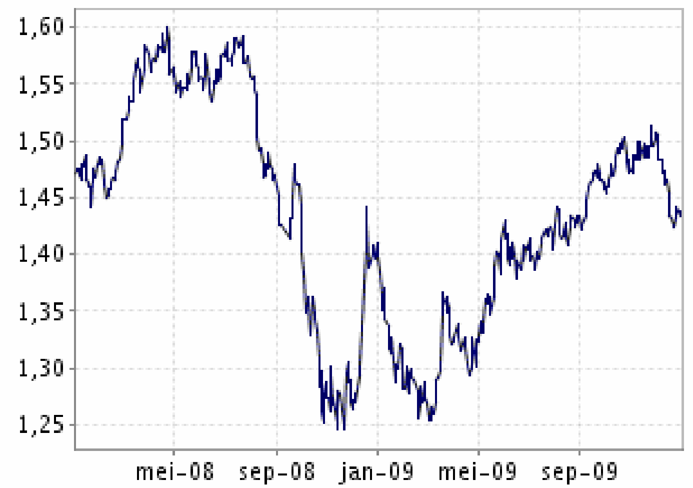

Illustration 4. USD >< EURO

Differences within EU

If we look at the three production areas Denmark, Holland and France it shows that the average yield of winter wheat is different. See illustration 7.

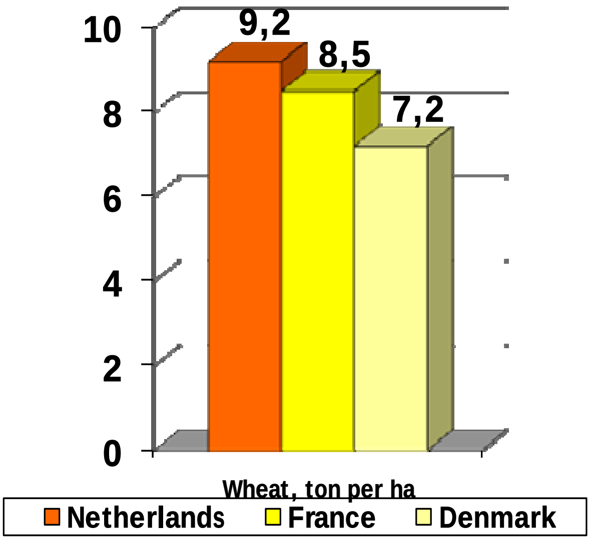

Illustration 5. Level of wheat yield

With the high seed yields in Denmark this means that Danish farmers have an comparable advantage in producing grass and clover seed compared to Dutch and French as the cereal yield are higher in these countries.

Seed production in Denmark

More or less all fields for grass and clover seed in Denmark are established with a cover crop. In most cases the cover crop is a cereal like barley or winter wheat. Protein peas are also used. This makes it less risky as the first year crop as cover crop is more secure compared to grass and clover. At the same time this allows for a longer period for establishment and development of the grass or clover crop.

In illustration 6 the small plants of Perennial Ryegrass can be seen in between the rows of spring barley.

Illustration 6. ***Lolium perenne*** **sown in spring barley**

In illustration 7 the development of Red Fescue can be seen after the removal of the cover crop spring barley.

Illustration 7. ***Lolium perenne*** **after harvest of cereal cover crop**

All the grasses produced, with the exception of Smooth–Stalked Meadow-Grass, are wind pollinated. During windy days and with dry cloudy weather a strong pollination can be seen as the photo in illustration 8 shows.

Illustration 8. Red Fescue pollination

If ever possible the fields are harvested directly without swathing. This ensures the optimal development of the seed weight, thus the total yield. Working with direct harvest means that most seed is harvested with a moisture percentage of around 20% or more. The seed needs to be conditioned and dried immediately and all Danish seed grower are able to handle this on the farm before delivery to the seed company. Often the seed is stored on the farm until cleaning takes place at the seed company.

Illustration 9 and 10 shows swathing and direct harvest.

Illustration 9. Swathing

Illustration 10. Straight combining

All seed is produced on contract

Only certified seed can be produced within the EU and all seed is produced on a contract basis. The contract stipulates the conditions.

A speciality by DLF-TRIFOLIUM is that each variety is paid according the seed yield. This means that varieties with a lower seed yield will need a premium price in the market. As the market in general is not willing to pay very high premiums, low seed yielders will disappear regardless of the quality.

Proizvodnja semena u Evropi sa posebnim osvrtom na Dansku

M. T. Jensen

Rezime

Postoje tri glavna područja u svetu u kojima se proizvodi seme trava i detelina. To su Severna Amerika sa Viljamete Dolinom u Oregonu i Dolinom reke Pis u Kanadi kao najveća, EU sa Danskom kao najvažnija i Novi Zeland. U EU, veći deo produkcije semena je tokom godina preseljen u Dansku zbog povoljnih, blagih, primorskih klimatskih uslova, veštine odgajivača, industrije i zbog povoljne cene proizvodnje. U većini slučajeva, proizvodnja trava i deteline je u sukobu sa proizvodnjom žitarica. Potreba za planiranjem na duže u proizvodnji semena trava i dateline, gini je nestabilnom u odnosu na promenu cena u proizvodnji žitarica. Postoji tendencija da cene konkurentskih produkcija ne budu u nivou cena proizvodnje trava i deteline. Proizvodnja semena u Danskoj se razvijala tokom dugog perioda i rezultat je posvećenih uzgajivača semena, kombinacijom doradnih linija velikog kapaciteta i rukovođenja poljskim dobrima.

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SEED PRODUCTION OF PERENNIAL FORAGE GRASSES IN SERBIA

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Abstract: Serbian grass seed production is low, with unsatisfactory yield. Despite favourable ecological conditions for seed production, due to low price and insufficient application of contemporary technology, it does not cover local seed demand, and large seed amounts are imported. Scientific researches indicated that the high seed yield and quality could be obtained with optimal establishment on suitable vegetation area, using fertilizers in accordance with soil and plant demands, and by mechanized harvest in the optimal time.

Key words: seed production, perennial forage grasses, yield, quality

Introduction

Grass seed plays an important role in production chain of safe and high quality animal food, primarily in sowing grasslands establishment in hilly-mountainous areas. Approximately 66% of total Serbian area is situated in the hilly-mountainous part. The extensive agricultural production, which represents the main or additional source of income for most inhabitants, is applied in these areas. It is necessary to provide cheap, high quality forage bases in order to make way for sustainable agriculture, especially animal production. A higher level of production intensification of animal feed, especially at higher altitude, is achieved on sown grasslands. An appropriate amount of grass seed is required for establishing wider areas of sown grasslands. Grasses provide highly productive and cheap animal feed of high quality. Costs of animal diet per kilo of gain are the lowest with green forage and the highest with concentrated food.

The development of grass seed production in Serbia dates back to the Second World War and the first scientific papers on grass seed growing date back to 1950s, which is in accordance with increased interest in providing its own resources for human and animal feed. The first scientific article of *Mijatović (1956)* investigated the technology of seed production of the two important grass types, *Lolium perenne* and *Arrhenatherum elatius*. Immediately before that, the first textbooks and manuals *(Đorđević, 1951; Kvakan, 1952)* were published. The specialized scientific institutions were established for researches in this scientific area. (1961, Institute for Forage Crops, Krusevac).

Mijatović (1956) pointed out in the introduction of the paper that concerning grass seed production in Serbia the needs are great, but the production is small and insufficient. The situation has not changed significantly since then. Grass seed production increased until 1960s, so Italian ryegrass was even exported, but after that it started decreasing rapidly. The maximum of seed production and consumption was recorded in 1970s and 1980s, and it can be divided into two periods: the first one (1971-1978) when mainly imported seed varieties and domestic seed from populations were used and the second one (1978-1989) when demand and supply were mainly from domestic varieties *(Miladinović et al., 1989)*. The most consumed ones were imported varieties which were on Serbian list of new varieties, whereas in domestic production the selections of domestic institutes were present. Serbia approximately spent in those years 600t of grass seed which was about 30% of the then SFRY consumption. The grass seed production varied from 480 t (1971) to 17.4 (1986), and the cause to this low and irregular grass seed production was cheap import of seed from East Europe. As a result, the low seed prices offered to our producers, the deficit of needed equipment for production, the deficit of staff and unfavourable conditions of granting credit ensued.. Serbia produced more than 50% of total production in SFRY, 147 tons in average.

Perennial grass species are mostly sown at hilly and mountainous area on poor soil *(Lazarević, 2007)*, where forage production is variable and unpredictable. The greatest decrease was noticed in cattle production during the last fifteen years at higher altitudes, which had inevitably negative effect on forage grass seed use, as well as on the seed production. Grass seed production farms in Serbia are more diversified and smaller than farms in the European countries. According to *Nikitović and Radenović (1996)* local seed production covered only 30% of total seed demand, and rest of seed needed was imported. Some species, as *Poa pratensis*, *Agrostis alba*, *Bromus inermis* and *Festuca ovina* was completely compensated by the import, i.e. domestic seeds were not produced.

Therefore, nowadays the perennial grass seed production in Serbia, from the point of view of country need is insignificant although there are ideal agro-ecological conditions for production. Grass seed was imported mostly from Poland and former German Democratic Republic.

The highest harvested seed amounts in EU in the year of 2000 were: ryegrasses (perennial - 72.6 thousand tons, Italian - 28.9 thousand tons, hybrid - 3.8 thousand tons) and fescues (red - 40.5 thousand tons, meadow - 5.3, sheep - 3 and tall - 6.9 thousand tons). There were significant amounts of Kentucky bluegrass seed (8.4 thousand tons), cocksfoot (6 thousand tons) and timothy (4 thousand tons). Leading countries with regard to seed production were Denmark, Germany and Holland *(http://ec.europa.eu)*. There were not significant changes by expansion of EU, ryegrass seed production was predominant in the total production (in the year of 2006 - 57 thousand tons of perennial and 37.6 thousand tons of Italian ryegrass, and the third one was red fescue with 13 thousand tons).

Over 60% of the world and 90% of the USA supply of temperate forage and turfgrass seed are produced on approximately 187 000 ha in northwest region USA, in the Oregon's Willamette Valley (*Steiner et al. 2006*). Mild and moist winters with dry summers favouring seed development and harvest make the Valley an ideal place to produce high quality seed.

The produced grass seed yield is lower than potential one, but it is possible to make the difference less significant step by step, using better agro-mechanization during the development of seed crops. The seed yield in the world is now about 50% higher than 40-50 years earlier, but the increase depends on the grass species *(Nordestgaard and Andersen, 1991)*. The higher yield increase was achieved in small-seeded grasses (for example, *Festuca rubra* and *Poa pratensis*), whereas the lower one in ryegrasses, which are easier for growing.

The science and practice should address the serious task of providing sufficient amounts of high quality grass seed. Previous technology of grass seed production is not complete and standardised, because there are different opinions and criteria, especially regarding mode and density of sowing, purposes and grass crops fertilising, intended for seed production.

Natural conditions and grass seed production

Climatic conditions, temperature and precipitation, played a major role in determining the level of management practice impact on grass seed yield during production. The grass seed production has specific demands regarding natural conditions, of which the most important are weather conditions, including amount and distribution of precipitation. Taking into account low demand for high-quality soil in comparison with legume seed production, grass seed production could be organized at poor soil, using obligatory application of fertilisers. Grasses are less sensitive than perennial forage legumes to coldness and temperature minimum, which is for most of them 0^0C *(Griffith and Chastain, 1997)*. Grass seed crops in Serbia have a critical temperature period during winter, when low temperatures together with black frost can cause damage and poorer crop growth. High temperatures in summer period are favourable for maturing and harvesting of most grown seed grasses in our conditions.

Serbia has a mild continental climate with cold winters and warm summers *(www.hidmet.sr.gov.rs)*. The north of Serbia and the upland regions have a continental climate, with the typical cold winters and hot summers. The summer months of June to August offer a hot climate and a small amount of rainfall.

Climate of Serbia can be described as moderate-continental with more or less pronounced local characteristics. Spatial distribution of climate parameters are caused by geographic location, relief and local influence as a result of combination of relief, distribution of air pressure of major scale, terrain exposition, presence of river systems, vegetation, urbanisation etc. Average annual air temperature for the period 1961-1990 for the area with the altitude up to 300 m is 10.9°C. The areas with the altitudes of 300 to 500 m have average annual temperature of around 10.0°C, and over 1000 m of altitude around 6.0°C. Absolute temperature maximum in the period 1961-1990 were measured in July and ranged in the interval from 37.1 to 42.3°C in lower regions, while in mountainous areas it is ranged from –35.6 to –20.6°C.

Annual precipitation sums increase averagely with altitude. In lower regions annual precipitation is ranged from 540 to 820 mm. Areas with the altitude over 1000 m have in average 700 to 1000 mm of precipitation and some mountainous summits in the south-western part of Serbia have heavier precipitation up to 1500 mm. A major part of Serbia has continental precipitation regime with higher quantities in warmer part of the year, except for south-western parts where the highest precipitation is measured in autumn. June is the rainiest with the average of 12 to 13 % of total annual precipitation. February and October have the lowest amount of precipitation. Snow cover occurrence is characteristic for colder part of the year, from November to March, and the majority of days with snow cover are in January. Grass seed crops must be planted on time to become established before the onset of severe cold, or hot and dry weather conditions. In most cases, grassland sowing for seed production is in the same time as used for forage production. The most relevant factor which influences grass seed production is distribution and amount of precipitation *(Chastain, 2000)*. Critical periods for grass seed crop, when abundant precipitation is needed, are the times of sowing and during the intensive growth in spring, when generative organs are formed. Considerable amounts of precipitation in the phase of maturing, followed by the wind, can cause lodging, and before the harvest they can cause fall and losses at harvesting. The perennial forage grass seed production mostly takes place in arid areas in the eastern part of Serbia. This area is unfavourable for more intensive crop cultures, but it is favourable for grass seed harvesting which is done mostly during June.

Grass species grown

The most important grass species in Serbia are *Lolium italicum* A.Braun., *L. perenne* L., *Arrhenatherum elatius* L., *Festuca rubra* L., *Dactylis glomerata* L., *Festuca arundinacea* Schreb., *Festuca pratensis* Huds., *Poa pratensis* L., *Phleum pratense* L. and *Agrostis alba* L. A Serbian list of registered varieties comprises 32 grass varieties (of 12 species) - 9 domesticated, 12 domestic and 11 imported (*www.sorte.minpolj.gov.rs*). Recently, 7 varieties out of 6 grass species were obliterated, for overdue of 15 years until they had been registered. The most represented species is *Lolium perenne* with 6 varieties, following *Arrhenatherum elatius*, *Festuca pratensis* and *rubra* with 4 varieties, *Lolium italicum* and *Phleum pratense* with 3 registered varieties; there are species with 2 varieties: *Dactylis glomerata*, *Lolium hybridum*, and some species with one variety: *Agrostis alba*, *Bromus inermis*, *Festuca arundinacea* and *Poa pratensis*.

Seed production management

The major factors of the slow spreading of grass seed production in our country are low and unstable yields due to an inadequate cultivation technology *(Vučković et al. 2003)*. Preparation is essential to provide an environment favourable for immediate seed germination and growth.

Seed crop establishment

The spatial arrangement of plants at seeding has been shown to have a strong influence on the yield and persistency of seed stands of perennial grasses, but such differences tend to disappear during the later stages of development *(Simić et al., 2009)*. Very dense crop stand and/or inadequate environmental conditions can play an important role in maximizing grass seed productivity. Recommended seeding rates and row spacing for important Serbian perennial grasses vary considerably, indicating that at very low seeding rates, differences could affect early season yields, while at high rates seedling competition for resources may actually reduce yields. According to *Tešić-Jovanović (1968b)* lower cocksfoot seeding rates shown advantage for seed production, and the best sowing time was in autumn by using the seeding rates of 5-10 kg ha^{-1}. Later sowing would require an increase of seed for sowing.

Tešić-Jovanović (1970) showed that the sowing time affected the seed yield in the following years of cocksfoot growth. The highest seed yield during the three years was obtained with sowing date 1March. The greatest annual seed yield (804 kg ha^{-1}) was achieved with the seeding rate of 5 kg ha^{-1}. It was found out that for the central part of Serbia the best spring sowing time of cocksfoot for seed production was the sowing period from 1st to 10th of March by using seed rate of 5 kg ha^{-1}. Later sowing requires an increase of seed for spring sowing of cocksfoot. *Vučković et al. (1998a)* achieved the highest perennial ryegrass seed yield with plants grown in 20 cm spaced rows using 20 kg ha^{-1} of seeds (751 kg ha^{-1} and 560 kg ha^{-1} during two years)

In the agroecological conditions of the eastern Serbia, set of trials conducted in order to determine the optimal management practice for seed production of important forage grasses *(Stanisavljević et al. 2007, 2008, 2009a, 2009b, 2010b)*. Researches focused on vegetation area and fertilizing practices. Thus, the seeding rate and row spacing (25 kg ha^{-1} with spacing 12,5 cm and 12,5 kg ha^{-1} with spacing 25 cm) were studied in order to determine the effect on yield components, yield and quality of cocksfoot seed (Table 1). It has been found that the crop established with a smaller seeding rate and higher row distance increased the cocksfoot grass seed yield, while the examined treatments did not show influence on seed quality *(Stanisavljević et al., 2010b)*. During 2 years period, the seeding rate and row spacing (30 kg ha^{-1} with spacing 12,5 cm and 15 kg ha^{-1} with spacing 25 cm) with different amounts and application time of NPK fertilizers were studied on the seed yield of tall fescue (tab. 1). Optimal seeding rate and row spacing was 15 kg ha^{-1} with spacing 25 cm, with spring NPK application of 90:60:60 kg ha^{-1} *(Stanisavljević et al., 2009b)*. Also, two fescues (tall and meadow) were examined in order to determine their panicle branches influence on seed yield *(Stanisavljević et al., 2007)*. Lower and intermediate panicle branches affected seed yield much more than upper branches in both species.

Some recent studies tested Italian ryegrass for seed under the agro-ecological conditions of Western Serbia *(Simić et al., 2005, 2006, 2007, 2008, 2009a)*. Italian ryegrass cv. Tetraflorum was established with three row spacing: 20, 40 and 60 cm, using four seeding rates: 5, 10, 15 and 20 kg ha^{-1} *(Simić et al., 2009a)*. The inter-row spacing of 40 cm was found to be the least uncertain for seed production. While the increase in seed rates (15-20 kg ha^{-1}) in stand establishment provided higher seed yield in years with unfavourable weather conditions, the seed rate had either no impact on seed yield, or decreased the yield of seed as a result of ryegrass lodging following seed shedding in the years with favourable weather conditions (Table 1).

Fertilizing Many authors tried to find optimal rates and ratios of NPK fertilizers for grass seed production in Serbia. Thus *Mladenović and Tešić-Jovanović (1983b)*, investigated three grass species: cocksfoot, red fescue and timothy and seed production in tested conditions (Vlasina, 1200 m a.s.l), in order to find optimal rates of NPK fertilizers. For cocksfoot optimal rate was 120:80:80 kg ha^{-1} NPK, for red fescue 80:80:80 kg ha^{-1} NPK and for timothy 90:50:50 kg ha^{-1} NPK.

Mladenović and Tešić-Jovanović (1983c) studied the effect of the application of NPK fertilizers in autumn and in spring and split rates (50% in autumn and 50% in spring) on the grass seed yield. Using spring fertilization the highest seed yield was obtained by *Phleum pratense* (330 kg ha^{-1}) and *Agrostis alba* (350 kg ha^{-1}). Autumn fertilization gave the highest seed yield in *Bromus inermis* (318 kg ha^{-1}), *Festuca rubra* (590 kg ha^{-1}) and *Poa pratensis* (263 kg ha^{-1}). Split fertilization gave the highest seed yield in *Dactylis glomerata* (414 kg ha^{-1}), *Arrhenatherum elatius* (610 kg ha^{-1}), *Festuca pratensis* (520 kg ha^{-1}), *Festuca arundinacea* (810 kg ha^{-1}) and *Trisetum flavescens* (286 kg ha^{-1}). The highest economical effect in the cocksfoot seed production, on the poor soil, has shown potassium and smaller amount of the phosphoric and NPK - fertilizers *(Tešić-Jovanović, 1969)*.

Results obtained by *Tešić-Jovanović (1968c)* show that fertilization with nitrogen in the amount of 50 kg ha^{-1} nutrient increased 100% the seed yield of cocksfoot, compared with nonfertilized standard. Nitrogen application at postharvest period increased meadow fescue seed yield in the next harvest year *(*Mladenović and Tešić-Jovanović 1978).

Mladenović and Tešić-Jovanović (1985) concluded by trials on poor soil that the optimal NPK fertiliser ratio was 120:80:80 kg ha^{-1} for high seed production of meadow fescue (seed yield 694 kg ha^{-1}) and tall oatgrass (751 kg ha^{-1}), while timothy reached 371 kg ha^{-1} with 60:60:60 kg ha^{-1} NPK. Stable and economical seed yield of tall oatgrass and meadow fescue could be obtained by application of 80:40:40 kg ha^{-1} NPK.

Simic et al. (2005) noticed that spring N-application did not influence the seed yield of Italian ryegrass and concluded that high seed production could be obtained with minimum or without spring N-application on good quality soils.

Irrigation Possibility to produce more seed, in Italian ryegrass in two harvests, is studied in trials with irrigation applied *(Tomić and Sokolović, 2000)*. Both diploid and tetraploid cultivars of *Lolium italicum* showed high annual seed yield in two harvests (577 kg ha^{-1} and 740 kg ha^{-1}, respectively), but seed yield in second harvest was not profitable (between 130 and 170 kg ha^{-1}). In the same investigation different application times of fertilizers were investigated and there were not noticed significant differences in seed yield of Italian ryegrass.

Mowing frequency *Tešić-Jovanović (1968a)* did not recommend utilizing the cocksfoot for seed production with more than two mowings in the first year of its growth, because of unfavourable influence on the seed production in the succeeding year.

Herbicide application Grass seed fields should be free of noxious weeds. In freely tillering grasses the main merit of a dense sowing is a weed-control measure, since the effect of tillering on sward development will increase competition, leading to plant death and consequently, the maintenance of a smaller population of plants *(Jewiss, 1972)*. Weed control is very important for successful seed production. Many weed species have good conditions for growth in inter-row space of established grass seed crop. *Jovanović (1975)* examined hormone herbicides application on Italian ryegrass seed crop and concluded that combined herbicides with high efficiency controlled broad-leaved weeds. *Savić (1985)* obtained on the Šara Mountain (1600 m a.s.l.) red fescue seed yield of 422 kg ha^{-1} with high total germination (85.7%) by reduced tilling (harrowing and herbicide application). The results achieved by testing efficiency of herbicides in cocksfoot seed crop cv. K-rana suggested the highest weed reduction (Ke=97%) by combined hormonal herbicide *(Tomić et al., 1998)*. A recent study *(Simić et al., 2008)* tested hypothesis that *Galium aparine* in Italian ryegrass seed field decreased growth of established species and consequently decreased seed yield. The experiment was conducted with four establishing methods: narrow and broad row spacings (20 and 60 cm) with low and high seeding rates (5 and 20 kg ha^{-1}). Control treatments were sprayed with herbicide Starane-250 to eradicate *G. aparine.* Italian ryegrass was affected by *G. aparine* in three of four establishing methods. The experiment showed that seed yield reached 973 kg ha^{-1} at control treatments and 956 kg ha^{-1} with *G. aparine.* Italian ryegrass straw yield was in a linear correlation with seed yield.

New challenges Perennial grasses grown for seed are prone to lodging at the high nitrogen fertility rates used to maximize seed production. Lodging of the crop can cause increased problems from disease and can decrease pollination, seed set and seed yield. *Simić et al. (2009b)* conducted trials with Italian ryegrass for seed production and plant growth regulator was used to prevent spring overgrowing, especially due to nitrogen application. Plant growth regulator decreased plant length, while other seed production parameters were not affected significantly at Italian ryegrass.

Seed yield

Mladenović and Tešić-Jovanović (1983a), according to the productive potential of grasses in Serbia (Kruševac) classified them in three groups: production potential of four years included *Poa pratensis* (average yield 375 kg ha^{-1}) and *Agrostis alba* (476 kg ha^{-1}), the second group included grasses with five-year potential – cocksfoot (482 kg ha^{-1}), meadow fescue (700 kg ha^{-1}) and timothy (432 kg ha^{-1}), and the third group included grasses with six-year production potential - tall oatgrass (581 kg ha^{-1}), tall fescue (751 kg ha^{-1}) and red fescue (728 kg ha^{-1}). The highest seed yield in Serbia achieved by Italian ryegrass, which could be reached at microplots more than 2 000 kg ha^{-1} in favourable conditions of Western Serbia *(Simić and Vučković, 2006)*. All forage grass species are characterised with a high variability of agronomic traits *(Tomić, 1997; Tomić et al., 1999a; Sokolović et al., 2002; 2004)* and seed yield components *(Sokolović et al., 2006, 2007; Stanisavljevic et al. 2010a)*, which provides them with great adaptability to different environmental conditions.

Table 1. Average seed yield of perennial grasses in trials *(Stanisavljević et al., 2009b, 2010b; Simić et al., 2009a)*

	Seed yield (kgha^{-1})		
Vegetation area	Cocksfoot	Tall fescue	Italian ryegrass
seeding rate 25 kg ha^{-1}, spacing 12.5cm	376	526	-
seeding rate 12.5 kg ha^{-1}, spacing 25 cm	414	632	-
seeding rate 15 kg ha^{-1}, spacing 40cm	-	-	1007

Seed quality

Seed quality was covered by minor part of forage grass seed researches in Serbia. *Jovanović and Tešić-Jovanović (1972)* examined seed germination of the most important grass species in Serbia: red, meadow and tall fescue, cocksfoot, timothy, smooth brome, tall oatgrass, perennial and Italian ryegrass. All seed showed high quality by germination rate and total germination, with differences during ripening characterised for species. The lowest germination has smooth brome (bellow 80%) and the highest one – Italian ryegrass, approximately 100%.

Đorđević and Tešić-Jovanović (1969) investigated cocksfoot germination energy and total seed germination by direct exposure to the sun, in different time intervals. According to their results, it would be advantageous to expose the seed to the sun's radiation at least for 9 hours, for the improvement of cocksfoot seed quality.

According to *Vučković et al. (1998a)* row spacing and sowing rate had no effect on percentage of perennial ryegrass seed germination, while differences between production years much more influenced ryegrass seed quality. High germination was achieved in both experimental years (>90%).

Recent researches were focused on actual problems connected with seed quality, important for seed production and trading. Thus, *Stanisavljević et al. (2007)* noticed that lower and intermediate panicle branches influenced seed quality of tall and meadow fescue much more than upper branches. Forage grass seed ripens in Serbia mostly in June, when it is harvested. There is a great percent of seed dormancy in that time and germination is reduced. There can be some problems during after-ripening period concerning grass seed germination prior to autumn sowing (August – September), depending on grass species. Grass seed dormancy may play a positive role in natural meadow associations. *Stanisavljevic et al. (2010a)* examined the effect of the dry after-ripening period on seed germination and the seedling growth in meadow (*Festuca pratensis* Huds.), tall (*Festuca arundinacea* Schreb.) and red fescue (*Festuca rubra* L.), as a prerequisite of grassland establishment. Seeds were after-ripened for 0, 30, 60, 90, 120, 150, 180, 210 and 240 days after harvest (DAH). The final count in all three species amounted to 60-65% immediately after harvest, suggesting a medium level of embryonic dormancy. Obtained data can serve for the determination of a proper sowing period (autumn/spring) of tested fescue species under agro-ecological conditions of Serbia. According to research results, study suggested that early fall is the best sowing period for red and tall fescue while early spring is the best for meadow fescue in Serbia.

Nevertheless, during five-year period viability and germination of grass seed decreased *(Tomić et al., 1999b)*. It can be concluded that storage keeping period should not be too long.

Italian ryegrass seed quality was not changed in seed crops, under different seeding rates, inter-row spacing or herbicide application *(Simić et al. 2005, 2006, 2007, 2009a, 2009b)*, but produced seed from the first harvest cut was mostly of an excellent quality, with high germination rate and total germination, whereas seed produced from the second harvest cut had lower germination and TSW, of unsatisfactory healthy state and low yield *(Simić et al., 2007)*.

Conclusions

Serbia has good agro-ecological conditions for perennial grass seed production. On the contrary, seed production does not cover local seed demands. Results and experience from research trials and studies should be helpful in restoring seed production of the most important grasses in Serbia: ryegrasses, fescues, cocksfoot, tall oatgrass and timothy. In many ways, managing grass for seed production is radically different than forage production on pastures and meadows. Seed crop establishment requires special conditions and preparation is essential to provide a favourable environment for rapid seed germination and growth. Supported by recent studies, Serbian grass seed production can be expected to continue developing its own capacities.

Semenarstvo višegodišnjih krmnih trava u Srbiji

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Rezime

Proizvodnja semena trava u Srbiji je mala, sa nezadovoljavajućim prinosom. I pored povoljnih ekoloških uslova za semensku proizvodnju, zbog niske cene i nedovoljne primene savremene tehnologije, proizvodnja ne pokriva domaće potrebe i velike količine semena se uvoze. Naučna istraživanja ukazuju da se visok prinos i kvalitet semena mogu ostvariti pravilnim zasnivanjem semenskog useva na odgovarajućem vegetacionom prostoru, korišćenjem đubriva u skladu sa potrebama biljke i zemljišta, kao i mehanizovanom žetvom u optimalnom vremenu.

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FORAGE CROP PRODUCTION ON ARABLE LAND IN SERBIA

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Abstract: This study gives a review of acreages and yields of forage crops grown in the Republic of Serbia over a period of twenty-two years (1988-2009) along with the results of regression analysis and the calculated coefficient of correlation, providing a clear insight into the current situation and future prospects with respect to the forage crop production on arable land in the country. Key cultural practices employed in growing forage crops on arable land are outlined through an overview and analysis of major crop growing operations used in the process, from cultivar choice through harvest. The current situation with respect to agricultural (growing) practices in forage crop cultivation on arable land is rather severe. This fact, coupled with weather conditions and the situation in the livestock production sector, has resulted in substantial yield declines. There has been a huge gap between research findings and their practical application. It is only under potentially more favourable economic conditions that the level of forage crop cultivation practices can be enhanced and that the gap between research findings and their practical application can be reduced.

Key words: forage crops on arable land, acreages, yields, production.

Introduction

Serbian livestock production has experienced unfavourable circumstances and frequent crises for a long time now *(Mijatović and Mišković, 1983; Krajinović et al., 1989; Lazarević, 1994; Erić et al., 1995, 1996, 2000)* being induced, inter alia, by the overall situation and current trends in livestock feed production. Continuous increases in the prices of agricultural inputs (seed, mineral fertilisers and pesticides), labour, and energy costs have led to a significant increase in fodder production, hay - and silage - making and storage costs. Diet costs for some livestock types account for as much as up to 80% of the total costs, depending on the diet system used. Therefore, increasing the level of forage crop production and reducing the related costs can serve as a primary solution to the above problems to be used within the set of measures aimed at intensifying livestock production, primarily cattle and sheep production *(Mišković and Erić, 1988; Erić et al., 1995, 2000).*

Moreover, all changes, fluctuations, and ups and downs in livestock production are rapidly reflected in forage crop production as well *(Mijatović and Mišković, 1983; Erić et al., 1995)*. Being an agrarian-oriented country, Serbia has suffered tremendously from the economic embargo being imposed on it over a long period of time i.e. since the breakdown of the former Yugoslavia in 1990, resulting in a substantial reduction in the cattle stock and loss of export markets for livestock products that have not been regained yet. New standards for the export of livestock products, though introduced in livestock production and meat processing industries, have not been met. This has led to a further deterioration of the current livestock production situation to such a degree that Serbia fails to satisfy the EU-approved export beef quotas.

The decline in cattle stock has induced a reduction in forage crop acreages. Moreover, the grave overall situation in crop production being faced by Serbia has also caused decreasing forage crop yields. Forage crops cultivated on arable land provide basic feeding material for livestock in the lowlands and, to a substantial extent, in the uplands.

In view of the above, the objective of this study was to identify the current situation and evaluate the acreage and yield trends of the forage crops grown on arable land in Serbia. The study also aims to analyse further prospects, based on potential resources (including climate, soil, genetic, human, technical and material resources), in terms of increasing the related acreage and yield per unit area at minimum production costs incurred, complying with safe fodder production and environmental protection standards.

Materials and Methods

Data on the total acreage and yield of forage crops (Statistical Yearbook of Serbia - SYS) for a period of twenty-two years (1988-2009) were collected, a regression analysis was conducted and the coefficient of correlation calculated in order to evaluate the situation and analyse further trends in forage crop production.

The situation with respect to forage crop production on arable land

Table 1. Forage crop acreage (ha) in Serbia (SYS, 2008; p. 210)

Land use categories	Year				
	2003	2004	2005	2006	2007
AGRICULTURAL LAND USE	5079	5075	5074	5066	5053
ARABLE LAND AND GARDENS	3345	3344	3330	3318	3299
FORAGE CROPS (FIELD-GROWN CROPS+GRASSLANDS)	1,883,000	1,885,000	1,902,000	1,906,000	1,912,000
GRASSLANDS, total (meadows + pastures+other grasslands)	1,420,000	1,421,000	1,441,000	1,448,000	1,455,000
Grasslands (meadows+pastures)	1,402,119	1,401,267	1,418,206	1,427,986	1,353,495
Meadows	590,592	595,884	604,624	601,152	613,907
Pastures	811,527	805,383	813,582	826,834	739,588
Other grasslands	17,881	19,733	22,794	20,014	101,505
FIELD-GROWN FORAGE CROPS (crops on arable land+othercrops)	463,000	464,000	461,000	458,000	457,000
FORAGE CROPS GROWN ON ARABLE LAND	351,563	352,410	354,206	350,260	350,072
Alfalfa (in ha)	187,952	190,305	191,620	187,928	186,268
Red clover (in ha)	122,938	121,750	121,822	121,015	118,876
Common vetch (in ha)	7,419	7,411	7,697	7,974	8,132
Field pea (in ha)	3,797	4,147	4,655	4,773	5,137
Fodder maize (in ha)	24,048	23,442	23,044	23,285	26,302
Fodder beet (in ha)	5,409	5,355	5,368	5,285	5,357
Other field-grown forage crops	111,437	111,590	106,794	107,740	106,928

*Data for Kosovo and Metohija not included

Field-grown forage crops in the Republic of Serbia covered 457,000 ha in 2007 (SYS, p. 210), accounting for as low as 9.04% of the total agricultural land use or 13.85% of the acreages under arable land and gardens. Generally, given the said parameter, forage crops grown on arable land covered small acreage. However, notwithstanding their substantial contribution to the choice of rotation crops and high-quality fodders, they play only a minor role in organic matter production on a global scale. In other words, the total grassland acreage (meadows + pastures + other grasslands) in 2007 accounted for as much as 28.79% (1,455,000 ha) of the total agricultural land use (5,053,000 ha).

Field-grown forage crops covered 23.90 % (457,000 ha) of the total forage crop acreage (field-grown forage crops + grasslands, giving a total of 1,912,000 ha), and grasslands accounted for 76.10% (1,455,000 ha, including 620,000 ha meadows and 835,000 ha pastures).

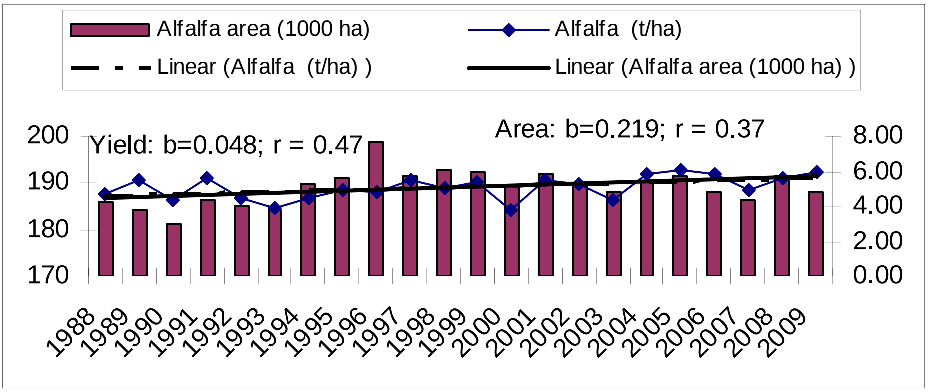
The six plant species (alfalfa, clover, common vetch, field pea, fodder maize and fodder beet) outlined in Tab.1 were grown on 76.60% and 18.31% acreage under field-grown forage crops and forage crops, respectively, during the period of the study, and serve as a good indicator of the overall situation of the forage crops grown on arable land *(Starčević et al., 1995; Erić et al., 1995, 1996 and 2000).*

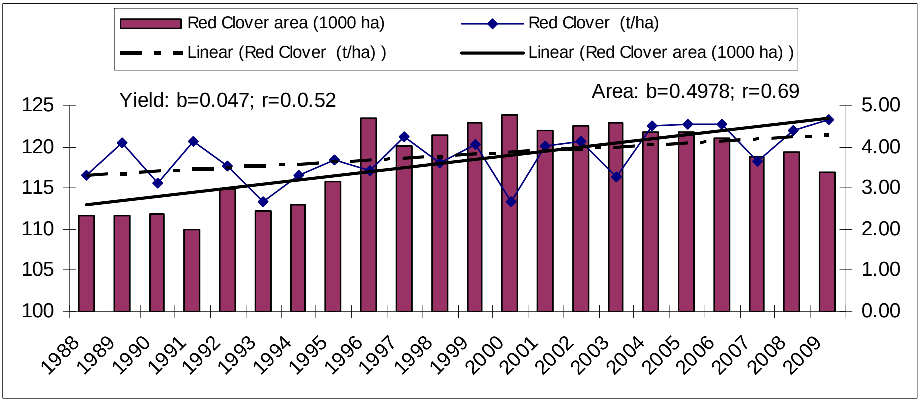
Table 2. Production of livestock feed (t) from six major forage crops grown on arable land and grasslands in Serbia (2007)

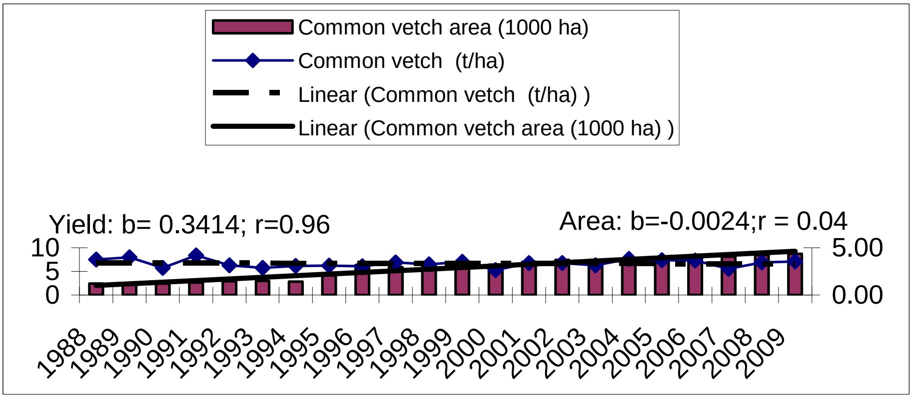
Plant species	Production (in t)	In % of total fodder production
Forage crops (grasslands+field-grown forage crops)	3,156,943	100.00
Grasslands (meadows+pastures)	1,266,817	40.13
Forage crops grown on arable land (6 species), including:	1,890,126	59.87
1. Alfalfa	904,838	28.66
2. Red clover	435,499	13.79
3. Fodder beet	51,910	1.64
4. Fodder maize	460,329	14.58
5. Common vetch	22,537	0.72
6. Field pea	15,013	0.48

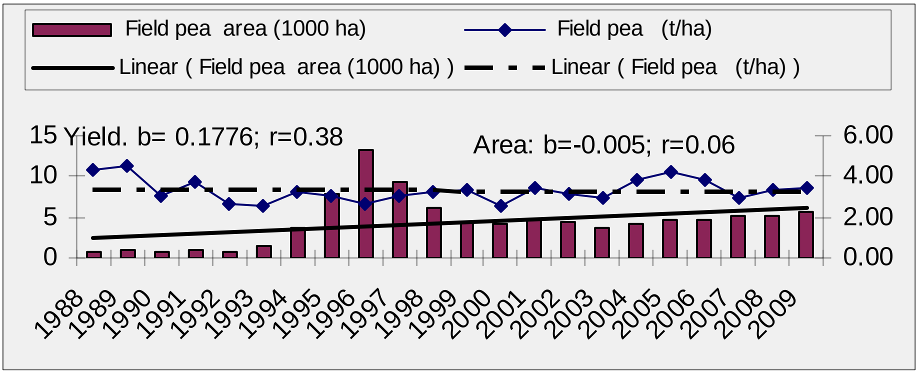
However, irrespective of the fact that forage crops grown on arable land cover less than 1/5 of forage crop acreage (Table 1) in Serbia, they provide larger quantities of livestock feed (59.87% or 1,890,126 t, as produced by the six major forage crops grown on arable land) as compared to grasslands (40.13% or 1,266,817 t). Only alfalfa and clover crops combined (28.66 + 13.79 = 42.45%) produce more fodder than grasslands do (40.13 %) in Serbia (Table 2). This serves as both a confirmation of the alfalfa alfalfa crop being considered "the queen of forage crops" and a reminder of the low level of exploitation of natural grassland resources.

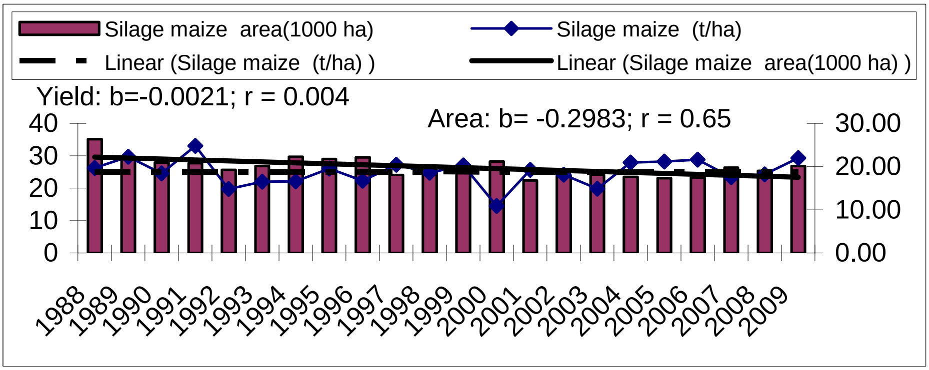
An increase in alfalfa acreage was observed during 1988-2000, followed by a steady mild decline until nowadays (2010). Similarly, red clover acreages mildly The major forage crops grown in the Republic of Serbia show very different acreage and yield trends (Figures 1-6), as monitored by official statistics. Increased from 1988 until 2000, but stagnated in the following 4-5 years, only to drop sharply until nowadays. Common vetches and field peas were the only forage crops to show a progressive, though slight, increase in acreage almost throughout the whole period under observation (1988-2009).

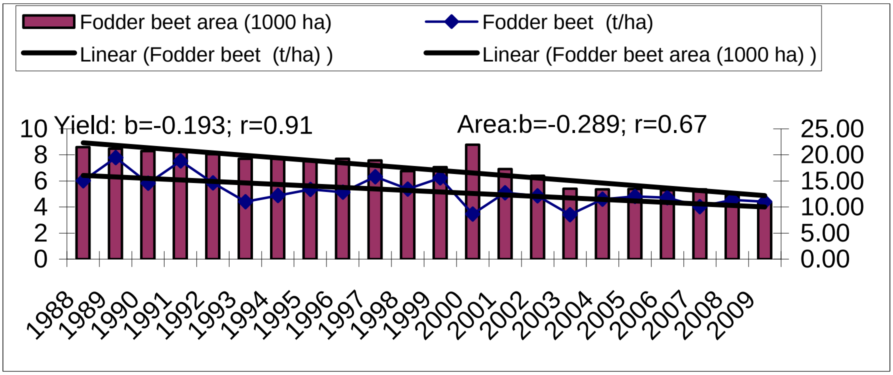

Alfalfa area (1000 ha)
Alfalfa (t/ha)
Linear (Alfalfa (t/ha))
Linear (Alfalfa area (1000 ha))
Yield: b=0.048; r = 0.47
Area: b=0.219; r = 0.37
200
190
180
170
8.00
6.00
4.00
2.00
0.00
1988 1989 1990 1991 1992 1993 1994 1995 1996 1997 1998 1999 2000 2001 2002 2003 2004 2005 2006 2007 2008 2009


Red Clover area (1000 ha)
Red Clover (t/ha)
Linear (Red Clover (t/ha))
Linear (Red Clover area (1000 ha))
Yield: b=0.047; r=0.0.52
Area: b=0.4978; r=0.69
125
120
115
110
105
100
5.00
4.00
3.00
2.00
1.00
0.00
1988 1989 1990 1991 1992 1993 1994 1995 1996 1997 1998 1999 2000 2001 2002 2003 2004 2005 2006 2007 2008 2009


Common vetch area (1000 ha)
Common vetch (t/ha)
Linear (Common vetch (t/ha))
Linear (Common vetch area (1000 ha))
Yield: b= 0.3414; r=0.96
Area: b=-0.0024;r = 0.04
10
5
0
5.00
0.00
1988 1989 1990 1991 1992 1993 1994 1995 1996 1997 1998 1999 2000 2001 2002 2003 2004 2005 2006 2007 2008 2009







Figures 1-6. Acreage and yield trends of alfalfa, red clover, field pea, common vetch, fodder maize and fodder beet in the Republic of Serbia during 1988-2009

The acreages under silage maize and fodder beet, two major row forage crops in Serbia, showed a mild decline, which was considerably sharper in fodder beet than in fodder maize. A slight increase throughout the period of the study was observed in the hay yield of alfalfa and clover, the major forage crops in Serbia, the increase being 48 and 47 kg hay, respectively. Hay yields of common vetches and field peas stagnated, and silage fodder yields of fodder maize and root yields of fodder beet mostly decreased.

Low fodder yields and their high fluctuations were specific of long-season spring forage crops grown on arable land (including fodder beet and fodder maize), due to dry weather generally occurring during the summer months.

The production of forage crops on arable land in 1988-2009 was generally marked by the following: decreasing acreage trends in all forage crops (excepting the slight increase in common vetch and field pea acreage), low yields and high variations in fodder yield across the years and a negative fodder yield trend in all the species analysed. Similar findings were reported by *Janković (2004)*.

The produced average yields of major forage crops, particularly in the past dozen years, were below the European average, in addition to being far below the average values for Western European countries practising intensive livestock production.

The degree of exploitation of the genetic yield potential of forage crops is best illustrated by the degree of exploitation of the genetic yield potential of alfalfa, ranging from 19.2% (without irrigation) to 32% (irrigation included), *(Erić et al., 1995a)*.

The forage crop production in Serbia was affected by a number of factors, with the drop in livestock production being the most important. The decline in livestock numbers per unit area had altered the crop acreage structure to the detriment of forage plants, legumes in particular, such as common vetch, field pea, alfalfa, clover, etc. *(Marko, 1989)*.

Natural resources (climate and soil) used in forage crop production on arable land

In general, temperate continental and continental types of climate are found in Serbia. A large number of factors, particularly altitude, along with Mediterranean and continental air masses coming from the south and north, respectively, contribute to the diversity of climatic conditions, not so much in terms of the total annual precipitation as in terms of the uneven distribution of precipitation, in particular during the growing season, as also induced by global climate change. Therefore, the climate of Serbia, specifically the Vojvodina region, is tending towards semi-arid, rather than semi-humid as previously considered *(Molnar and Milošev, 1995)*.

Another major natural factor, apart from deficit precipitation, affecting fodder production on arable land is soil. Soil is a natural factor likely to be most affected by humans, through an adequate use of cultural practices or necessary amendments. Moreover, a large number of distinct types, subtypes and varieties of soil having different yield potentials are found in Serbia.

However, despite the huge diversity, the present climatic and edaphic conditions definitely favour the production of a wide range of forage crops on arable land.

The different conditions, both climate and soil, affect the forage crop production structure and fodder yield per unit area, eventually inducing diversity within the livestock production structure in some parts of Serbia.

Forage crops cultivated on arable land provide basic feeding material for ruminants, particularly cattle, in the lowlands and, to a substantial extent, in the uplands. Hence the special importance is being given to the topic.

Current issues with respect to forage crops grown on arable land for fodder

Cultural or growing practices for forage crops involve a set of operations employed during cultivation, crop management and harvest of fodder or seed, including plot selection, crop rotation (crop sequence), tillage (primary and secondary tillage operations), fertilisation (the use of organic and mineral fertilisers), sowing (cultivar selection, seed quality, sowing date, sowing method, plant number per unit area, planting depth, seeding rate), crop management operations (disease, pest and weed control – mechanical, chemical, biological and integrated management) and crop harvest (identification of optimal harvest-cutting dates, harvesting methods etc.).

The main objective of all of the above cultural practices is to create the most favourable conditions for plant growth and development viz. yield potential exploitation. Additionally, the cultural practices, management ones in particular, are aimed at bridging or reducing the gap between plant requirements and specific agro-environmental conditions in that the timely and adequate use of certain cultural operations will mitigate the negative effect of different factors, primarily edaphic (pedological) and climatic ones *(Drezgić, 1972; Starčević et al., 1990. and 1995).*

Basically, the main components of the cultural practices used for forage crops are similar or identical to those employed for field and vegetable crops, with forage crops, however, showing certain peculiarities.

Ruminant (polygastric animal) nutrition is based on fodder from arable land and grasslands. The contribution of the two forms of fodder production to livestock nutrition in Serbia is quite varied, depending on the production region. In the lowlands and uplands, fodder from arable land and fodder from both arable land and (native and sown) grasslands are, respectively, major contributors to livestock nutrition. In alpine regions, the main and often the only source of fodder are native grasslands *(Mijatović and Mišković, 1983).*

Roughage particularly when fed as green fodder should be the predominant feedstuff used in daily feed ration formulation for ruminants. Green fodder is the most cost-effective source of feed due to substantially reduced yield and quality losses accompanying the fodder preparation and preservation processes; minimised preparation costs and absence of storage costs. The consumption of green fodder by grazing will result in no preparation costs and will facilitate higher milk production as compared to the preserved fodder diets *(Pavličević et al., 1994*).

Moreover, livestock exhibit the highest nutrient conversion efficiency from green fodder due to its high digestibility *(Erić et al., 1998)*, making room for the additional feed intake *(Pavličević et al., 1999)*. The high water intake through green feed absorbs the heat produced in the digestive tract, contributing to mitigating the unfavourable effect of high temperatures *(Pavličević et al., 1999)*. Therefore, ruminant diets during the growing season should be based on green fodder viz. on the year-round green fodder production system.

What are the reasons for the failure to develop and practise the year-round green fodder production system countrywide notwithstanding its numerous important advantages?

The answer to this question should be sought in the following facts: a number of different forage crop species are being introduced, resulting in complicated crop production; the year-round green fodder production system requires a well-organised management plan, adequate technical equipment, high dynamism and the strict observance of both work and technological discipline. This is strengthened by the fact being faced by Serbia that the personnel involved in the process are insufficiently stimulated to work harder and that there is a lack of personnel, primarily those trained in fodder production. The above stated serves as a clear explanation for the avoidance of introduction of the year-round green fodder production system even at highly developed model farms. Moreover, there is strong opposition among nutritionists emphasising (quite rightly) the negative effect of frequent alterations of feedstuffs on the physiology of the livestock digestive system, eventually resulting in a slight decline in milk yield or daily weight gain within 1-2 days following feed intake. At the same time, however, the importance of green fodder for livestock health and the substantial use of antibiotics to maintain it are being disregarded *(Erić et al., 1998)*.

Major forage crops grown for fodder throughout lowland Serbia, the region of Vojvodina in particular, include the following:

a) alfalfa, grown primarily for hay, with hay-making and hay storage causing the greatest harvest losses. Its secondary use is as green feed, and its minor as hayage or silage and dehydrated alfalfa processed into alfalfa meal, briquettes and pellets;

b) fodder maize, grown primarily for silage and, secondarily, for green fodder, with minor use as dehydrated meal (in Vojvodina);

v) forage sorghum, used primarily as silage or, to a minor extent, as green fodder; and Sudan grass, in reverse order, grown primarily for green fodder and to a minor extent for silage;
g) field pea and common vetches, grown for green fodder and typically planted as a mixture with small grain crops (oat, barley, rye, wheat);
d) forage brassicas, primarily fodder kale, fodder rape, turnip rape, Perko PVH and Tifon (*Brassica campestris* f. *biennis* DC. x *B. rapa* L.) hybrids, grown for green fodder, and
e) fodder beet, used as a fresh feedstuff fed during the winter feeding period.
Roughage supplements include fresh or, rarely, dry shredded beet pulp, brewery spent grains, sugar beet tops, etc.

Major forage plant species used for fodder production in the Serbian uplands are as follows:
a) species fed within feeding stalls:
- red clover and birds foot trefoil, fed as green fodder and hay
- short-term clover/grass mixtures, fed as green fodder, silage and hay
- fodder maize, hybrids in early FAO maturity groups, fed as silage, and
b) species used for grazing – native and sown grasslands.

Crop rotation plays an indisputably important role in improving forage crop production. However, its role and value have changed throughout the historical development of field crop production, becoming more significant for the extensive production system than for the intensive system.

Crop rotation holds a special position in biological husbandry theory. It is seen as being an important phytosanitary practice as it prevents the development of crop-specific diseases, pests and weeds, and reduces or eliminates the use of pesticides *(Milojić, 1990)*. As a biological measure, crop rotation has a positive effect on soil physical, chemical and biological properties. Particular focus is being placed on growing forage intercrops *(Ćupina et al., 2004 a, b)* and green manure crops *(Erić et al., 2000)* contributing to increasing the humus content of soils, enhancing soil structure, and reducing weed infestation, disease and pest risks, but more particularly on growing soil biocleaning forage plants (nematodes), *(Erić, 1996; Erić et al., 1994, 1997 and 2006)* etc.

The disregard of crop rotation is the result of the unfavourable field crop planting structure made up, for the most part, of row crops (56%), followed by small grains (27%) and forage crops (14%), in particular high-density stands of field (arable) crops (annual and perennial legumes), which eventually often leads to catastrophic consequences in semi-arid and arid environments *(Starčević et al., 1995)*.
Soil cultivation is also being given particular importance within biological husbandry. It is being analysed from the biological and environmental standpoint, as well as in terms of production, economy and related techniques.

Soil tillage system and tillage depth are largely dependent upon the preceding crop used and time of tillage. Soil tillage depth for forage crops aimed at soil moisture conservation is generally determined more by tillage date viz. planting (post-planting and stubble planting) date than by the actual requirements of the plant species. Therefore, an appropriate choice of preceding crop and adequate primary (deep) tillage assume an added importance. As a result, arable plot establishment and regeneration system *(Todorović, 1957)* serves as a commonly accepted method of soil tillage for forage crops. The deep ploughing of arable land accompanied by manure fertilisation would be practiced on a permanent basis for a small number of forage crops (alfalfa, silage maize, fodder beet) sown during the main planting date. As for the other forage crops, the prolonged effect of the preceding deep tillage operation would be used.

As all forage crops excepting annual fodder legumes and fodder maize have small seeds, much greater attention than hitherto should be given to the quality of seedbed preparation.

There is no need to elaborate on fertilisation and the importance of organic and mineral fertilisers. However, the manufacture and use of mineral fertilisers in Serbia were inadequate, the average not exceeding 105 kg ha^{-1} NPK during maximum application period (1982-1986). The highest application rates of mineral fertilisers in Vojvodina were 215-240 kg ha^{-1} on state-owned land and 140-170 kg ha^{-1} NPK on privately-owned land, being 25% and 50-60% lower, respectively, than the optimal rates for maximum production *(Manojlović, 1989)*. Further detailed studies of Vojvodina soils confirm the suitability of the soils for safe food production *(Kastori et al., 1997)* in as much as they are not loaded with harmful substances introduced through mineral fertilisers. The use of mineral fertilisers has been even more limited in forage crops or frequently lacking in annual forage legumes. There has been a complete absence of organic (manure) fertilisation in forage crops due to the lack of manure and the need to apply fertilisers in more intensive field and vegetable crop production systems.

The N-min method has been widely used lately as an aid in specifying optimum nitrogen fertilisation rates. It involves maximum plant utilisation of soil mineral nitrogen, resulting in the judicious use of nitrogen fertilisers and, hence, high savings in nitrogen fertiliser inputs and environmental protection against the high rates of unused nitrogen from mineral fertilisers. Accordingly, nitrogen rates are adjusted to suit the nitrogen requirements of the crop *(Ubavić, 1996)*. Fertilisation of forage crops, excluding perhaps alfalfa and silage maize, is conducted based on experience instead of on fertility control, including the control of soil nutrient levels and nutrient removal by crops. This does not enable precision mineral fertilisation as either insufficient or excessive rates are applied, being equally harmful to crops. The nutrient requirements of minor forage crops have not been precisely defined yet (being within a wide range of value) or have been based on the low productivity of old cultivars.

In view of the significant number of forage crops being legumes, the potential of using biological fertilisers has not been exploited. Serbian microbiologists have isolated in pure culture the most productive strains of nodule bacteria and can undergo their cultivation for each forage legume crop *(Govedarica et al., 1995).*

It is not by chance that a traditional proverb warns “As you have sown, so shall you reap.” Accordingly, sowing-planting must receive due attention. The most common failures are associated with the time of sowing (late sowing and long sowing period), seeding rate (enormously high rates) and, hence, plant density, seeding depth, etc. The sowing period for small grains is impermissibly long, often lasting for even two months, inevitably resulting in declining yields *(Spasojević and Malešević, 1989).* In regard to sowing of forage plants, priority has always been given to sowing wheat in order to provide ’our daily bread’ and daily sustenance, particularly during the period of economic embargo and sanctions imposed on Serbia. It is even in the best of times that winter forage crops are not sown, but are being compensated for by increased acreage of spring forage crops. This leads to green fodder diets being fed for a shorter period of time. Planting dates for forage crops should be strictly adhered to during the post-planting and stubble planting dates. Crops suffer a 3% reduction in yield for each day of delay in stubble planting *(Vučić, 1981)*. Forage crop seeding rates are impermissibly high, particularly for small-grain species, among others. Increased seeding rates cannot make up for the failure to employ adequate growing practices. The seeding rate for most forage crops can be reduced to half the normally used rate through appropriate seedbed preparation, timely planting and the use of precision planters (seed-plant).

A number of forage crops can be planted as transplants (fodder kale, swede, stubble turnip and fodder beet), which can substantially contribute to their spread into the uplands where the growing season length is a limiting factor to their cultivation. This particularly refers nowadays to fodder kale grown in home gardens *(Erić et al., 1996)*.

Plant protection measures help maintain plant density following crop germination and emergence and secure optimum conditions for plant growth and development throughout the life of the plant from the emergence stage through harvest-cutting.

The use of pesticides is a double-edged sword. Pesticides are poisons and foreign to living beings. Therefore, special focus within biological farming systems is being placed on pest, disease and weed management. A new approach to pest, disease and weed control is now being applied, involving the combined use of cultural and biological methods, apart from the chemical control measures being also employed. Accordingly, the use of pesticides serves only as a correction factor for cultural practices.

Biological pest and disease control agents are also used. Apart from crop rotation, there are no biological methods of weed control. Cultural practices such as crop rotation, hoeing and cultivation provide the most effective methods of weed control. A number of questions arise: What are the ways to fight weeds in high-density stands (of alfalfa, clover, grassland, common vetch, field pea, etc.) where hoeing is excluded? What are the ways to fight weeds in row crops (fodder beet, swede, stubble turnip, fodder maize) when hoeing is not included, being an operation that requires a high energy and labour demand, leading to an increase in the cost of cultivation of these crops? Are cultural operations alone sufficient to control weeds, in view of the fact that weed seeds are often being incorporated with the organic fertilisers applied?

Another means of controlling pests, diseases and weeds is the cultivation of resistant plant species, cultivars and hybrids. However, notwithstanding the above, chemical control will remain the predominant type of control in crop production for a long time. Nevertheless, herbicide selectivity, persistence and residual effects should be closely monitored.

Green fodder cutting or harvesting is an important step in roughage production, typically carried out as next to the last or as the last operation (depending on the purpose of the crop to be used as immediate fodder or preserved for storage). Forage crops are genetically predisposed to grow vigorously during the growing season, and most of them have an indeterminate growth habit. It is for this reason that the most common mistakes are made when determining the optimum cutting time, for grasses in particular. Cutting time is dependent primarily on the crop purpose (green fodder, hay, silage, dehydration, pasture). The abundance of fodder species does not permit a detailed analysis of this cultural operation. Importantly, cutting time is determined by crop yield and quality for crops grown for green fodder and hay, crop quality (CP content) for crops cultivated for dehydration, dry matter content for crops grown for silage, and plant growth for pasture crops. Hence the following optimal cutting stages for forage crops: plant height of 25-30 cm for pasture crops, stage of budding for crops grown for dehydration, onset of flowering for crops cultivated for green fodder and hay, and full bloom or blossom fall stage for silage crops, excepting silage maize, fodder sorghum and Sudan grass reaching their optimal harvest time during the transition from the milk to the dough development stage. Fodder cutting-harvesting is followed by major preparation, preservation and storage operations.

The production of fodder crops on arable land should be oriented towards environmental protection and safe food production to help ease growing concerns caused by both the development of modern industrial production technologies and the increased use of chemical and biological agents in livestock farming, aiming at making the production more efficient *(Čobić et al., 1990).*

The basic problem in ruminant nutrition is the degradation of the lignocellulose complex, a major causal agent of the insufficient roughage utilisation ability. Since there are organisms, such as termites, capable of utilising lignin, the gene responsible for the ability can be isolated and introduced by genetic engineering into rumen micro organisms in order to increase the utilisation efficiency of roughages in ruminants *(Čobić et al., 1990)*.

The development of biological disciplines, biotechnology and information systems will significantly contribute to enhancing field crop production and, hence, the development of forage crop production on arable land. Improvements in the production of forage crops on arable land will remain closely associated with improvements in livestock production. Livestock production is expected to increase to meet the growing food demand in human nutrition, both in terms of quantity and quality (change in dietary structure).

In the light of the above, further improvements in crop production, particularly forage crops on arable land, will involve the following:

a) actions aimed at increasing, zoning and cost reduction of forage crop production on arable land;

b) focusing greater attention on fodder quality and environmental protection;

c) increased and more effective use of natural resources, soil in particular;

d) introduction of new species, cultivars and hybrids of forage plants grown on arable land;

e) efforts to reduce the dependence of fodder production on the vagaries of weather, primarily deficit rainfall;

f) greater use of feeds as green fodder;

g) higher stability of the seed production of forage plants grown on arable land and the availability of a wider range of forage seeds;

h) design and introduction of roughage feed production and marketing standards, primarily for alfalfa hay.

Conclusion

The current situation with respect to agricultural (growing) practices in forage crop cultivation on arable land is rather severe. This fact, coupled with weather conditions and the situation in the livestock production sector, has resulted in substantial yield declines.

There has been a huge gap between research findings and their practical application. It is only under potentially more favourable economic conditions that the level of forage crop cultivation practices can be enhanced and that the gap between research findings and their practical application can be reduced.

Proizvodnja oraničnog krmnog bilja u Srbiji

P. Erić, B. Ćupina, V. Stevović

Rezime

Kroz prikaz dvadesetdvogodišnjih površina i prinosa u Republici Srbiji (1988/2009), urađenu regresionu analizu i koeficijent korelacije dat je uvid u stanje i perspektivu oraničnih krmnih biljaka. Predstavljena je agrotehnika oraničnih krmnih biljaka kroz prikaz i analizu primene osnovih tehnoloških operacija od izbora sorte do ubiranja krme. Sadašnje stanje u agrotehnici (tehnologiji proizvodnje) oraničnih krmnih biljka je na veoma niskom nivou, što je pored vremenskih uslova i stanja u stočarstvu, značajno uticalo na smanjenje prinosa. Veliki je raskorak između dostignuća u nauci i njihove primene u proizvodnoj praksi. Republika Srbija ima prirodne resurse i kadrovske potencijale da se ova proizvodnja podigne na znatno veći nivo proizvodnosti i kvaliteta. Samo u ekonomski povoljnijim uslovima moguće je podići nivo agrotehnike oraničnih krmnih biljka i smanjiti jaz između naučnih dostignuća i njene primene u praksi.

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GRASSLAND USE IN EUROPEAN UNION AND SLOVENIA

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Abstract: In European Union (EU) grasslands are an important land use with essential functions for livestock farming systems and ecosystem service supply which all depend on the activity of biological organisms and processes. Permanent grassland represents 13% of EU-27 territory. There is of course large spatial variability in both grassland systems and productivity between EU countries. Grassland most abundant countries (Ireland and United Kingdom) lead with 45%, while Greece and Finland have only 1% grassland of total area. From total area Slovenia has 14% permanent grassland. Grassland ecosystems hold an important part of EU's biodiversity. Especially permanent and semi natural types offer ideal conditions for a vast diversity of habitats and species. Threats to grasslands in EU are changes in land use as conversion to arable land mainly driven by increased demands for agricultural product or land abandonment as an absence of traditional activities. The paper discusses about the past and current status of grassland management in EU with emphasis on future challenges in managing it in both directions. The search for the balance between the forage productivity with intensification (use of mineral fertilizers, pesticides and re-seeding) and environmental functions of grasslands will continue not only on the regional but also national scale.

Key words: grasslands, productivity, environment, land use, biodiversity, management

Introduction

Grasslands can be defined as terrestrial ecosystems dominated by herbaceous and shrub vegetation and maintained by fire, grazing, drought and/or freezing temperatures. This definition includes vegetation covers with an abundance of non-woody plants and thus lumps together some savannas, woodlands, shrub-lands, and tundra, as well as more conventional grasslands. Globally speaking, grasslands are found most commonly in semi-arid zones (28% of the world's grasslands), followed by humid (23%), cold (20%), and arid zones

(19%) *(White et al., 2000)*. In recent times definition of grasslands as highly dynamic ecosystems provide goods and services to support flora, fauna, and human populations worldwide also was emphasized. They are one of the world's major ecosystems groups and over the last century their use has changed from being volunteer leys, or a resource on non-arable land, to a productive resource equal to any crop and managed as such *(Kemp and Michalk, 2007)*. In European Union (EU) grasslands are an important land use covering more than a third of the EU agricultural area *(Eurostat, 2009)*. They are an integrated part of pastoral and mixed-farming systems. Grasslands have a basic role in feeding herbivores and ruminants and provide important regulating ecosystem services (e.g. reducing erosion by supporting slope stability; regulating water regimes; purifying water from fertilizers and pesticides). Grassland also support biodiversity and cultural services, e.g. contributing to a region's cultural heritage and to recreational values *(Hopkins and Holz, 2005)*. Permanent and semi natural types offer ideal conditions for a vast diversity of habitats and species, and are especially important for birds and invertebrates, providing vital breeding grounds *(Weigelt et al., 2009)*. Later type need to be maintained through farmers' grazing or cutting regimes. The objectives of the paper are to get the insight in the distribution of grassland and its use in EU, understand past and present situation on this type of agricultural land and predict the future use of most valuable land use in EU.

Origin of grassland in EU and its definitions

Most grassland in EU is generally considered to be of anthropogenic rather than natural origin, having developed from former forested land by the removal of trees and shrubs by burning, felling and controlled grazing, or from the draining of marshes. Until the early 20th century, virtually all grassland, with the exception of short-term forages such as red clover, was permanent, having developed from natural vegetation under human influence. However, the emergence of improved grass and legume varieties and the recognition that grass could be managed as a crop led to the development of sown grassland *(Wilkins et al., 2003)*. But there are also other explanations which say that grasslands in the temperate zone usually persist due to moderate disturbance: grazing, mowing, or fires. During the last millennia temperate EU grasslands have been mostly managed by grazing of domestic animals or by haymaking. This is the reason why this ecosystem has often been 'semi-natural' *(van Dijk, 1991)*. This fact does not imply that EU temperate grassland as an ecosystem is man-made. It means that large grazing mammals are an integral part of this ecosystem, be it wild game or domestic livestock. In this respect, temperate European grasslands are not different from grasslands in other parts of the world with temperate climate *(Pärtel et al., 2005)*.

Table 1. Types of grasslands in EU (Adopted from Annex 1 of the Habitat Directive)

Grassland name	Description
Natural grasslands	includes nine grasslands habitats that thrive without direct human intervention and are limited by specific ecological, soil and climate conditions, e.g. Alpine grasslands
Semi-natural dry grasslands and scrubland facies	includes 12 grasslands habitats that are to some extent managed, ranging from Mediterranean grasslands to Pannonic steppe and Fennoscandinavian grasslands.
Sclerophillous grazed forests (dehesas)	includes only one grassland habitat known in Portugal montado and in Spain as dehesas – semi-natural savannalike open woodlands with scattered oak trees and extensive grazed grasslands.
Semi-natural tall-herb humid meadows	includes six grasslands habitats that have some soil water presence.
Mesophile grasslands	includes three grasslands habitats comprising all meadows.

Grassland systems in EU are diverse, ranging from extreme Taiga vegetation in the far North to dry Mediterranean grassland in the South. To distinguish between grassland systems across EU, Eurostat, the Statistical Office of the European Community, has developed a classification for forage and grassland types (Table 1).

Spatial distribution of grassland in EU

Grassland in EU is utilized predominantly by grazing in summer or by conservation as silage or hay for winter feeding. Grass and forages are the main feed supply for domestic livestock that provide mankind with high quality food (e.g. meat and milk) *(Breymeyer and Snaydon, 1987)*. However, animal feeding systems differ across Europe. In NW part of EU, grasslands meet 70–75% of the nutrient requirements of the ruminant livestock population and about 70% of the grassland contribution comes from grazing *(Mayne et al., 2000)*. In other parts of EU (e.g. Mediterranean countries and Scandinavia), forage crops and temporary grasslands are more important *(Lee, 1988)*. Utilised agricultural area represents 41% of the whole EU-27 territorial area. The size of UAA varies greatly from country to country, from only 7% in Finland and Sweden to more than 70% in the United Kingdom *(Čop, 2006; Eurostat, 2009)*.

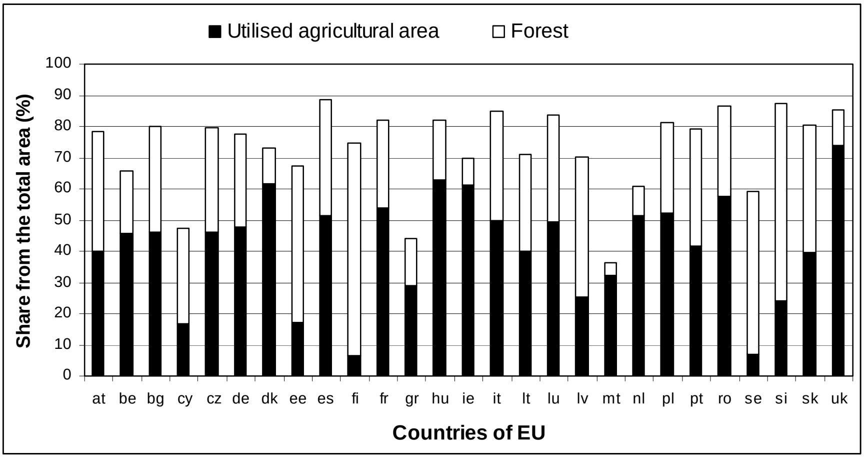

Figure 1. Share of utilized agricultural area (UAA) and forest with other wooded area cover from total area in EU countries in 2008.

The second most common type of land use in EU is forestry. Forests and other wooded land cover 42% of the land area and are one of the most valuable multifunctional and renewable natural assets we have. The most densely forested member states are Finland, Sweden and Slovenia, whereas the least forested are Malta, Ireland and the Netherlands *(Eurostat, 2009)*.

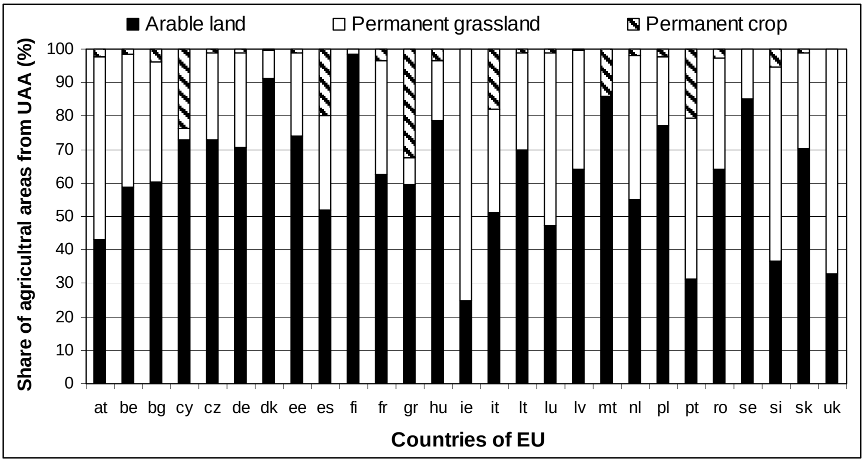

Figure 2. Share of different agricultural areas from UAA in EU countries in 2008.

The land used for arable production, grassland management and permanent cropping and their proportions of the utilized agricultural area (UAA) are presented in Figure 2. Permanent grassland covers about 12,8% of the total EU land surface and 30,8% of the utilized agricultural area (Figure 1). On average, about 10% of the total grassland area is temporary grassland, but in certain countries, e.g. Scandinavian or Baltic countries, the proportion of temporary grassland can be significantly higher. There is little variation in land use between years, although there are signs of an increase in temporary grassland.

In the Nordic countries, woodland is the dominant land cover type and grasslands form only a small part of the total land area (<3%). There are distinct country differences in the proportion of grassland on UAA. In Central EU, grasslands are especially important in mountainous areas, e.g. Austria, Slovenia (>40% of UAA), while in the lowlands, grassland covers just 20–25% of the UAA. In the Mediterranean, grassland is an important land use (30–40% of the UAA), and is mainly grazed by sheep and goats.

Productivity and biodiversity of EU grassland

In EU there are various types of grasslands, ranging from almost desert types in south-east Spain through steppe and mesic types to humid grasslands/meadows, which dominate in the north and north-west. The highest productivity, about 10 t ha^{-1}, is achieved in the Atlantic zones (Lusitanean, Atlantic Central and Atlantic North) (Table 2).

Table 2. Estimated grassland productivity for permanent grassland for the 12 principal Environmental Zones of Europe *(Smit et al., 2008)*

Environmental zone	Grassland productivity (t ha^{-1})	Environmental zone	Grassland productivity (t ha^{-1})
Alpine North	4.04	Atlantic Central	6.96
Boreal	3.09	Pannonian	1.9
Nemoral	3.36	Lusitatnian	5.2
Atlantic North	7.42	Mediterranean Mountains	2.19
Alpine South	3.25	Mediterranean North	1.90
Continental	4.29	Mediterranean South	9.20

These regions comprise North Western Spain, Western France, Ireland, Wales and England, the Benelux and the North of Germany *(Smit et al., 2008)*. Ruminant production in the UK is largely dependent on grassland: approximately 52% of UK land is (improved) grassland and rough grazing. Herbage production from improved grassland can be over five times higher than that of indigenous swards and these pastures account for the majority of ruminant (meat and milk) production from grassland in the UK *(Scollan and Moorby, 2008)*. The highest yields recorded are in the Netherlands, which is due to the combination of a suitable climate and highly intensive pasture use in this country *(van Bruggen,* *2006)*. Aside from the favourable climatic conditions, the high fertilization rate is a major determinant of the attained yields. The grassland species in these ecosystems are usually *Lolium perenne* and *Poa* spp. The regions with the lowest productivity are located in the Mediterranean (Table 2). Here, grassland is subject to severe moisture stress, with annual yields limited to 1.5 t ha^{-1} or even less *(Vidrih and Batič, 2002)*. Slightly higher yields are attained in more mountainous areas, which are more mesic, e.g. Pyrenees and Sierra Nevada (in Spain), the Balkans and the North of Greece. When irrigation is applied, much higher yields are achieved (>15 t ha^{-1}) *(Lee, 1988)*. Mediterranean grasslands are highly diverse ecosystems, consisting of grass species, annual plants and herbaceous species. The North of the continent, with its tundra systems, forms another low productivity zone in EU. Among these, the Scandinavian countries have a slightly higher productivity, because of a more intensive management that involves more frequent grass sowing. In Scandinavia, *Phleum pratense* is often used. The Central EU countries reach fairly high yields. Germany and the northern part of Austria reach yields of 6 t ha^{-1} and higher. However, yields in the upper parts of the Alps are low (around 2 t ha^{-1}). Grassland yields in Poland, Czech Republic and Slovakia are around 4 t ha^{-1}. These systems are less intensively managed and involve large areas of meadows. Yields in the steppe conditions of the Hungary, Bulgaria are lower (around 1.5 t ha^{-1}) due to severe moisture stress. Grass species used in Central EU are often *Festuca* or *Agrostis* spp.

In EU, agricultural intensification, particularly ploughing and reseeding and a shift from hay-making to silage production over the last 50 years, has resulted in the loss of biodiversity (flora and fauna) associated with semi-natural grasslands. While remaining species-rich meadows have been the focus of nature conservation effort, trials have been made to restore semi-natural grasslands on ex-arable soils *(Soussana and Duru, 2007)*. Grasslands are among the most species-rich habitats in Europe. Indeed calcareous (chalky) grasslands are EU's most species-rich plant communities (up to 80 plant species m^{-2}). This extremely high plant diversity gives rise to high arthropod diversity (e.g. butterflies) and can support grassland-adapted birds – bustards, falcons, etc – and other species such as rodents (souslik, voles, etc) *(Silva et al., 2008)*.

Land use in Slovenia

With 20,273 km^2 of surface and nearly 2 million inhabitants, Slovenia can be considered as one of the smallest EU countries. Slovenia is a distinctively Central European country, located between the Alps, the Adriatic and the Pannonian Plain. In spite of its geographically small size, it is a convergence point of a range of different landscapes: Alpine and Mediteranean, Pannonian and Dinaric, each of which has its own characteristics and unique features *(Klopčič and*

Huba, 2006) . Diverse natural conditions are reflected in the structure of the land utilisation, too. Slovenia is one of the most forested countries in the EU, with 65% being behind Finland and Sweden. Slovenian agriculture is characterised by small parcels of agricultural land, each farm on average only cultivation 5,6 ha of land area. Sixty percent of farms cultivate up to 5 ha, 25% from 5 to10 ha and 2% more than 20 ha of land *(Surs, 2008)*. The professional level of agriculture, and thus farm productivity in Slovenia are therefore still quite low. The share of agriculture and fishing in total domestic product is around 5%. Slovenia is a net importer of agricultural products, importing cereals, sugar, oils, cattle fodder and exotic fruits. There are surpluses in milk, poultry, hops and wine.

Spatial distribution, productivity and biodiversity of grassland in Slovenia

Slovenia is a small country with a wide range of climatic, topographic and edaphic conditions. In many regions the climate and topography are unsuitable for arable crops and the landscape is dominated by grassland. Because of the predominantly hilly and mountain regions Slovenia is undoubtedly one of the EU's countries with the least favourable conditions for conventional forms of agriculture. A much as three quarters of agricultural land lies in less favourable areas, and two third of the rural population live and work on farms with less favourable conditions. The natural conditions dictate significantly the exploitation of grassland and therefore the keeping of livestock. Cattle breeding prevails in the structure of agricultural production. Two thirds of all the cattle is bred and more than half of milk and the meat is produced in these less favourable areas. In Slovenia grassland is spread all around the country *(Vidrih et al., 2009c)*. It appears as the most important part of mosaic cultural landscape. Grassland in each phyto-geographical region has its own characteristics as in productivity (Table 3) as in biodiversity.

Tabel 3. The main types of grassland in Slovenia according to the management intensity

Management intensity	Utilisation frequency	Stage at the 1st cut	Fertilisation rate	Main grasses
Extensive	very low	after flowering	nil	*Bromus erectus, Briza media, Agrostis capillaris, Nardus stricta*
Low intensive	low	flowering	low	*Arrhenatherum elatius, Trisetum flavescens, Holcus lanatus, Cynosurus cristatus, Festuca rubra*
Fairly intensive	intermediate	after heading	intermediate	*Dactylis glomerata, Alopecurus pratensis, Festuca pratensis, Poa trivialis, Holcus lanatus*
Intensive	high	before heading	high	*Lolium multiflorum, Lolium perenne, Poa pratensis, Alopecurus pratensis, Poa trivialis*

The most productive and for good forage quality one is in pre-alpine region. In lowland regions, with mild winter temperatures, the climatic conditions are very favourable for grass growth. Even on permanent grassland, the annual dry matter production can reach 9.5 t DM ha^{-1}, as in the best grassland regions of north-western Europe. On the other side *Čop et al. (2009)* and *Vidrih et al. (2009a)* in their studies revealed opportunities for herbage production on the *A. elatius* grassland and the *M. caerulea* fen meadow in order to meet agricultural and environmental targets by applying modifications to management which means moderate intensive management. Later target namely does not put in front of all the quality and quantity of produced forage. The most rich in plant species grasslands are those existing on limestone and maintained by small ruminant grazing *(Vidrih et al., 2009b; Vidrih et al., 2009c)*, where on a plot size of 80 m^2 even more than 100 species could be find (Figure 3) with high diversity index (Figure 4).

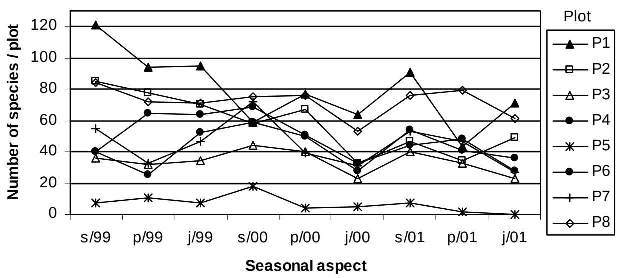

Figure 3. Species richness on plots (P1-P8) according to sheep grazing and vegetation aspects (s-spring, p-summer, j-autumn) between 1999 and 2001 in karst pasture *(Vidrih et al., 2009c)*

From the total area of grassland in 2008 (285,973 ha) 46,249 ha is harvested once, 118,623 is harvested twice, 88,935 ha is harvested three times and 23,104 ha is harvested four or more times per year *(Surs, 2008)*. The area of common pastures amounts to 9,602 hectares.

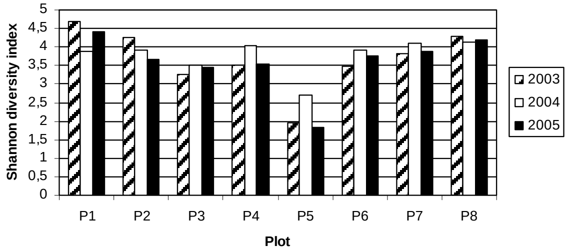

Figure 4. Shannon diversity index according to years and plots (P1-P8) in spring aspect in karst pasture *(Vidrih et al., 2009b)*

Future needs of grassland management

Grasslands of EU are an embedded part of pastoral and mixed-farming systems and have traditionally been used for livestock grazing and hay-making, or both. In broad terms, high biodiversity values coincide with low agricultural inputs, low stocking densities and often labour-intensive management practices *(Gibon, 2005)*. Particularly important are the small-scale farming systems that are responsible for creating and maintaining the species-rich semi-natural grasslands, which are often true hot spots for biodiversity. Generally speaking, livestock species, stocking densities, and timing of grazing and mowing have a strong influence on the grassland habitat and its wildlife. Too long grazing period is generally harmful to biodiversity, but the optimum livestock density and timing of grazing or mowing strongly depend on the local conditions and conservation targets.

Extensive or intensive

Many ecosystems with high nature values in EU depend on the continuation of specific forms of extensive agricultural land use. For centuries, the agricultural exploitation of large parts of EU consisted of extensive grazing and haying at low stocking rates, and low-input arable farming, although even centuries ago there already existed pockets of higher productivity *(Hopkins and Holz, 2006)*. From the middle ages onwards, the overall increase of the agricultural production in Europe occurred primarily through expansion of the agricultural area *(Hopkins and Holz, 2006)*. Natural lands were gradually put into use for agricultural production. Until about a century ago the overall intensity of land use increased only slowly. On the national level it can be demonstrated that in fact the important intensification of agricultural land use started only a few decades ago.

Extensive grassland management system is often charasterized as a type of agriculture that uses small quantity of inputs per land, like fertilizers, feeds, seeds and pesticides. It is frequently associated with low investments in machinery and buildings per surface unit and low land prices *(Peeters, 2008)*. In EU this kind of grassland management is mainly located at high latitudes and high altitudes, in Mediterranean dry rangelands as well as in hills and uplands of the British Isles *(Strijker, 2005)*. Temperate semi-natural grasslands, which are forming a large part of the biodiversity of the EU agricultural landscape *(Isselstein et al., 2005)* are an important biodiversity resource *(Pärtel et al., 2005)*. The proportion of such valuable grasslands in the EU declined during the last 50 years *(Rook et al., 2004)*. To prevent a further reduction of both bio-diverse grassland and the number of animals able to maintain it, systems enabling biodiversity maintenance and agricultural production have to be developed *(Isselstein et al., 2005)*.

Conclusion

Grassland and its use will continue to be of major importance throughout EU. The role of grassland for the provision of feed for ruminants may from time to time decreases some what through competition from concentrate feeds and other forage crops *(Lemaire et al., 2005)*. Research already indicates ways in which the use of nitrogen and phosphorus can be improved to both increase production efficiency and reduce losses to the environment. With increased adoption of organic farming, short-term grassland, including legumes, grown in rotation with arable crops will be important in maintaining the level of production of the complete systems. The use of grassland for environmental protection and enhancement will be of increased importance. The management of substantial areas will be targeted principally on biodiversity and landscape.

Grassland science faces new challenges which need to be addressed by better integrating the available knowledge in areas, such as biodiversity, climate change, carbon sequestration and farming systems, which too often have been considered separately. Grassland managers are confronted to multiple questions concerning e.g. the balance between environmental and production goals, the balance between food and energy (bio-fuels) supply, the mitigation of and the adaptation to climate change. Grassland science needs to help answer these questions by progressing towards decision support tools that are informed by the best available research and that allow for a quantitative integration of knowledge *(Soussana and Duru, 2007)*.

The importance of grassland as the basis for the ruminant livestock industries in the temperate zone of EU seems likely to continue for the foreseeable future, despite competing demand for land use *(Hopkins and Wilkins, 2006; Vidrih, 2007)*. The search for the balance between the forage productivity with intensification (use of mineral fertilisers, pesticides and re-seeding) and environmental functions of grasslands will continue not only on the regional but also national scale in EU.

Iskorišćavanje travnjaka u Evropskoj Uniji i Sloveniji

M. Vidrih, J. Čop, S. Trdan, A. Vidrih, K. Eler

Rezime

Travnjaci u EU zauzimaju značajane površine u strukturi ukupnog poljoprivrednog zemljišta. Na njima se zasniva stočarska proizvodnja, izbalansirana između održanja ekosistema i potreba životinja. Prirodni travnjaci zauzimaju prosečno u 27 zemalja EU 13%, ali se raspored teritorijalne

zastupljenosti razlikuje od zemlje do zemlje. Najzastupljeniji su u severnom delu Evrope (Engleska, Irska) oko 45% poljoprivrednih površina, dok su u Finskoj i Grčkoj zastupljeni na svega 1%. U Sloveniji prirodni travnjaci zauzimaju 14%. Travnjački ekosistemi čine značajan deo biodiveriteta u EU. Pretnja prirodnim travnjacima dolazi od povećane potrebe za poljoprivrednim proizvodima, a time i pretvaranjem travnjaka u oranice. Takođe napuštanje travnjaka u graničnim područjima i njihovo pretvaranje u druge ekosisteme predstavlja opasnost po njihov opstanak. U ovom radu je obrađeno stanje upravljanja travnjacima u prošlosti i sada, sa posebnim naglaskom na pravce promena u budućnosti koje se odnose na traženje balansa između mera u cilju intenzifikacije proizvodnje stočne hrane (primena mineralnih đubriva, pesticida, usejavanja). Takođe je obrađen ekološki značaj travnjaka ne samo sa lokalnog, već i sa šireg, nacionalnog značaja.

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THE FUTURE OF PLANT DIVERSITY IN GRASSLAND FARMING VEGETATION – A REVIEW OF DIVERSITY IN A STRONGLY TRANSFORMED AGRICULTURAL LANDSCAPE

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Invited review paper

Abstract: Austrian agriculture is widely dominated by grassland management (ca. 60%). Almost half of this area is used for forage production from meadows or as pastures. In case of extensively managed low-productivity grassland, a species rich community of specialised plant and animal taxa leads to highly differentiated biotopes. Many of these cultivated sites are nowadays exposed to changing cultivation practice due to the intensification process and also to temporary abandonment under unfavourable conditions. In fact, within the last four decades more than 300,000 hectares of low-productivity grassland got lost in Austria as a result of afforestation or by giving up cultivation. In this review we outline, that the number of plant communities along with the number of species decreases constantly and significantly with increasing land use intensity and on fallow land. For instance, alpine pastures decline as Austrian Alps are an area which is difficult to manage. Extensively-used meadows have more than three times as many species on average, than intensively-used meadows. Despite utilising traditional management, remaining habitat fragments may still loose specialist species due to intensification in the surrounding farmland. For these reasons, traditional management of semi-natural habitats is today a common conservation measure in Europe.

Key words: grassland farming, alpine pastures, meadows, traditional management, species richness, Austria

Introduction

In Europe, the present-day landscape has been strikingly dependent on a strong impact of human activities for several millennia. Consequently much of the recent vegetation composition is the result of long-term interactions between species requirements, environmental gradients and anthropogenic management *(Cousins and Eriksson, 2008)*. In Europe the long lasting traditional grassland management has created grassland habitats with small-scale species richness *(Myklestad, 2004; Pykäla, 2005; Angeringer and Karrer, 2008; Niedrist et al., 2009)*. In these semi-natural grasslands, the total exclusion of synthetic fertilizers or ploughing may promote high species diversity (*Cousins and Eriksson, 2008*). Generally, the number of species in Austria is impressive, allowing for estimates which include more than 21,000 plant and fungi species so far *(Sauberer, 2008)*. Among all the habitats low-productivity grassland" is simply the most species-rich habitat of our homeland“ *(Pils, 1994; Mohl and Bogner, 2008)*. Not only is the high number of species remarkable, but in addition, low-productivity grasslands host many habitat specialists and endangered species *(Hassler and Bernhardt, 2007; Bernhardt and Mühlbauer 2009; Bergamini et al., 2009)*. Therefore, the European Union Habitats Directive has considered this cultural landscape a priority habitat for conservation.

In the modern agricultural landscape most of the traditionally managed semi-natural grasslands have been vanished as a result of drainage, altered cultivation, fertilisation and afforestation *(Bernhardt and Kropf, 2006; Cousins and Eriksson, 2008)*. These man-made affections are followed by a spatial decrease in heterogenicity as well as in species richness *(Griffin et al., 2009)*. In the last four decades, more than 300,000 hectares of low-productivity grassland got lost in Austria *(Mohl and Bogner, 2008)*, mainly as a result of abandoned utilisation and successive establishment of woody vegetation or by afforestation. Additionally, the closely linked socio-economic situation of farmers promotes that low-productivity grassland is highly endangered in Austria *(Bernhardt and Kriechbaum, 2006; Hassler and Bernhardt, 2007; Holzner 2007, Mohl and Bogner, 2008, Koch et al., in press)*. Since biodiversity is strongly dependent on land use, the establishment of species rich communities is threatened by alteration of cultivation practice or total abandonment.

As one consequence specialists of nutrient poor grassland may suffer from isolation and altered abiotic conditions associated with periphery effects from continuous land use change in the close proximity, from nutrient spill-over by more intensively used areas as well as from atmospheric nitrogen deposition *(Lienert et al., 2002; Hooftman et al., 2003; Galeuchet et al., 2005; Bernhardt and Mönninghoff, 2006; Bossuyt, 2007; Bergamini et al., 2009)*. For instance, alpine pastures and meadows in Austrian Alps harbour high species diversity *(Tappeiner et al. 1998; Tasser et al. 2003; Bernhardt and Mönninghoff, 2006; Holzner, 2007; Marini et al., 2007; Blaschka et al., 2008; Niedrist et al., 2009)*. These grasslands, however, are nowadays endangered due to the fact, that more and more farmers give up the traditional extensive management. Thus, many low-productivity grasslands are transformed into woodlands either by natural succession or forced by afforestation.

In the present review, we will outline two recent examples of land use change in grassland farming systems. The studied habitats are located in the Eastern Austrian lowland and in alpine pastures, respectively. Both demonstrate a clear loss of biodiversity (species richness and habitat or management richness) as a result of changed agricultural practice.

General situation of grassland farming in Austria

With almost two-third (61%) of the total farming area, the Austrian agriculture is widely dominated by grassland management (1.8 Mio ha). Approximately one half of this area is used for forage production from meadows or as pastures (*Angeringer and Karrer, 2008)*. In key areas of farming in the Austrian Alps, permanent grassland and field forage culture occupy 80 - 100% of the total agricultural area (*Buchgraber and Gindl, 2004)*. Among grasslands, meadows which are mowed more than two-time per year amount to 44%. The area of farmland used as mountain or alpine pastures is in decline and covers 41% of the total area *(Buchgraber and Gindl, 2004; Holzner, 2007)*. On the other hand in Eastern Austria seasonal flooding meadows are still traditionally used. Along the river "March" (community of Marchegg) for example, grassland types persist which correspond to wet meadows or flooding meadows. Most of these sites are no longer cultivated or are used extensively in conservation management programs.

Materials and Methods

To study the change in diversity of habitat types, historical maps ("March") and aerial photographs (Alps) were evaluated and compared with the present situation of vegetation composition and habitat structure. These historical documents were also considered to evaluate species richness in former times *(Werschonig, 2008; Lapin, 2010)*.

Results

Example 1: Marchegg in Lower Austria

Between 1821 and 1946, 39% of the agricultural area in the community of Marchegg was used as meadows on average (Figure 1). In 1996 and 2009 the percentage of meadows decreased nearly to 12% in this region. Reasons of this decline are drainage, afforestation and abandonment *(Lazowski, 1990)*. Between 1821 and the first part of the 20th century, grasslands in Marchegg were important for the use as litter meadows *(Kuyper et al., 1978)*. This kind of grassland usage became essential due to increase of stable stock farming. For the production of litter for cowsheds, poorly decomposable grassland vegetation on wet and nutrient-poor soils was mown late in the course of a year. Analyses of different biotopes in the period from 1821 to 1946 show, that the natural landscape was formed by establishing extensive meadows and pastures. The dynamics of the lowland along the river “March” significantly affected the shape of the landscape until its regulation in 1914. In addition, the construction of a flood dam in 1930 altered the region’s characteristics by protecting the area from flooding and affecting the structure of aquatic habitats. The legal consolidation of agricultural lands in the 1950’s furthermore influenced the formation and structure of biotopes in the region.

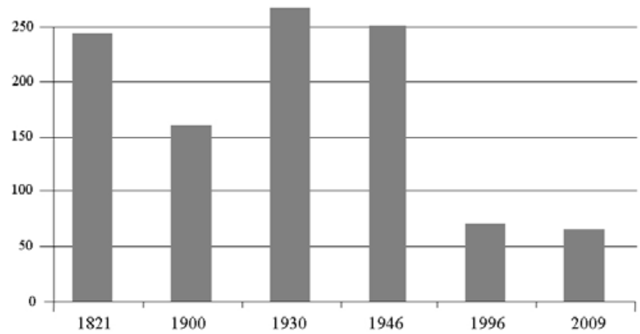

Figure1. Fluctuation in the area size of grassland (in ha) used as meadows in Marchegg between 1821 and 2009

In line with this, extensive meadows which shaped and formed the landscape diminished and became isolated over recent decades (Figure 2). To avoid further decline, such grasslands should remain environmentally protected as many are no longer used and thus lie fallow. These changes to the landscape caused by the expansion of agricultural lands (i.e. drainage) were exacerbated within a period of 50 years.

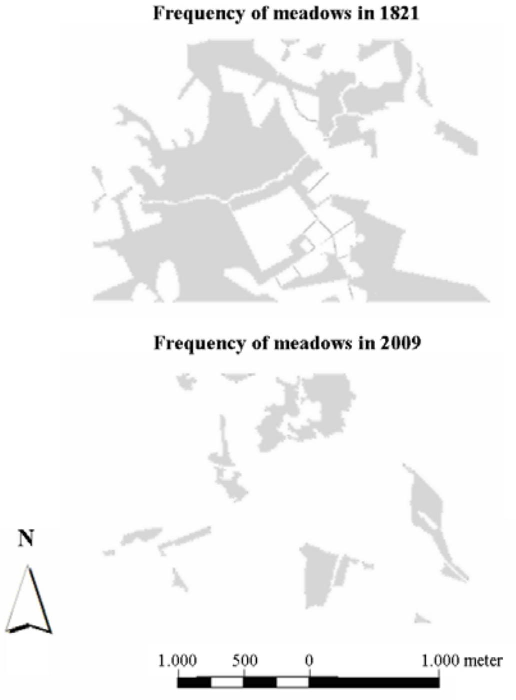

Figure 2. Comparison of the distribution of meadows in the community of Marchegg

Extensively managed hay meadows of the “Marchwiesen”, which were two times mown and not artificially fertilized, have been used over the last 200 years. A lot of specialised species like *Clematis integrifolia, Cnidium dubium, Iris sibirica, Eryngium planum* and *Allium angulosum* are endangered today because of the constant loss of habitats. The investigation of historical herbar material (1890 – 1940) elucidated a wide and frequent distribution of the mentioned species to that time. The current change of land use, however, has led to a decline of wet or flooded meadows followed by a loss of habitat specialists.

Example 2: National park “Gesäuse” in the Eastern Alps

Historical and recent aerial photographs of the surroundings of abandoned alpine farms “Eggeralm” and ”Wolfbauernhochalm” provide the opportunity to compare vegetation patterns of 1954 and 2003 and to monitor the progress in succession. The performed vegetation studies revealed, that the ”Eggeralm” is nowadays characterised by a high percentage of meadows classified as *Deschampia caespitosa*-type, Polygono-Trisetion and Caricetum ferrugineae (Table 1). On the whole research area “Eggeralm”, a dominant coniferous woodland (*Adenostyles alliariae-Alnetum*) with almost exclusive occurrences of *Picea abies* and *Larix decidua* was recorded. While meadows were more frequent on “Eggeralm”, the “Wolfbauernhochalm” was used as a forest pasture in the past. Today this research area has the highest percentage of woodland in our survey. Meadows which belong to Polygono-Trisetion (Table 1) community occur around the ancient alp buildings (Figure 4). Additionally, some small patches of Caricetum ferrugineae-meadows were detected between the forest areas (Table1), creating a characteristic mosaic with the woodland.

Table 1. Recorded plant communities based on phytosociological survey of the vegetation on “Eggeralm” and”Wolfsbauernhochalm“ in the subalpine region ”Gesäuse“.

Plant community (class)	Sub-classification
Molinio-Arrhenatheretea R. Tx. 1937 em. R. Tx. 1979	*Molinietalia Koch 1926* Deschampsia caespitose-(Molinietalia)-society *Poo alpinae-Trisetetalia Ellmauer et Mucina 1993* Polygono-Trisetetion Br.-Bl. et R. Tx. ex Marschall 1947 nom. inv. Trisetetum flavescentis Rübel 1911 Astrantio-Trisetetum Knapp et Knapp 1952
Calluno-Ulicetea Br.-Bl. et R. Tx. et Klika et Hadač 1944	*Nardetalia Oberdorfer ex Preising 1949* Nardio-Agrostion tenuis Sillinger 1933 Homogyno alpinae-Nardetum Mráz 1956
Seslerietea albianctis Oberdorfer 1978 corr. Oberdorfer 1990	*Seslerietalia coeruleae Br.-Bl. et Jenny 1926* Caricion ferrugineae G. Br.-Bl. et J. Br.-Bl. 1931 Caricetum ferrugineae Lüdi 1921
Mulgedio-Aconitetea Hadač et Klinka in Klinka et Hadač 1944	*Adenostyletalia G. Br.-Bl. et J. Br.-Bl. 1931* Adenostylon alliariae Br.-Bl. 1926 *Rumicetalia alpini Mucina in Karner et Mucina 1993* Rumicion alpini Rübel ex Klinka in Klinka et Hadač 1944 Rumicetum alpini Beger 1922
Erico-Pinetea Horvat 1959	*Erico-Pinetalia Horvat 1959* Erico-Pinion mugo Leibundgut 1948 nom. inv. Pinus mugo-society Penetum cembraea Bojko 1931 Laricetum deciduae Bojko 1931
Vaccinio-Piceetea Br.-Bl. in Br.-Bl. et al 1939	*Athyrio-Piceetalia Hadač 1962* Chrysanthemo rotundifolii-Piceion (Krajina 1933) Březina et Hadač in Hadač 1962 Adenostylo alliariae-Abietetum Kuoch 1954

The vegetation research and the analysis of aerial photographs of the years 1954 and 2007 in the two alpine regions exposed a lot of changes in the vegetation patterns during this period (Figure 3; Figure4). On western parts of the "Eggeralm", former tall herb communities have altered to scrub and forest stages and meadows with young tress have changed into low density forests over the past five centuries (Figure 3).

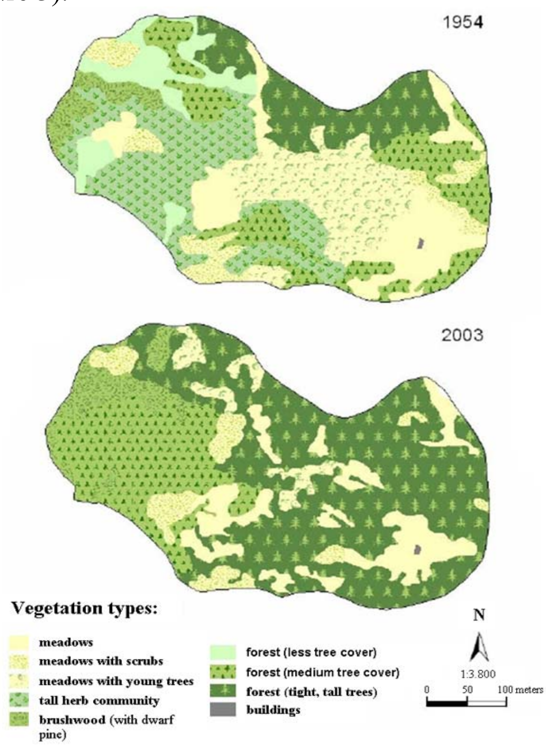

Figure 3. Changes in vegetation composition in the study area "Eggeralm" between 1954 and 2003

The "Wolfbauernhochalm" was already characterised by a higher frequency of trees in the past and a general increase of forest coverage could be observed in this study. More drastic changes occurred on sites which have been used as meadows in 1954 but are now in a succession stage of young low density forests. Taking together, an obvious decline of former grasslands was recorded in both study areas. Primarily, because more and more farmers give up the traditional extensive management and many low-productivity grasslands are thus turning into woodlands by succession due to the abandoned utilisation.

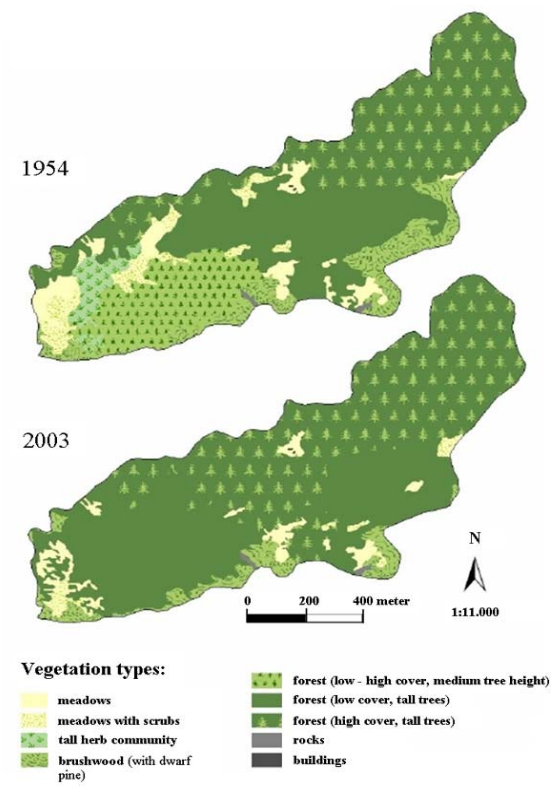

Figure 4. Changes in vegetation composition in the study area ”Wolfsbauernhochalm“ 1954 and 2003

Conclusion

Low-productivity grasslands are extremely valuable as they represent habitats for many specialised plant and animal species. However, in the last four decades more than 300,000 hectares of low productivity grassland got lost in Austria due to afforestation or simply abandonment of land *(Mohl and Bogner, 2008)*. As low-productivity grassland can be farmed only partly with modern machines its continuing usage is strongly related to traditional methods *(Wallner et al., 2007)*. These methods are time-consuming and depend on traditional knowledge and skills *(Mohl and Bogner, 2008)* and thus, land use changed in the Austrian Alps in the last two decades. As a consequence, more and more alpine farms with mountain meadows and subalpine pastures disappear, since they are no longer managed. These abandoned areas, correspond to grass respective scrub phrases, a plant community which is normally considered typical for advanced successional stages in the subalpine region *(Tasser et al., 2007)*. Moderately fertilized, once-mown alpine meadows have much lower average number of species. Due to faster and higher growth in fertilized areas, most of the sunlight is already absorbed in the upper canopy layer while only a small amount of light reaches the lower layers *(Niedrist et al., 2009)*. As a result, a few highly competitive species are favoured to dominate, whereas stress-tolerant species disappear *(Marini et al., 2007)*. The apparent trend of decreasing species number with increasing intensification of land use was observed in flooding meadows in the lowland "March" region, too. The call for conservation strategies, however, is not only due to a decrease in total species richness, but it is also the loss of habitat specialists which demands protection measures.

To include the temporal dimension of changes in vegetation patterns within cultural landscapes, we examined historical maps and aerial photographs in our study. In fact, the importance of historical landscape patterns and fragmentation as well as the change of habitats has become more in focus in recent landscape studies *(Lindberg and Eriksson, 2004; Lunt and Spooner, 2005; Helm et al., 2006; Gustavsson et al., 2007; Cousins and Eriksson, 2008)*. Since it is important to clearly state how large the habitat extent has been in the past and to estimate the time since the land use change accompanied by fragmentation, has initiated *(Cousins and Eriksson, 2008)*, historical documents certainly represent valuable tools to monitor changes in the vegetation and design promising conservation measures for threaded habitat types such as low-productivity grasslands.

From a conservation point of view it is interesting to investigate the biodiversity potential of grassland fragments in ordinary landscapes as they have a great importance to act as reservoirs of plant species richness in most agricultural landscapes. Thus, maintaining low-productivity grassland management is essential and restoration of large abandoned grasslands may conserve and improve species richness at a local landscape scale *(Spiegelberger et al., 2006; Cousins and Eriksson 2008)*.

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Buduće stanje biljnog diverziteta travnjnačke vegetacije – pregled diverziteta na veoma izmenjenim poljoprivrednim površinama

K.-G. Bernhardt, K. Lapin, E. Werschonig

Rezime

Poljoprivreda Austrije se u velikoj meri oslanja na upravljanje travnjacima (oko 60%). Skoro polovina od ukupne površine pod travnjacima koristi se za proizvodnju krmnog bilja sa livada i pašnjaka. U slučaju ekstenzivno iskoriščavanih travnjaka niske produktivnosti dolazi do visoke diferencijacije biotopa na staništima bogatim specijalizovanim taksonima biljaka i životinja. Na mnogim lokalitetima primećuje se promena u primeni agrotehničkih mera usled procesa intenzifikacije korišćenja, ali i privremenog napuštanja u slučaju nepovoljnih sredinskih uslova. U stvari na području Austrije u toku poslednje četiri decenije je preko 300,000 hektara slabo produktivnih travnjaka izgubljeno kao posledica pošumljavanja ili odsustva iskoriščavanja. U ovom radu se ističe da broj biljnih zajednica zajedno sa brojem vrsta konstantno i značajno opada sa rastom intenzifikacije iskoriščavanja travnjaka i krčevina šuma. Tako na primer, površine pod alpijskim pašnjacima se smanjuju, jer je u zoni Austrijskih Alpa njihovo korišćenje teško izvodljivo. Ekstenzivno korišćene livade ispoljavaju u proseku više nego tri puta veći specijski diverzitet u odnosu na one intenzivno korišćene. Uprkos primeni tradicionalnih mera iskoriščavanja na travnjacima, fragmenti preostalih staništa još uvek mogu izgubiti svoje vrste specijaliste zbog intenzifikacije upravnjanja na okolnim površinama. Iz tih razloga, tradicionalni načini korišćenja na poluprirodnim travnjacima se danas smatraju zajedničkom konzervacionom merom u čitavoj Evropi.

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PRODUCTION OF LIVESTOCK FOOD ON NATURAL AND SOWN GRASSLANDS

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Invited review paper

Abstract: In this paper the synthesis of results of long lasting researches of production capacities of natural and sown grasslands in lowland and hilly-mountainous regions of Serbia is presented. Grasslands represent significant resource in forage production and livestock production on whole. This resource has not been exploited to the extent ensured by the potentials. Productivity of natural grasslands is extremely low, but with adequate fertilization it can be increased considerably, even by 10 times. The greatest contribution to the increase of production have nitrogen fertilizers where 1 kg of applied fertilizer realized the production of 9,6-59,7 kg of hay per 1 kg of applied N. Impact of PK fertilizer is not so considerable (2,7- 19,1 kg of hay per 1 kg of applied nutrient), but these fertilizers are also necessary. Most of research studies on meadow associations confirmed that ratio of N:P:K nutrients should be 2-3:1:1, with basic rates of 40-50 kg, i.e. $N_{80-150}P_{40-50}K_{40-50}$. Fertilizers have greater impact on more productive associations. Research that has been carried out for many years confirmed that sown grasslands represent exceptional potential for forage production. It was determined in these researches that it is possible to realize production of up to 20 t ha^{-1} in lowland regions and 10 t ha^{-1} in mountain region. Forage production and quality is in positive correlation with ratio of leguminous plants in the mixture. Sustainability of leguminous plants in the mixture during exploitation is often very serious problem, primarily on higher altitudes and soils of poorer quality. Solution of this problem should be objective of research programs in future period.

Key words: Natural grassland, sown grassland, quality, production

Introduction

Grasslands in Serbia represent natural resource and great production potential in development and improvement of livestock production, especially in hilly-mountainous regions, which has not been exploited to the full extent by far. This is confirmed by numerous results obtained in investigations that have been conducted for many years, and which show that production on the grasslands can be considerably increased in very short time without disruption to established natural balance and unfavourable impact on the environment. According to the latest statistical data, grasslands extend over the land area of 1.454.000 ha – natural grasslands, and approx. 150.000 ha – sown grasslands which makes 31% of total agricultural land. Recently, the neglect of natural grasslands is occurring, they are covered with weeds and becoming forest associations. Similar tendencies are also present in Europe, especially marginal areas of hilly-mountainous regions *(Peeters, 2008)*.

Although the productivity of grasslands is low, primarily of natural grasslands (meadows 1.9 t ha^{-1}, pastures 0.6 t ha^{-1}), it is possible to produce from generated biomass 2.000.000 t of milk or 200.000 t of meat.

It should be pointed out that present total production of milk is by 30% less than the production which could be realized only on grasslands as they are today. So, their utilization is minor. If, in addition, potentials of the livestock production on grasslands are stated, opportunities which open for total improvement of agricultural production are obvious.

The fact that every third hectare of agricultural land is under grasslands, and that in the hilly-mountainous regions they are the main or even single source of forages, confirms that no program of livestock development and improvement can be initiated without activities on the grasslands.

For this purpose, research results and some examples realized in practice, as well as potential approach in improvement of production of livestock on grasslands will be presented in the paper.

Natural grasslands

Natural grasslands take the greatest part of agricultural land area in Serbia (approx. 28%). They are of different production potential depending on the association or even the components of the same association. Differences in agro-ecological conditions have induced the incidence of more associations which in regard to the way they were formed can be divided into primary (climax) associations formed on high mountain massifs above the forest zones or in marsh/swampy or salty soils, and secondary associations formed as consequence of the human actions and in the forest zones and which are changeable, i.e. have tendency to return to primary associations which are mainly forest associations. *Kojić et al. (2004)* pointed out that in Serbia until now 273 associations have been registered, but in the most recent researches and systematization it was established that they can all be divided into 48, and the following 10 have the highest economic importance: *Molinietum coeruleae, Alopecuretum pratensis, Cynosuretum cristati, Agrostietum vulgaris, Danthonietum calycinae,* *Chrysopogonetum grylli sirbicum, Chrysopogonetum grylli pannonicum, Festucetum vaginatae, Poetum violeceae* and *Nardetum strictae*. Great majority of these associations, with the exception of those located in high mountain regions above forest zone are closely related and in correlation to activity of the humans.

From numerous researches which lasted for several years and focused on the possibility and ways to improve production of livestock food on natural grasslands it results that yields can be increased significantly and over short period of time, with adequate utilization of mineral fertilizers. Application of $N_{100}P_{100}K_{100}$ in association *Danthonietum calycinae* on Goč, *Stošić (1974)* managed to realize average increase of yield by 422%, and $N_{200}P_{150}K_{100}$ by 675%, and compared to parcel without fertilization where production was very low (0,74 t ha^{-1}). There is not the same fertilizer effect on associations . *Lazarević et al. (2003)* pointed out that more productive associations such as *Chrisopogonetum grylli* and *Agrostietum vulgaris* have relatively lower increase of production (200 and 214%, respectively) compared to less productive associations such as *Koelerietum montanae* where with application of $N_{120}P_{60}K_{60}$ production was increased by even 1024%. However, in absolute sense, $N_{150}P_{50}K_{50}$ resulted in 7.64 t ha^{-1} of hay in association *Chrisopogonetum grylli* and 4.96 t ha^{-1} in *Agrostietum vulgaris*, whereas $N_{120}P_{60}K_{60}$ resulted in 2.56 t ha^{-1} of hay in association *Koelerietum montanae.* This can clearly be seen in the Figure 1.

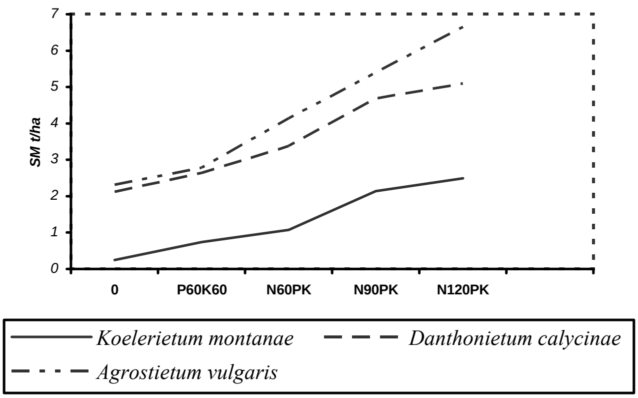

Figure 1. Effect of different N rates at equal level of PK on yield of grasslands on the same location (Radočelo) ***(Kojic et al., 1992)***

In addition, same association in different locations does not react in the same way to application of mineral fertilizers which can be seen from the Table 1.

It is noticable from presented results that the nitrogen fertilizer has the highest contribution to the increase of yield. Dominant share of species from the family *Poaceae* and their excellent utilization of nitrogen influence the intensive growth and increase of production by 5 to 6 times compared to unfertilized plots. Increase of nitrogen fertilizer rate influences the increase of production, but with higher rates increase is less, especially in more productive locations. For instance, on location Javor 50 kg of N increased production by 40%, subsequent 50 kg (N_{100}) by 31% and finally increase to 150 kg by 8%. It is even more distinct on location Radočelo 77, 12 and 5%, respectively.

Table 1. Yield of dry mass of ass. *Danthonietum calycinae* on different locations in hilly-mountainous regions of Serbia *(Stošić et al., 1999)*

Fertilization	Pešter			Goč			Javor			Radočelo		
	t ha^{-1}	%	kg hay per kg N	t ha^{-1}	%	kg hay per kg N	t ha^{-1}	%	kg hay per kg N	t ha^{-1}	%	kg hay per kg N
Control	0.57	100		0.83	100		1.33	100		2.05	100	
$P_{50}K_{50}$	0.84	147		1.30	157		3.24	244		2.55	124	
$N_{50}P_{50}K_{50}$	1.32	232	9.6	2.61	314	26.2	4.55	342	26.2	4.51	220	39.2
$N_{100}P_{50}K_{50}$	2.31	405	14.7	4.02	484	27.2	5.97	449	27.3	5.06	247	25.1
$N_{150}P_{50}K_{50}$	2.90	509	13.7	5.14	619	25.6	6.42	483	21.2	5.33	260	18.5
Lsd 5%	0.22	39		0.46	55		0.78	59		0.55	27	
1%	0.30	53		0.63	76		1.04	78		0.76	37	

The greatest effect of the nitrogen fertilizer on less productive associations is realized with use of N_{100}, whereas in more productive associations even with lower amounts of N fertilizer. Similar data is presented also by *Vučković et al. (2005a, 2005b).*

Production of plant mass per one kilogram of applied fertilizer depends on the production potential of the association itself, which can be seen from data presented in the Table 2.

Table 2. Effect of 1 kg N and PK nutrients on dry matter production (kg) *(Lazarevic et al., 2003)*

Association	**PK***	N_1PK	N_2PK	N_3PK	N_4PK
Chrysopogonetum grylli (Rudnik)	13.5	33.2	40.0	41.9	
Lolio-Cynosuretum cristati (Javor)	19.1	26.2	27.3	21.2	
Danthonietum calycinae (Goč)	4.7	26.2	27.2	25.6	
Danthonietum calycinae (Pešter)	2.7	9.6	14.7	13.7	
Festuco-Agrostetum (Kopaonik)	12.0	22.0	15.0	16.6	
Agrostietum capillaris (Radočelo)	3.8	45.3	43.6	43.0	37.5
Danthonietum calycinae (Radočelo)	4.3	24.6	34.0	27.3	23.0
Koelerietum montanae (Radočelo)	4.1	11.0	23.3	19.4	17.2
Festucetum rubrae (Pešter)	3.9	37.3	21.2	20.7	20.7
Festucetum vallesiaceae (J. Kučaj)	2.4	59.7	40.8	33.0	30.2

*$P_{50-60}K_{50-60}$ N_1- 30-50, N_2 – 60-100, N_3 – 90-150, N_4 - 120

As presented, 1 kg of nitrogen fertilizer realized production of 9,6-59,7 kg of hay, and 1 kg PK resulted in 2,7- 19,1 kg. Most of researches done so far on most of meadow associations confirm that nutrient ratio should be: $N:P_2O_5:K_2O$ or N:P:K 2-3:1:1 and 4,7-9,2:1:1,9 *(Stošić and Lazarević, 2007)*. Amounts depend on association, soil fertility and weather conditions. If the grassland is more productive, in that case it has greater capacity of utilizing greater amounts of nutrients and if the amount of precipitation is high, more fertilizer can be applied. Considering that natural grasslands are present in hilly-mountainous region where vegetation period is shorter or in lowland regions, which is mainly Vojvodina, where the second part of the summer is very dry, all planned rates of mineral fertilizers should be applied in spring, before the start of vegetation *(Stošić, 1974; Stošić et al. 1996)*. Nutrients for natural grasslands in Serbia should be within following limits: N 40-120, P_2O_5 and K_2O per 20-50 $kgha^{-1}$.

Application of only phosphorus or potassium increases insignificantly yield and only after several years of application. It affects the change of floristic composition because it influences the increase of leguminous species ratio *(Lazarević, 1994; Stošić et al., 1999, 2004)* and increase in this way indirectly the yield and directly improve the quality of plant biomass.

Sown grasslands

Forage production on sown grasslands has been introduced more intensively and with great success at the end of sixties of the last century. Establishing of sown grasslands was most important in hilly-mountainous regions. This way of forage production represents connection between field and livestock production, because sown grasslands occur as independent, long lasting culture or fields within crop rotation. They have special importance on slopes and lighter soils, which is general characteristic of higher altitudes. They expanded and took broader land areas which were used for crop production, eliminating the need for frequent soil cultivation and thanks to that played decisive role in protection of soil from erosion. In lowland regions sown grasslands were grown on border soil types such as pseudoglay, saline soils, brown forest soil and smonitsa or waterlogged areas. It is rare occurrence that they are formed within farm systems as very intensive production form. It is considered that during the period of the greatest expanding of sown grasslands, they were cultivated on 150.000 ha, but today, the land area under sown grasslands has ben significantly reduced, primarily due to extreme decrease of livestock fund. Sown grasslands in mountainous region are mainly established instead of natural ploghed through grasslands, because of decline in the intensity of production and utilization they are gradually transformed into new plant associations which are looking more like natural grasslands. Therefore, or division of grasslands in natural and sown should be adjusted to the new situation giving us the basis to introduce categories similar to the English system.

Scientific and research results, application of results and experience in forage production on sown grasslands in our country had several stages over the period of fourty years, which differ in their duration and gradually crossing from one to another. Fact is that the most of the activities took place in Kruševac, and this is still the same, considering that specialized research institution (Department, Centre, Institute for Forage Crops) and most of the experts are located in this city. For the purpose of better visibility, in the continuation research results according to subject/topics will be presented which at the same time represent also chronological entry.

Composition of mixtures: Biological diversity and polymorphism of species in the families *Fabaceae* and *Poaceae* which are components of the mixtures ensure numerous combinations according to different traits and purposes. The most important are life duration and vertical (underground and above ground) distribution of the vegetative mass. For the beginning of researches in this field, during the fifties and sixties of the last century, when the knowledge of biological traits of most of the species was not so extensive, especially grasses, characteristic was composing of mixtures with great number of species, often more than ten *(Đorđević et al., 1968)*. When certain species were studied in more detail, especially their competing abilities, and with the increase of production intensity, especially with fertilization, number of species was significantly reduced *(Lazarević et al.,1998)*. Also, there are cases where individual species were used, especially in farm production system. In the third stage, with the decrease of the production intensity, number of species increased to 4-6 *(Stošić and Lazarević, 2007)*.

Key question in determining of composition of every mixture intended for forage production is selection and ratio of leguminous species to grasses. Desire to have as much possible of leguminous plants in the mixture, because of their quality and nitrogen fixation, is significantly limited by ecological conditions of hilly-mountainous regions, where sown grasslands are mainly located, and poor soil types where nitrogen fixations is impeded. In our conditions mainly alfalfa, red clover and birdsfoot trefoil are used, since for white clover there were no adequate types (ladino), and sainfoin had no adequate place in the researches or practice. The greatest limitation for higher share of alfalfa in mixtures and its longer sustaining is acid soil, for red clover short life cycle and for birdsfoot trefoil low competitive ability. Therefore, the most frequent case is that after the first, and especially the second year of exploitation, leguminous-grass mixtures become only grass mixtures. For this purpose researches were initiated in Serbia to study limiting factors for sustaining of alfalfa in mixtures, but these studies were insufficiently systematic and were not finished. This is one of the important tasks for science in future period, and in order to get to know it better and realize it efficiently, it is necessary to organize multidisciplinary approach to the study.

Main orientation in selection of leguminous component should be the following: on deeper, more fertile, neutral or mildly acid soils up to 1.000 m a.s.l., alfalfa should have the advantage, and on dry, sandy and acid soils – birdsfoot trefoil. Red clover can be grown on all types of soil, only provided that they are not too dry, or in combination with alfalfa or birdsfoot trefoil, but always bellow 20%. In hilly-mountainous regions, white clover appears spontaneously in mixtures. Since this is prostratum type, it is not significant, especially in predominant system of exploitation – cutting, because cutting machines don't catch it.

Contrary to leguminous plants with limited number of species, and extensive limitations in regard to environment conditions, in case of grasses the selection of species is greater and also great selection of grass cultivars in the world. This enables composing of mixtures precisely according to set characteristics in regard to duration of exploitation, purpose, quality, production intensity, frequency of exploitation, etc. In general, it can be recommended for intensive systems, including into mixtures of high quality species with shorter exploitation period (Italian and perennial ryegrass), for waterlogged areas tall fescue, and for more dry and sandy soils smooth brome, for lighter soils in hilly region tall oatgrass, for deeper and more fertile soils cocksfoot and meadow fescue, and for mountain region timothy *(Lazarević et al., 2006, Stošić and Lazarević, 2007)*. This would be the leading grass components in the mixture, and other species, among which are low plants (red fescue, redtop, Kentucky bluegrass) as accompanying plants for better filling of the space *(Radojević and Stošić,1975)*. Choice of leading grass component and its cultivar are of key importance and time of beginning of exploitation depends on it. Adequate combination can be used to organize continuous production for longer period, which is extremely important for grazing system of exploitation. As an example we can use results obtained on mountain Kopaonik (1200 m above sea level) where use of first cut started on May 20th (red fescue), and ended on June 25th (timothy), which means that in the period of 35 days there was continuous exploitation of vegetative mass *(Tomić et al.,1989)*.

Based on research carried out so far and acquired experiences in broad production, it can be concluded that leguminous components should participate with 20-30% of sward in the mixture. This was always very important question since it was necessary to find balance between production, quality and longer sustaining of projected mixture. *Pflimlin and Journet (1983)* stated that share of white clover in mixture of 30% provides balance between high production and quality of biomass. Similar data are presented by *Murphy (1987)* stating that 35% of white clover is necessary. Share of leguminous component cannot be same with all species. In case of higher species (alfalfa, red clover) can be greater in comparison to lower species (white clover, birdsfoot trefoil), which is indicated by results of *Stošić and Jeremić (1981).* Less than that would have no importance for the quality of livestock food and production rationalization, and more would be uncertain since after withdrawel of leguminous component there is a lot of empty space which grasses cannot fill even with more intensive clustering. Therefore yields are decreasing and quality is worsening since the empty space is being filled by weeds.

Production of sown grasslands: Sown grasslands can realize extremely high productivity of good quality biomass, depending on the region/area where they are established and on management. In lowland region even up to 20 t ha^{-1} *(Lazarević et al., 1998, 2001, 2005, 2006)*, whereas in the mountain region production is significantly lower, on altitude of 1000m a.s.l. up to 13 t *(Lazarević et al., 2004)*, and on higher altitudes up to 10 t ha^{-1} *(Stošić i Lazarević, 1997; Lazarević et al., 2006)*.

Mixtures as a rule result in increased production of grassland compared to individual leguminous plants and grasses fertilized with 100 kg ha^{-1} N *(Lazarević et al., 1998, Soegard and Nielsen, 2000)*. *Morrison (1988)* stated that mixture compensates 200 kg N, i.e. the mixture is expected to realize 80% of grasslands fertilized with 300-400 kg N ha^{-1}. *Bax and Thomas (1992)* pointed out that share of leguminous plants of 25% increases the consumption of food by animals by 10% and that mixtures realize yield the same as grasses fertilized with 180-200 kg ha^{-1} N. Results obtained at field trial of the Institute for Forage Crops from Kruševac show that mixtures have more yield than individual grasses and leguminous plants, as presented on Figure 2.

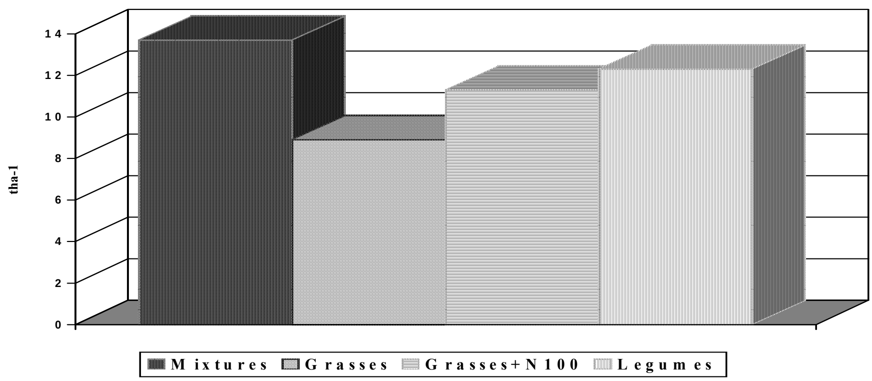

Figure 2. Yield of individual grasses and leguminous plants and their mixtures

Production of grassland, ratio of species in mixtures and exploitation period are closely depending on the management system of all changes on grasslands during exploitation period. Fertilization has decisive role in this regard as well as system of grassland exploitation which have immediate influence on floristic changes *(Lazarević et al., 1999a)*, and fertilization on productivity *(Stošić and Radojević, 1980; Lazarević et al., 2006)*.

Fertilization of sown grasslands: Grasslands are mainly established outside crop rotation, on poor, more acidic, shallow and skeltoid soils. Therefore, it is possible, even without fertilization, to organize forage production, and also on soil of higher quality where they are established after field crops – grains. Only when grasses are subsequent to potato, because of abundant fertilization, nutrient rate can be reduced considerably or even leave out, but only in year of establishing of grassland.

Determination of the nutrient ratio and rates is a very delicate task, considerably more difficult compared to any other plant culture. Considering that the composition of mixture changes in every subsequent year of its utilization, types and rates of fertilizer must be constantly adjusted to the new situation. Point is to subordinate fertilization completely to leguminous component, in order to sustain it as long as possible in the mixture. Therefore, in the year of establishing of grassland and during exploitation, as long as leguminous plants are participating in the projected or even lower percentage, the most attention must be directed to phosphorus and potassium. Rates of these two nutrients is limited only by the treshold of rationality. In the establishing they should not exceed 60 kg ha^{-1} since the mixture is not ready to utilize so much, and fertilization "reserves" have no economical justification or agro-economical, considering that these are mainly light soils with risk of nutrient leaching. If the soils are coloid rich, there is risk of immobilization by fixation, especially of phosphorus *(Stošić and Jeremić,1981)*.

Nitrogen as decisive nutrient in production of vegetative mass, in the first stage should be administered very carefully in order to stimulate development of grass components to the extent not leading to the supression of leguminous plants. Only with the significant reduction of absence of leguminous component, can nitrogen, phosphorus and potassium ratio be the same as for natural grasslands - 2-3:1:1. Contrary to natural grasslands, for sown grasslands total amount of necessary nutrients are higher since production capacities of sown grasslands are higher. Practically, in the first year – year of establishing, nitrogen rate should be 30-50 kg ha^{-1}, and in subsequent years, depending on the botanical composition, 40-80-100 kg ha^{-1} *(Stošić and Lazarević,1997, Vučković et al., 2002)*. Decline of share of leguminous plants depending on the fertilizer rate can clearly be seen in Figure 3 *(Stošić, 1990)*.

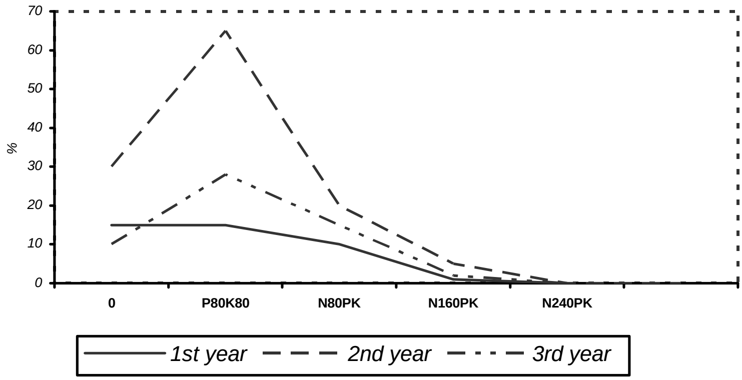

Figure 3. Influence of quantity and ratio of NPK on part of legums in mixture with grass

Nutrition of sown grasslands depends on more circumstances, but duration of vegetation period and botanical composition are the most important. Certain regularity applies here: with the longer vegetation period also the need for crop nutrition increases and with the increase of share of leguminous plants, the need for nutrition reduces. This means that in regions where three or more cuts are realized, one fourth or one third of foreseen of nitrogen rate is applied after first cut, but not after the second or subsequent cuts. If the share of leguminous plants is over 30-40%, no nutrition is applied or not more than 30-40 kg ha^{-1} N is used.

Time of cutting: Dynamics of development of biomass on sown grasslands and its distribution during vegetation period depend on several factors. The most important are ecological conditions, altitude, biological traits of leading components, fertilization, rates and distribution of precipitation, time of first utilization, etc. It is rule which applies grasslands that, regardless of the differences among them, it is the feature of the grassland that accumulation of organic substance goes on continuously from the beginning to the end of vegetation period. However, this process is not equal: at the beginning it is very intensive, and than it drops to minimum during dry season (end of July, August) and than it increases again at the beginning of September. It was established that the most intensive forming of the yield takes place in the second decade of the month of May *(Stošić,1988)*. However, simultaneously to increase of yield, the contrary tendency occurrs: decline in quality. Therefore it is a big problem how to determine time of the first cutting in order to obtain the highest rates of available nutritive substances for livestock production. Determination of the time of first cutting is hence more important if the first cut participates more in the total annual production, which is directly connected to altitude. In lowland region, the first cut makes approximately half, in hilly regions about two thirds, and in the mountain region over 90% of total production *(Stošić and Radojević,1980)*. Therefore, the first cut in the lowland region should be done at the beginning of inflorescence forming, in hilly region in the middle of inflorescence forming, and in mountain region in time of full inflorescence forming of the leading grass component. This approach enables that in the valley regions faster regeneration is achieved and additional cut, in hilly region also that the third cut is ensured and in the mountain region the second cut. Somewhat later cutting on higher altitudes affects decline of the quality but that is necessary compromise since there is no sufficient time for regeneration and obtaining of higher yields.

Quality of biomass: Sown grasslands, in addition to pure leguminous crops, represent areas on which the best quality forage can be produced. Quality of biomass from sown grasslands depends on several factors: share of species in mixtures, especially share of leguminous plants in biomass, plant development stage, fertilization, conservation and exploitation method, etc. *(Dinić et al., 1994a).*

In regard to quality of biomass, especially important are leguminous plants. Because of the high protein, mineral and carotene contents, *(Dinić et al., 1994b)* they represent excellent addition/supplement which have lower protein content but are richer in carbohydrates. Adequately balanced grass and leguminous plants ratio in the mixture provides quality diet to animals from the aspect of energy and protein needs. Researches carried out near Kruševac *(Lazarević et al. 1999/b)* determined that grasses fertilized with 100 kg ha^{-1} N had higher protein content by 4,4% compared to unfertilized grasses, mixtures 60,9%, and pure leguminous plants by 82% (Figure 4) Similar data are presented also by *Vučković et al. (2003).*

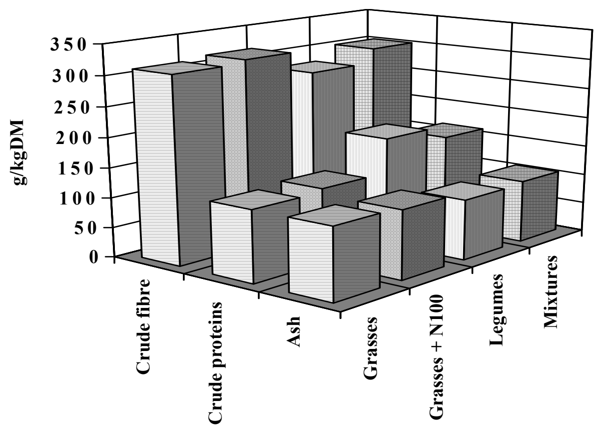

Figure 4. Contents of crude protein, fibre and ash in grasses, legumes and their mixtures

Conclusion

Undoubtedly grasslands represent significant resource in forage production and livestock production on whole. This resource has not been exploited to the extent ensured by the potentials. Productivity of natural grasslands is extremely low, but with adequate fertilization it can be increased considerably, even by 10 times. The greatest contribution to the increase of production have nitrogen fertilizers where 1 kg of applied fertilizer realized the production of 9.6-59.7 kg of hay. Impact of PK fertilizer is not so considerable (2.7- 19.1 kg of hay per 1 kg of nutrient), but these fertilizers are also necessary. Most of research studies on meadow associations confirmed that ratio of N:P:K nutrients should be 2-3:1:1, with basic rates of 40-50 kg, i.e. $N_{80-150}P_{40-50}K_{40-50}$. Fertilizers have greater impact on more productive associations.

Research that has been carried out for many years confirmed that sown grasslands represent exceptional potential for forage production. It was determined in these researches that it is possible to realize production of up to 20 t ha^{-1} in lowland regions and 10 t ha^{-1} in mountain region. Productivity and quality of produced forage is in positive correlation with share of leguminous plants in the mixture. Sustainability of leguminous plants in the mixture during exploitation is often very serious problem, primarily on higher altitudes and soils of poorer quality. Solution of this problem should be the objective of research programs in the future.

Acknowledgment

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Proizvodnja stočne hrane na prirodnim i sejanim travnjacima

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Rezime

U radu je data sinteza rezultata dugogodišnjih istraživanja proizvodnih mogućnosti prirodnih i sejanih travnjaka u ravničarskom i brdsko-planinskom području Srbije koji predstavljaju značajan resurs za proizvodnju stočne hrane, a

time i stočarstva u celini. Taj resurs nije ni izdaleka iskorišćen koliko mu mogućnosti za to dozvoljavaju. Produktivnost prirodnih travnjaka je izuzetno niska, ali se adekvatnim đubrenjem može višestruko uvećati, čak i do 10 puta. Najveći učinak na povećanje produkcije imaju azotna đubriva gde 1 kg primenjenog hraniva ostvaruje proizvodnju od 9,6-59,7 kg sena. Učinak PK đubriva je manji (2,7- 19,1 kg sena po kg hraniva), ali su i ona neophodna. Najveći broj dosadašnjih ispitivanja na većem broju livadskih asocijacija potvrđuje da odnos N:P:K hraniva treba da bude 2-3:1:1, sa osnovnim količinama od 40-50 kg, odnosno $N_{80-150}P_{40-50}K_{40-50}$. Đubriva imaju veći učinak na produktivnijim zajednicama. Dugogodišnjim istraživanjima je utvrđeno da sejani travnjaci predstavljaju izuzetan potencijal za proizvodnju stočne hrane.Istraživanjima je utvrđeno da se može ostvariti produkcija do 20 t ha^{-1} u ravničarskom i 10 t ha^{-1} u planinskom području. Produktivnost i kvalitet proizvedene stočne hrane je u pozitivnoj korelaciju sa učešćem leguminoza u smeši. Održivost leguminoza u smeši tokom perioda eksploatacije je često ozbiljan problem, pre svega na većim nadmorskim visinama i lošijim zemljištima. Rešavanje ovog problema je cilj istraživačkih programa u narednom periodu.

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BIODIVERSITY OF NATURAL GRASSLANDS OF SERBIA: STATE AND PROSPECTS OF UTILIZATION

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Abstract: Natural and semi-natural grasslands of Serbia represent one of the major resources in agriculture occupying about 1.4 millions of hectares, and distributed in a wide range from lowland to highest alpine zone. Vegetation of meadows and pastures of Serbia comprises more than 300 described phytocoenoses, although some newest revisions of this vegetation indicate an existence of only 48 plant associations classified into 23 alliances of 15 rows and 7 classes, out of the classes *Molinio-Arrhenatheretea, Festuco-Brometea* and *Nardo-Callunetea* are of the greatest importance both from the aspect of biodiversity and the utilization. The highest floristic biodiversity was determined for plant communities of the class *Festuco-Brometea* (1194 species, accounting for about 42% of the total flora), followed by *Molinio-Arrhenatheretea* (895 species), *Festucetea vaginatae* (681) and *Festuco-Seslerietea* (673). Distribution, quality and floristic composition of meadows and pastures is highly dependent on manners of their management, whereas extreme utilization, such as overgrazing and lack in utilization (abandonment) cause the most adverse effects. The study provides a review of floristic and vegetation diversity of (semi) natural grasslands of Serbia and estimation of major factors affecting their biodiversity and quality.

Key words: biodiversity, flora, vegetation, use, quality

Introduction
Needs for conservation of grassland's biodiversity

Grasslands are integral part of the semi-natural landscape of Europe and of major importance for biodiversity in agricultural landscapes *(WallisDeVries et al., 2002; Tscharntke et al., 2005),* providing a wide range of habitats supporting a high biological diversity *(Eriksson et al., 1995).* Grassland's semi-natural communities are listed as a priority habitat for biodiversity conservation in European Union Habitats Directive (92/43/CEE). Grassland ecosystems have also attracted considerable scientific and policy interest because of their potential role as sinks or sources for atmospheric carbon dioxide. Conversion of arable land into permanent grassland is one measure that is believed to have a considerable carbon sequestration potential *(Ammann et al., 2007).*

Plant species-richness and floristic composition of grasslands are structured not only by current site conditions, mainly topography and soil *(Janssens et al., 1998; Sebastiá, 2004),* but also site history and ancient land use and practices that may have ceased long time ago *(Waldhardt and Otte, 2003).*

Decline of grassland diversity throughout Europe within the last decades is threatening biological diversity and is a major conservation problem. There is an urgent need to determine the underlying factors that control vascular plant species richness and composition in managed grasslands *(Klimek et al., 2007)*. Most of grasslands are composed of semi-natural vegetation as a consequence of a long history of human use. Different types of historical and current grassland use have created different plant and vegetation diversity patterns. Traditionally managed semi-natural grasslands (hay meadows and pastures) are known to support a rich flora and are recognized by high species diversity in different regions of Europe *(Norderhaug, et al., 2000; Kojić et al., 2004; Matevski et al., 2008).* Their continued existence depends upon correct management such as mowing or grazing. However, many grassland communities are now being threatened by rapid changes in agricultural practices, especially related to effects of land abandonment *(Pykälä et al., 2005; Dajić Stevanović et al., 2008a)* or eutrophication *(Stevens et al., 2004)* as opposite management practices.

Effects of key management practices on grassland's biodiversity

Agricultural activities such as tillage, drainage, intercropping, rotation, grazing and extensive usage of pesticides and fertilizers have significant implications for wild species of flora and fauna *(Mc Laughlin and Mineau, 1995).*

There is evidence that grassland management by grazing livestock at moderate levels contributes to the maintenance of plant diversity by reducing the abundance of competitive dominant species *(Collins et al., 1998)*. In addition, grazing has been supposed to have a profound influence on small-scale grassland heterogeneity by creating disorder in the soil and the sward structure, enabling species establishment through preference of particular niches *(Adler et al., 2001).* The 'rest-rotation' grazing system has been found useful for ameliorating some of the adverse impacts of season-long grazing on wild birds and mammals primarily because certain areas of pasture are left undisturbed at least part of the time *(Anderson and Scherzinger, 1975)*. Mowing at moderate cutting intensity maintained plant species richness in grasslands *(Fischer and Wipf, 2002).* In general, a more diversified grassland management would promote more species of grassland plants, as shown by *Gustavsson et al. (2007).* The addition of growth-limiting nutrients to herbaceous plant communities frequently leads to an increase in plant production and a corresponding decline in plant species diversity as a consequence of increased competition for light *(Abrams, 1995),* as well as, the accumulation of plant litter, especially at high levels of plant productivity *(Bonaomi et al., 2006).*

Floristic and vegetation diversity of grasslands of Serbia

Serbia is characterized by high plant diversity containing about 40% of all European plant species, despite the fact that its territory contributes for only 2% of the European land. According to last reviews, there is total of 3662 plant species and subspecies of 766 genera and 141 plant families *(Stevanović et al., 1995).* Nevertheless, the most recent reports appointed even higher number of vascular plant taxa, i.e. total of 3730 species and subspecies *(Tomović, 2007).* Vegetation diversity is also high with approximations ranging from about 600 to 1200 plant communities *(Lakušić, 2005).* Regarding grasslands in Serbia, the most presented and used are semi-natural grasslands (in local literature also known as secondary), formed in the forest zone, (as a consequence of deforestation), and natural or primary ones, distributed as final vegetation stage on places inappropriate for forest development, including high-mountainous (above timberline) zone, flooded land in lowland valleys and xeric steppe and/or salinized habitats in the northern part of the country (Vojvodina). Natural and semi-natural grasslands of Serbia represent one of the major resources in agriculture, occupying about 1.4 millions of hectares.

Intensive research of grassland biodiversity in Serbia started in fifties of the last century and was conducted upon Braun-Blanquet methodological approach, resulting in many remarkable reports on floristic and vegetation diversity of different geographic areas of the country *(Kojić et al., 2004).* Simultaneously, several studies focused on relations between management practices and grassland biodiversity *(Mrfat-Vukelić, 1991; Lazarević, 1995; Djordjević-Milosević, 1997; Peeters and Dajić, 2006),* as well as eco-physiological features of particular grassland flora *(Dajić et al., 1997, 2000a)*

According to recent review of grassland vegetation, it consists of 48 plant communities (mainly treated as “sensu lato”) classified into 23 vegetation alliances of 15 rows and 7 classes, previously described as more than 300 associations of 46 vegetation alliances of 24 rows and 10 classes *(Kojić et al., 2004).*

Analysis of the alpha diversity (total species number) of the highest grassland’s syntax (classes) of Serbia showed that the highest and lowest floristic richness was found for classes *Festuco-Brometea and Salicetea herbaceae*, respectively (Table 1). The latter class was used as an example of the high-alpine primary grassland vegetation, although it couldn't be treated as meadow or pasture in literal sense. In addition to the Salicetea herbaceae, the classes *Festuco-Seslerietea* and *Thero-Salicornietea* haven't been listed in reviews of *Kojić et al. (1995, 2004)* relating vegetation classification of meadows and pastures of Serbia. Nevertheless, we considered these vegetation classes, knowing that some of their associations were or still are grazed *(Dajić Stevanović et al., 2008b)*.

The highest alpha diversity, determined for the most developed and most used grasslands of *Festuco-Brometea* class (followed by those of *Molinio-Arrhenatheretea*), indicates significance of semi-natural grasslands as biodiversity resources, as shown in many recent studies *(Garcia, 1992; WallisDeVries et al., 2002; Poschlod et al., 2005; Klimek et al., 200;, Dajić Stevanović et al., 2008a).*

Table 1. Alpha diversity of grasslands of Serbia after *(Lakušić, 2005)*

Vegetation class	Species number	% (of total plant species of Serbia
Festuco-Brometea Br.-Bl. et R. TX. 1943	1194	41.84
Molinio-Arrhenateretea R. Tx. 1937	895	31.36
Festucetea vaginatae Soó 1968 emend. Vicherek 1972	681	23.86
Festuco-Seslerietea Burbero et Bonim 1969*	673	23.58
Juncetea trifidi Hadač 1944	441	15.45
Nardo-Callunetea Preising 1949	333	11.67
Phragmitetea communis R. Tx. et Preising 1942	290	10.16
Festuco-Puccinellietea Soó 1968	246	8.62
Thero-Slicornietea Pignatti 1953 emend. R. Tx. 1955	77	2.70
Salicetea herbaceae Br.-Bl. et al. 1947	46	1.61

Importance of grasslands of Serbia in terms of biodiversity preservation could be evaluated upon presence of endemic and critically endangered species (Table 1). Endemic species of vascular plants are the basic group used to identify national biodiversity hotspots over large scales *(Myers et al., 2000).* The highest number of Balkan's endemics was shown for grasslands of the class *Festuco-Brometea*, followed by those of class *Festuco-Seslerietea*. Regarding grasslands of the highest economic value, such as semi-natural meadows and pastures of the lowland valley (class *Molinio-Arrhenatheretea*) and hilly-mountainous region (class *Festuco-Brometea*), the participation of Balkan's endemic species accounts for near a half of total listed endemic species found in all surveyed classes of grassland vegetation. It should be quoted that out of 46 local endemics ("steno endemics") determined for Serbia *(Tomović, 2007)*, 29 appear within grassland vegetation. Among them, more than a half is associated to communities of the Festuco-Brometea. The highest number of critically endangered taxa was recorded for grasslands of Festuco-Brometea (e.g. *Artemisia pancicii, Crambe tataria, Astragalus exscapus subsp. exscapus, Bulbocodium versicolor, Sysimbrium* *polymorphum, Achillea ochroleuca, Artemisia austriaca, Herminum monorchis, Helichrysum plicatum subsp. plicatum, Aster oleifolius, Cachrys cristata, Crocus pallasii subsp. pallasii, Opopanax hispidus, Alkanna pulmonaria, Crocus olivieri subsp. olivieri, and Herminium monorchis*). However, critically endangered species weren't listed for associations linked to primary grassland vegetation of the classes Nardo-Callunetea and Juncetea trifidi, which might be a consequence of history of use and management.

Table 2. Some relevant biodiversity patterns of grasslands of Serbia (species treated as species and subspecies)

Class	Balkan endemic species	Local endemic species*	Stenoendemic species**	CR species***
Phragmitetea communis	2	-	-	3
Molinio-Arrhenatheretea	91	15	1	5
Festuco-Brometea	367	53	16	17
Festucetea vaginatae	0	-	-	10
Thero-Salicornietea	0	-	-	3
Festuco-Puccinellietea	2	-	-	3
Festuco-Seslerietea	267	34	7	6
Juncetea trifidi	107	15	3	-
Nardo-Callunetea	83	9	2	-
Salicetea herbaceae	33	6	-	2
Total in Serbia	547	165	45	121

*- Local endemics treated as species found in only one of three Balkan provinces: the east, west and south Balkan (mainly refers to species recorded in Serbia and in neighboring countries); **- Stenoendemics treated as species growing in Serbia only (usually of very limited distribution); ***- adopted after Stevanović, 1999

Some studies suggest that species at risk may be associated with patterns of total species richness *(Lawler et al., 2003),* whereas some other reports suggest that rare and threatened species have special habitat requirements limiting a coincidence with areas of high total species richness *(Aubry et al., 2005).*

Impacts of use and management of grasslands in Serbia on its biodiversity and quality

It is well accepted that variations in grassland floristic composition were dependent on historical management practices, including mowing, spring ranking, late grazing, grazing every second year, and livestock moving among the sites *(Garcia, 1992; Honnay et al., 2006).*

The first land use types were grazing of forests and ‘‘alternate husbandry’’, a system that involved alternating arable fields and pasture. Since that time the diversity of land use types increased until the 19th century resulting in a high biodiversity of habitats and species. Some specific early types of land use caused loss and/or degradation of particular grassland habitats (either regionally or locally), such as forest pastures, pig pastures, wooded meadows or pastures *(Poschlod et al., 2005)*. It was shown that certain ‘‘relic’’ species still indicate the former type of land use in calcareous grasslands *(Alard et al., 2005)*.

Transhumance was for centuries the traditional system of livestock production in the Balkan Peninsula. Livestock and herders moved seasonally between the northern and the southern parts of the Peninsula. Once State borders stopped these large movements, these transhumant movements were reorganized between lower and higher elevations. Forests were progressively cleared in mountain areas leaving the place for species-rich grasslands. Rustic, local breeds were used in this extensive system of livestock production. During the seventies great grassland areas were used either for cutting or for grazing, where the latter included even grasslands of the highest altitudes *(Vidanović-Sazda, 1955)*. According to the relief features, a spontaneous use of natural grasslands was performed, where grasslands on the smallest slopes were used for cattle grazing, rocky slopes for goats, and south-facing slopes and distant sites for sheep *(Djordjević-Milošević, 1997)*. Insufficient grazing area near the villages (hilly and lower mountain zone) was compensated by hay and forage crop production. Grasslands of higher altitudes weren’t properly managed as well, mainly because of improper schedule of grazing and insufficient use. The consequence was under-grazing, whereas the highest grazing zone has not exceeded the altitude of about 1600 m *(Djordjević-Milošević, 1997)* declining at present to about 1000-1200 m (personal observations). Most of cutting meadows haven’t been fertilized at all (at least fertilized insufficiently or in an improper way) neither in the past nor at present, and are mowed too early or too late *(Djordjević-Milošević and Trenkovski, 1992)*. In general, both overgrazing and under-grazing, as key management practices in former period of significantly higher grassland utilization, led to biodiversity loss as a result of grassland degradation, successions and spreading of undesirable species and grassland communities of low quality.

Similarly, current management practices (mainly related to extensive use and insufficient and/or inadequate treatment) cause further decline of floristic and vegetation diversity, as well as additional degradation of grasslands, either related to reduced yields or expansion of undesirable species (Table 3). The potential of huge grassland areas of the hilly and mountainous areas of Serbia is not sufficiently exploited. Moreover, considerable area under grassland habitats exposed to unfavorable environmental conditions, including drought, over-flooding, extremely shallow, acid, alkaline or saline soils couldn’t be accounted for efficient and sustainable exploitation *(Dajić, 200; Dajić Stevanović et al., 2008b).*

Table 3. Review of impacts of main existing management practices on biodiversity of grasslands in Serbia

Zone	Key change	Major threats for biodiversity
Lowland	Improper management : Fertilization (insufficient/absent/inadequate) Cutting schedule (inadequate) Overgrazing in areas near villages Inter-conversion: arable land ⇔ grasslands cutting meadow → under-grazed grassland Abandonment of agricultural area	Successions: Good quality grasslands into those of bad quality, such as Deschampsietum coespitosae Floods and erosion Expanding of undesirable species: woody species and arable weeds
Hilly	Improper management: Fertilization (insufficient/absent/inadequate) Cutting schedule (inadequate/insufficient cutting) Under-grazing Overgrazing in areas near villages Inter-conversion: Abandoned cutting meadow → under-grazed grassland Abandonment	Successions: Good quality grasslands into those of bad quality, such as: Brachypodietum pinnatae, Nardetum strictae Degradation: Loss of species and decrease in quality Expanding of undesirable species: trees, shrubs and herbaceous plants
Mountain	Improper management: Under-grazing Abandonment ↓ Loss of grasslands	Successions: Spreading of low quality grasslands: Nardetum strictae, Festucetum spadiceae, Calamagrostietum arrundinaceae Grasslands → Shrub vegetation → Forest Degradation: Loss of species Expanding of undesirable species: trees, shrubs and herbaceous plants Invasion of dwarf juniper → successions of grassland communities into Vaccinio-Juniperetum nanae ↓ Loss of species and ecosystem diversity (loss of grassland communities)

It is well established that productivity does not respond predictably to species richness or vice versa. This was also shown for semi-natural grasslands of Serbia using indirect measurement of grassland productivity and quality based upon total coverage value and pastoral value, respectively (parameters were calculated according to floristic diversity and related abundance) (Table 4). The highest pastoral value was recorded for valley association, the Arrhenatheretum elatioris, and the lowest for subalpine community *Poetum violaceae*, followed by lowland wet grassland community *Deschampsietum coespitosae*. The highest and the lowest species number were recorded for the *Agrostietum vulgaris*, the community of relatively high productivity and *Deschampsietum coespitosae* of low productivity, respectively. High coverage value (indirect parameter of productivity) was recorded for good quality grasslands, such as *Arrhenatheretum elatioris* and *Agrostietum vulgaris*, as well as for grassland of low quality, the *Nardetum strictae*. The consequences of changes in plant diversity for ecosystem functioning have been addressed in a large number of recent studies, showing that in mountain and alpine regions a diverse vegetation cover may maintain ecosystem functions such as productivity and vegetation cover and thus stabilize the ground *(Cardinale et al., 2006).*

Table 4. Floristic diversity and quality of some representative grassland associations of the Mt. Stara planina *(unpublished data)*

Zone	Ass	SP no	TC	PV	L (%)	G (%)	U (%)
L	1	28.7 ± 10.9	202.2	19.1	6.3	39.4	35.5
	2	41.6 ± 11.2	301.9	31.5	8.5	39.3	3.6
H	3	46.2 ± 12.3	237.7	25.2	12.4	54.3	14.9
	4	43.6 ± 9.8	255.5	21.5	9.2	40.6	14.9
	5	54.1 ± 8.3	283.6	26.5	20.9	31.7	6.6
M	6	36.8 ± 7.7	296.1	17.6	6.3	38.5	8.5
	7	34.1 ± 3.7	217.4	14.4	1.2	41.4	2.2

L- lowland; H- hilly; M- mountainous; SP no – species number; TC – total coverage; PV- pastoral value; L – participation of Legumes in average coverage; G- participation of Grasses in average coverage; U – participation of undesirable species in average coverage; Ass – plant associations: 1- Deschampsietum coespitosae; 2- Arrhenatheretum elatioris; 3- Danthonietum calycinae; 4- Koelerietum montanae; 5- Agrostietum vulgaris; 6- Nardetum strictae (*sensu lato*); 7- Poetum violaceae

In summary, grasslands of Serbia exhibits distinctive floristic and vegetation diversity and represent major natural resource for agricultural production. Therefore, they should be used in more efficient and sustainable way to maintain existing biodiversity, including diversity of genetic resources, such as forages, crop wild relatives and medicinal plants (the latter already reviewed by *Dajić et al., 2000b*). Application of adequate management practices could both prevent further degradation of grasslands and related biodiversity loss, and improve grassland quality and productivity. A detailed knowledge of community variation and related floristic spectra, together with estimation of productivity and quality of Serbian and Balkan semi-natural grasslands may provide the scientific basis for designing management plants that would be proper for maintaining the biodiversity of particular landscapes.

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Biodiverzitet prirodnih travnjaka Srbije: stanje i perspektive održivog korišćenja

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Rezime

Prirodni i poluprirodni travnjaci Srbije predstavljaju jedan od najvažnijih resursa u poljoprivredi, i zauzimaju oko 1.4 miliona ha, rasprostirući se od nizijskih predela, pa sve do najviših planinskih masiva. Vegetacija livada i pašnjaka na području Srbije obuhvata blizu 300 opisanih fitocenoza, pri čemu novije revizije ove vegetacije ističu postojanje 48 asocijacija, svrstanih u 23 sveze iz 15 redova i 7 klasa, od kojih najveći značaj, kako sa aspekta biodiverziteta, tako i iskorišćavanja, imaju travnjaci iz klasa *Molinio-Arrhenatheretea*, *Festuco-Brometea* i *Nardo-Callunetea*. Najveći floristički biodiverzitet utvrđen je u zajednicama klase *Festuco-Brometea* (1194 vrste, što je približno 42% od ukupne flore Srbije), *Molinio-Arrhenatheretea* (895 vrsta), *Festucetea vaginatae* (681) i *Festuco-Seslerietea* (673). Rasprostanjenje, kvalitet i floristički sastav livada i pašnjaka najdirektnije su uslovljeni načinima iskorišćavanja, pri čemu, preterana ispaša sa jedne, i njihovo napuštanje sa druge strane, imaju najveće negativne efekte. U radu je dat pregled vegetacijskog i florističkog biodiverziteta prirodnih travnjaka Srbije, procena uticaja načina iskorišćavanja na biodiverzitet i kvalitet najvažnijih travnjačkih zajednica, kao i analiza flore livada i pašnjaka sa aspekta kvaliteta i vrednosti genetičkih resursa krmnog i lekovitog bilja u cilju njihovog održivog korišćenja.

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ANIMAL FEED QUALITY – PAST AND PRESENT

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Abstract: The feeds are accepted as quality feeds when they have favourable sensory properties, optimal nutritive value, are hygienically suitable and harmless. Sensory properties of the feeds may be changed under a number of physical, chemical and biological factors, although those changes are not necessarily a sign of decreased nutritive value and usefulness. With the knowledge of the mentioned factors and different degrees of their control the maximal quality of the feeds, diets and mixtures may be obtained. The majority of animal feeds are of plant origin. There is a decreasing trend in the use of feeds of animal origin with a tendency to replace them completely with mixtures of plant feeds and synthetic amino acids. Contemporary achievements in the feed production technology and use of animal feeds are connected with maximal mechanization in the process of hay and silage production, the use of various additives and use of modern machines for production and agglomeration of concentrate mixtures. The recent machines in the feed mill industry are mostly performing heat or hydrothermal processing of feeds with the aim to increase nutrient utilization and eliminating anti-nutritive substances. Also, various machinery is binding and shaping concentrate mixtures, and increasing energy value by adding fats.

Key words: feeds, quality, conserving, processing, additives.

Introduction

Animal nutritive needs may be fulfilled by great number of feeds, and their choice depends on animal species and category, level of production, health, environment conditions and others. It is generally accepted that more than 2000 various feedstuffs are used in the world presently *(Đorđević et al., 2009)*. Nutritive values of various feeds can be very different, first because of their different nutrient composition second because of the method of processing or conservation, change of feed composition due to various factors, their interaction, presence of anti-nutritive components, contamination and other factor *(Esminger et al., 1990)*. The possibilities for reciprocal substitution or supplementation of their nutritive value have great significance for profitability in animal production, and for fulfilment of various needs of modern farmers *(Đorđević et al., 2003a)*. In addition to usual feedstuffs, various additives have great significance for intensive animal production. Based on nutritive, prophylactic, conserving or some other role, additives stimulate better utilization of diets of concentrate mixtures *(Đorđević et al., 2006a)*.

Not long time ago the main task in feed manufacturing industry was to produce quantity, but today it is the quality that is most strictly required. Animal feeds are exposed to many adverse factors. With the influence of physical, chemical and biological factors, one or several, the feed quality deteriorates. Harmful effects are demonstrated through decrease in production and health problems, and in the worst case in animal mortality. One of the big problems for nutrient utilization is the presence of anti-nutritive substances. Also, a big danger for human population potentially exists in mycotoxins present in feedstuffs *(Đorđević et al., 2006b)*. Ability to retail animal products on EU market demands the meticulous criteria in quality control, where presently the most often referred to is the HACCP control system *(Đorđević et al., 2007a)*.

Production and conservation of forages

The profitable cattle production is based on maximal utilization of forages because they are much cheaper when produced. However, the quality of forage feeds is very often unsatisfactory, and the decreased nutritive value is compensated with concentrates. That is increasing production costs and the risk of metabolic disorders in cattle. That is why farmers are always forced to find best, simplest and cheapest solutions for forage production of maximum quality *(Đorđević et al., 1996)*.

In the field of production of crops for animal feeding the interspecies hybrids are produced where they achieved positive qualities of parental lines. Today the greatest publicity is given to genetically modified plants. Those products of biotechnology enable crop production with reduced expensive agro technical procedures, but it is also a matter of great controversy and disagreements in the part of the world which has concerns about production of healthy and safe food. *(Hodgson, 1999)*.

In the counters with developed animal production the concept of use conserved forages throughout the year is accepted. This concept provides maximally stabile diet, and therefore the stabile animal production *(Đorđević et al., 2005)*. It is evident that in many countries there is a increase in the use of silage and decrease in hay production. Among others, it is a result of astonishing development in agricultural machinery, which used to be the limiting factor for spreading of the ensiling technology.

Modern principles of feed preservation have the aim to obtain maximum quality and highest degree of preservation of nutritive value, which is achieved with the use of novel machinery and chemical and biological additives. However, the nutritive value of crops used for silage production is depending mostly on botanical factors, such as plant species. The biggest quantity of silages is produced from grasses and maize, while legumes and various side products of crop production and food industry are much less common *(Wilkinson and Toivonen, 2003)*.

Whole plant maize silage is one of the most important energy sources in cattle feeding, considering the high green mass yields, relatively high energy content, high palatability and its importance in total mixed ration (TMR) formulation *(Forouzmand et al., 2005)*. Commercial maize hybrids are selected on the basis of grain yield and resistance to diseases *(Clark et al., 2002)*, while their nutritive value is mostly being neglected *(Bal et al., 2000)*. However there are large differences in nutritive value between silages produced from various maize hybrids which are a result of the diversity in their digestibility of nutrients *(Bagg, 2001)*.
Nutritive value of maize silage is defined by hybrid, degree of maturity and dry matter digestibility. In experiments with brown-midrib hybrids (bm3) the significantly lower level of lignin was determined and an increased *in vitro* digestibility of NDF *(Oba and Allen, 1999)* and therefore higher milk production in cows *(Keith et al., 1979)*. However, these hybrids are not suitable for commercial production since they have low yield of grain and biomass for ensiling. In recent years there was considerable number of investigations of leafy maize hybrids for silage. They have high production of leaves, more moisture in grains and softer texture of their cobs. *Dwyer et al. (1998)* reported that in North America about 16% of maize silage is produced from leafy hybrids. In order to increase nutritive value of maize silage various other hybrids are produced such as high-oil *(Weiss and Wyatt, 2000)*, waxy *(Akay and Jackson, 2001)* and others.

In recent years institutions that producers in Serbia are investigating digestibility of their maize hybrids because that parameter is very important for their utilization in animal production. The aim of this work was to investigate digestibility in a range of commercially available hybrids and to perceive its importance on maize silage use in fattening cattle production.

The presence of significant differences in performances when animal are fed with silages produced of different maize hybrids was determined in some previous experiments (Table 1).

The use of biological additives (bacterial-enzyme inoculants) is presently the most interesting field in the technology of silage production. They are used to intensify fermentation in plant matter that is hard to ensile, but also for improvement of aerobic stability in maize silage *(Đorđević et al., 2006c)*. In that sense the biggest attention is given to hetero fermentative lactic acid bacteria *Lactobacillus buchneri* which are incorporated in the latest generation of bacterial-enzyme inoculants *(Driehuis et al., 1999)*.

In order to simplify ensiling process for grasses, legumes or grass/legume mixtures the haylage preparing in bales is becoming more and more popular in Europe, while in America the silo bags ("sausage") are becoming accepted *(Dinić et al., 2004)*.

Processing feeds of plant origin

There are a small number of feedstuffs used in native form or on their own in an intensive animal production. With various methods of mechanical, thermal and biological treatments of feeds a number of improvements are achieved which result in better animal production performances *(Đorđević et al., 2008)*. Heat and hydrothermal processing is most often used today for grain processing. The aim of the processing is inactivation of anti-nutritive substances and starch gelatinization.

Soybean grains are very commonly heat treated in order to eliminate anti-nutritive substances, and also maize (very rarely other species) grain to achieve starch gelatinization and better utilization in feeding of young animals. Soybeans are commonly processed by extruders, modern machines that perform several mechanical and hydrothermal processes, and the final result is significant reduction of trypsin inhibitor and urease *(Sakač et al., 2001)*. Micronizers are used for cereal processing, which are working on the basis of infra red rays. This is influencing starch granules to swell and destroy the primary structure of starch, and at the same time it is reducing number of microorganisms, deactivating plant enzymes and has other effects *(Stojanović et al., 2004)*. *Grubić (1988)* and *Grubić et al. (1990)* confirmed that inclusion of heat processed maize (micronized and extruded) in mixtures for calf feeding results in a improvement of feed conversion ratio of 14-17%, decreases the energy conversion ratio per kg of gain by 10-12% and contributes to the better nitrogen retention.

However, the described methods do need substantial amounts of energy, which is why they are not commonly used in our country *(Stojanović, 2006)*.

Table 1. Digestibility (*in vivo* and *in vitro*) and feeding trial results *(Jovanović, 2007)*

Parameters	Hybrids			
	ZP677	ZP680	ZP735	ZP753
DM digestibility (*in vitro*),%	58.38[c]	67.57[a]	60.74[b]	61.91[b]
DM digestibility (*in vivo*), %	65.65[d]	76.10[a]	70.91[c]	72.78[b]
First experimental period (silage + concentrate, 0-53. days)				
Starting weight, kg	247.47	247.80	247.20	246.60
Final weight, kg	297.00[ns]	302.00[ns]	296.93[ns]	297.47[ns]
Average daily gain, g day^{-1}	934[b]	1021[a]	938[b]	959[ab]
Total gain, kg	49.53	54.20	49.73	50.87
Second experimental period (silage, 54-84. days)				
Final weight, kg	315.33[b]	322.06[a]	315.73[b]	316.80[b]
Average daily gain, g day^{-1}	591[b]	647[a]	606[b]	624[ab]
Total gain, kg	18.33	20.06	18.6	19.33
Whole experiment (0-84. days)				
Average daily gain, g day^{-1}	807[c]	884[a]	816[c]	836[b]
Total gain, kg	67.86	74.26	68.53	70.20

Explanations: a, b, c = values in same column (for some factor) with different letter are significantly different (P<0.05); ns = no significance

Industrial production of concentrate mixtures

Concentrate mixture production is a complex process which is enabling mixing of several different starting raw materials, into mixtures of desired shape and kind. Modern feed mills are a complex of constructions, machines and devices for receipt, measuring, cleaning, drying, keeping, cracking, grinding, dosing, mixing, pelleting, bricketing, adding liquids, and packaging *(Đorđević et al., 2006a)*. In concentrate mixture production all of the initial ingredients preserve its original characteristics, but through the process of mixing the mixture is obtained with desired quality *(Grubić and Đorđević, 2005)*. Raw materials are dosed and mixed in quantities that are optimally suited for certain species or categories of animals, based on scientific research and given as nutritive recommendations *(Božičković et al., 2008)*. The correct formula provides that final product – mixture, has better characteristics than any of the components, in terms of nutritive value and price *(Đorđević et al., 2007a)*.

Shaping concentrate mixtures, normally referred as pelleting, is also a method commonly used because of several advantages. As first, the dispersion of mixture id decreased, as second the intake is enhanced, but there some other good sides which result in better production performances. In our country the quantity of mixtures that are pelleted is comparatively small, and only fish feeds are usually pelleted. During the process of pelleting the decrease of total number of microorganism is occurring, which is improving hygienic quality of mixtures.

(Table 2). Since pelleting includes presence of steam, high pressure and temperature, changes in chemical composition occur, and the digestibility of starch, hemicellulose, cellulose and pentosane is increased.

The use of all feedstuffs of animal origin, with the exception of milk products, is forbidden in ruminant feeding due to danger of distribution of lethal disease *Bovine spongiform encephalopathy*–BSE, and their use is limited in non-ruminant nutrition (pigs and poultry). Instead of those feedstuffs various substitutions based on combined plant feeds and synthetic amino acids, or commercial fish meal substitutes are gaining popularity in non-ruminant nutrition *(Đorđević et al., 2007b)*.

Table 2. The influence of pelleting on microorganism numbers in various concentrate mixtures. *(Lević et al., 1998)*

Parameters	Mixtures for:		
	broilers	Pigs	cattle
Before pelleting:			
Temperature (°C)	30	30	30
Microorganism number:			
Saprophyte bacteria	1085000	982000	225000
Moulds	50000	26500	16500
After pelleting:			
Temperature of conditioning (°C)	83-85	75-78	80
Microorganism number:			
Saprophyte bacteria	10000	22500	15000
Moulds	-	3500	2000

Additives in animal nutrition

The most significant difference between different concentrate mixtures is that those for ruminants are just a supplementation in a diet consisting mostly of forages, while in non-ruminants they form the complete diet. Their nutritive value can be improved with various additives.

Mixtures for dairy cows are today supplemented with a number of additives to prevent and optimize certain physiological processes: buffers, alkalis, anionic salts, monensine, yeasts and moulds, protected amino acids and fats, mycotoxin adsorbents and others *(Grubić and Adamović, 2003)*. In recent times the additives based on organic zeolites are very often used as mycotoxin adsorbents *(Đorđević et al., 2003b)*. Feed contamination with moulds and their secondary metabolites – mycotoxins is very serious problem of contemporary animal science. In countries with cold and moderate humid climate the most common mycotoxins are: vomitoxin, zearalenone, ochratoxin, diacetoxiscirpenol (DAS) and T-2, while in hot and humid climate aflatoxin is present. The contamination with moulds is possible wile plant is growing in the field, during harvesting, storing and feed processing. All types of animal feeds, particularly. Non-nutritive adsorptive substances are used in order to decrease harmful effects of mycotoxins (zeolite, bentonite, various types of clays) and biological additives (yeasts). Zeolite is increasingly used because of its property to adsorb various toxins in animal feeds, and particularly aflatoxin and zearalenone *(Piva and Galvano, 1999)*. It was experimentally confirmed that the excretion of mycotoxins through milk is significantly reduced when mineral mycotoxin adsorbents are used (Table 3).

Various additives with nutritive, stimulation and preventing effects are used as conservants, antioxidants, enzymes, acids, aromas, colours and similar.

Considering the process of plant material digestion in non-ruminants, the problem occur with polysaccharides, and phosphorus complexes with phytic acid, which is solved with the use of appropriate enzymatic additives (Table 4). Organically bonded micro elements are also often used, which are part of the chemical complexes with peptides and amino acids (chelates), or carbohydrates (SQM and carbozanes). Such form of micro elements do not form insoluble complexes and is better utilized than their inorganic compounds. Also, the organic Selenium is much less toxic and more rapidly deposed and kept in tissues *(Todorović et al., 2004)*.

Table 3. Milk production per day and its composition *(Nešić, 2000)*

Parameters	A	B	C	D	E	Significance
Zearalenone and zeolite in concentrate						
Zearalenone in concentrate, mgkg-1	0.00	0.00	2.55	2.40	2.33	-
Average daily intake of zearalenone, mg	0.00	0.00	10.20	9.60	9.32	-
Zeolite in concentrate, gkg-1	-	2	-	2	5	-
Nutritive values						
NEL, MJ	124.72	124.68	124.72	124.68	124.56	-
Total protein, g	2,709.1	2,708.3	2,709.1	2,708.3	2,707.5	-
Total protein, gkg-1 DM	147.4	147.3	147.4	147.3	147.3	-
Lipids, gkg-1 DM	40.0	40.0	40.0	40.0	39.9	-
Calcium, gkg-1 DM	8.6	8.6	8.6	8.6	8.6	-
Phosphorus, gkg-1 DM	4.5	4.5	4.5	4.5	4.5	-
Milk production per day and its composition						
Daily milk yield, kg	22.48	23.44	21.85	22.12	23.54	NS
4% FCM, kgday^{-1}	20.18	21.09	19.17	19.86	20.72	NS
Milk fat, %	3.32	3.33	3.18	3.32	3.20	NS
Milk fat, kg	0.746	0.781	0.695	0.734	0.753	NS
Protein, %	3.09	3.08	3.01	3.11	2.97	NS
Protein, kg	0.695	0.722	0.658	0.688	0.699	NS
Lactose, %	4.89	4.81	4.75	4.81	4.84	NS
Lactose, kg	1.099	1.127	1.038	1.064	1.139	NS
Milk solids-not-fat, %	8.71	8.62	8.55	8.69	8.57	NS
Milk solids-not-fat, kg	1.958	2.021	1.868	1.922	2.017	NS
Zearalenone in milk, mgkg^{-1}	0.000b	0.000b	0.053a	0.019ab	0.004b	***

Table 4. The effect of adding phytase on the obtained results with the broilers *(Živkov-Baloš et al., 2005)*

Investigated traits	Control group	Trial group
Body weight (g)	2216.1±268.6	2259.5±312.8
Daily weight gain (g)	51.11±6.42	54.10±7.33
Feed conversion ration (kg)	1.76	1.73
Content of phosphorus in chyme (gkg^{-1})	2.080 ± 0.802^{a}	1.611 ± 0.301^{b}
Digestibility of phosphorus (%)	50.59 ± 12.90^{x}	75.27 ± 14.86^{y}
Content of calcium in chyme (gkg^{-1})	$2.274 \pm 1.247^{a.x}$	1.561 ± 0.590^{x}
Digestibility of calcium (%)	42.30±22.46	59.88±17.49

^{a,b}=P<0.05; ^{x,y}=P<0.01

Conclusion

Modern achievements in production and use of animal feedstuffs of plant origin are considered in following aspects:

Selection in order to increase yield of digestible matters;

Maximal mechanization of the production of hay and silage;

The use of modern machines for processing and agglomeration of concentrate mixtures;

The use of various additives.

It is evident that all of the above mentioned achievements are used in Serbia, first in scientific institutions and than in feed milling industry, which is one of the most developed industry branches. It is very important predisposition for further development of agriculture.

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Kvalitet hrane za životinje – nekada i sada

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Rezime

Pod kvalitetom hrane za životinje podrazumevaju se poželjna organoleptička svojstva, optimalna hranljiva vrednost i higijenska ispravnost i

neškodljivost. Organoleptička svojstva hrane mogu biti promenjena pod uticajem brojnih fizičkih, hemijskih i bioloških faktora, mada te promene ne dovode uvek i do smanjenja hranljive i upotrebne vrednosti. Poznavanjem navedenih faktora i različitim merama njihove kontrole može se postići maksimalni kvalitet hraniva, obroka i smeša koncentrata. Najveće količine hrane za životinje su biljnog porekla, sa daljom tendencijom smanjenja korišćenja hraniva animalnog porekla ili potpune supstitucije istih sintetičkim aminokiselinama. Savremena dostignuća u oblasti proizvodnje i korišćenja hrane za životinje odnose se na maksimalnu mehanizaciju procesa pripremanja sena i silaže, korišćenje različitih aditiva kao i upotrebu savremenih mašina za obradu i aglomeriranje smeša koncentrata. Savremene mašine u industrijskoj proizvodnji hrane za životinje najčešće obavljaju termičke ili hidrotermičke postupke obrade hraniva u cilju povećanja iskoristljivosti ili eliminisanja antinutritivnih materija. Osim toga, različitim mašinama se vrši aglomeriranje (uobličavanje) smeša, kao i dodatno povećanje energetske vrednosti dodavanjem masti.

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PROCEDURES FOR IMPROVEMENT OF THE QUALITY OF FERMENTATION PROCESS AND INCREASE OF NUTRITIVE VALUE OF SILAGES

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Invited review paper

Abstract: The significance of bio-mass in regard to the content of water soluble carbohydrates (sugars), buffer capacity and ratio between sugar and buffer capacity for the purpose to ensure successful lactic acid fermentation is presented in this paper. Increase of the level of dry matter in silo mass (wilting) is very important procedure for reducing of losses of nutritious substances and obtaining of good quality silage without the presence of butyric acid. Application of inoculants on the basis of homo and hetero-fermentative bacteria provides improvement of lactic acid fermentation and increase of the aerobic stability of silages. The cutting of crops in adequate stage contributes to obtaining of high quality forage feed of high nutritive value.

Key words: sugars, buffer capacity, wilting, inoculants, silage, nutritive value.

Introduction

Main problem reflecting on the stage of development of agriculture in Serbia, and especially of livestock production, is the size of agricultural holding. In year 2008 there were 363.642 registered holdings of different size of land property *(Stat. Yearbook, 2008*). Majority of holdings in Serbia are from 2.0 do 5.00 ha, i.e. 1/3, followed by ¼ of holdings from 0.5-2.0 ha and from 5.0-10 ha, which makes total of 84.45%. Size of holdings decreases from north part of the country to the south part. Serbia (without Kosovo and Metohija) disposes with 5.093.000 ha of agricultural land area, of which 3.099.000ha under plough land and gardens, and 466.000 ha under forage plants *(Statistical Yearbook, 2008)*. In regard to crops, corn is grown on the largest land area - 1.277.000 ha, followed by wheat - 488.000 ha, alfalfa - 192.000 ha, sunflower - 188.000 ha, soybean - 144.000 ha, red clover - 120.000 ha and barley - 92.000 ha. Based on said land area of 3 million ha, and if only ½ of this land area would be used for production of livestock food only for cattle, it would be possible to feed 1.5 million heads of cattle, which was number of cattle recorded in year 1991 (1.483.000). It is known that production of livestock food from 1 ha of land can ensure breeding of one dairy cow, although in more intensive production of livestock food even for more that 2 dairy cows per 1 ha.

Realized yields in production of livestock food in Serbia (2008) per unit of land surface/area are very low, which also contributes to livestock production which is not economically efficient. Number of livestock in Serbia in the period from 1991-2008 demonstrated tendency of decrease. Total number of cattle in Serbia in 1991 was 1.483.000 heads, and in 2008 1.057.000 heads. Significantly greater decline of number of heads is recorded in most important cattle category "cows". In year 1991 there was 847.000 cows, in 2008 - 578.000 *(Stat. Yearbook, 2008)*, i.e. 68,24% of the number of animals present in 1991. Decline in number of cows is very serious problem considering that Serbia used to be important exporter of beef and that presently it has no capacity to meet the export quota for beef to EU. Number of sheep also decreased significantly in relation to period 30 years ago. Recently, number of sheep has stabilized around 1.600.000 heads, although conditions exist for breeding of considerably higher number of animals. Number of goats is within 150-160 thousand heads.

Nutrition of high-yielding animals with conserved forage food throughout the year, which includes first of all use of ensiled food, is becoming increasingly modern and interesting globally *(Jovanović et al., 2002)*. In some countries, the technology of use of green food from fields during vegetation has been abandoned for long time, and replaced by stable nutrition using diets of similar composition throughout the year, which includes large quantities of high quality silage. Quality of forage food influences the economical efficiency of the milk and meat production.

Main forage cultures in Serbia which are used in very different ways are corn and alfalfa. In recent decades, greater quantities of these plant species are conserved by ensiling and in this way stable and uniform nutrition of ruminants is ensured, as well as stable milk production. High nutritive value of alfalfa can hardly be preserved in the form of hay because of considerable impact of weather conditions. Ensiling is better solution, but there are problems of high BC and insufficient quantity of fermentable sugars. Using modern inoculant based additives and limitation/suppression of proteolysis through optimum level of DM, it is possible to achieve maximum quality and nutritive value of alfalfa silage. In addition to corn and alfalfa, in production of livestock food, also other perennial grasses and leguminous plants, grains and other forage species are important *(Dinić et al., 2008)*.

Type of silage mostly prepared in certain country depends on geographical origin, i.e. possibility to grow certain plant species. For instance, in Great Britain, Ireland and Nordic countries, grass silage is predominant, and in Germany, France, Belgium, Spain and Italy, corn silage. In certain developed countries (England, Germany, Austria), production of silage is decreasing, but this is consequence of the decline in livestock fund, whereas in Bulgaria and Lithuania it is consequence of fundamental changes in the agro-industrial complex *(Wilkinson and Toivonen, 2003).*

Today, we don't have precise data on the quantity of silage prepared from whole corn plant in Serbia. It is considered to be bellow 5% of total land area under corn. However, in regard to silages and haylages produced from lower cultures (alfalfa, red clover, sown meadows, annual leguminous plants, grains, etc.), they are mainly prepared on big farms, and on smaller farms only sporadically. In certain regions in Serbia (Rasina district), where the concentration of cattle is high, also on small holdings significantly greater quantities of whole corn plant silage are prepared, as well as silages and haylages prepared from different bio-masses.

Objective of this paper was to present principles of conservation of livestock food by method of ensiling in order to have successful lactic acid fermentation, in order to choose the most suitable inoculants for obtaining of high quality silage, achieving the maximum anaerobic stability.

Suitability of bio-mass for ensiling

Silage is biologically fermented, or chemically conserved livestock food of plant origin intended for animal nutrition. Lactic acid is the most important product of lactic-acid fermentation in ensiled material, and at the same time efficient silage conservation agent / conservants. In addition to lactic acid, in ensiled material different quantities of acetic, butyric, propionic acids are generated, as well as ethyl alcohol, carbon dioxide and ammonia. However, these products are poor conservants, and at the same time indicators of negative processes in silage. Therefore, entire ensiling process is based on execution of measures which lead to maximum production of lactic acid and minimum production of other acids *(Đorđević and Dinić, 2003)*.

For successful conservation of the forage plant bio-masses and similar plant materials, it is necessary to establish the content of water soluble carbohydrates – sugars (S), buffer capacity (BC), ratio between S/BC, minimum content of dry matter (DM) and critical pH value *(Dinić et al. 1998)*.

Plant BC or their ability to oppose to change in pH is important factor in ensiling. BC is defined as quantity of lactic acid necessary for acceding of silo mass to the pH value of 4,00 *(Weissbach, 1967)*. It is expressed in meqv of lactic acid per 100 g of DM. In addition to stated method, often the Playne and McDonald method (1966) is used according to which chopped mass is first titrated using solution of 0,1mol/l per pH 3,00 for the purpose of removal of carbonates in the form of carbohydrates, and then repeated titration is carried out using solution

NaOH of the concentration 0,1mol/l to pH 6,00. BC is expressed in ml of base needed to change pH value from 4,00 to 6, and 00 per 1 kg DM.

If the content of DM in ensiled material is lower, in that case acidifying must be more intense in order to prevent forming of butyric acid. Value of pH needed to obtain stable silage with certain content of dry matter is called critical pH value *(Beyer et al., 1982)*.

In order for fermentation to proceed in wanted direction, S/BC ratio must be increase with the decrease of DM content. Minimum content of DM (Y) depends on the S/BC ratio (x) and it is expressed by formula Y (g kg^{-1}) = 450 - 80x *(Beyer et al., 1982)*. Same authors found that, in order for fermentation to be successful and silage to have good quality without presence of butyric acid, ratio S/BC must be 3.0 or higher.

Leguminous plants belong to forage plants which are difficult or more difficult for ensiling. Low sugar content, high BC value as well as extremely unfavourable ratio WSC/BC, indicate unsuitability of leguminous bio-mass for ensiling. For successful ensiling of alfalfa bio-mass, and in order to avoid synthesis of butyric acid, it is necessary to ensure DM content of approx. 400 g kg^{-1} by wilting, since WSC/BC ratio is bellow 1,00 *(Dinić, 1997)*, or add carbon hydrate feed when level of DM can be lower. Sugar content, WSC/BC ratio in bio-mass of red clover is much more suitable for ensiling compared to bio-mass of alfalfa and white clover (Table 1) and with good wilting it is possible to ensile it without the use of additives. Red clover is more suitable for ensiling compared to alfalfa because of lower degree of proteolysis of nitrogen substances *(Jones et al., 1995)*.

Alfalfa and corn are main forage cultures in Serbia. They differ from each other significantly in regard to the chemical composition and nutritive value, which is why they complement each other in diets for ruminants. Corn bio-mass is most suitable for ensiling, especially since WSC concentration is high and BC low, as consequence of low content of crude proteins and minerals.

Perennial grasses are much more suitable for ensiling than perennial leguminous plants. WSC/BC ratio is over 3.00, which is considered as desirable according to *Weisbassh (1967)* for successful ensiling. Confirmation of favourable WSC/BC ratio in perennial grasses is found in studies by *Knotek (1997)*. The most favourable ratio WSC/BC of 4.62 was established in the bio-mass of Italian ryegrass, followed by bio-mass of timothy – 4.32, and bio-mass of marsh fescue of 4.10. Extremely favourable WSC/BC ratio of over 5.00 in silo mass of French ryegrass was established by *Dinić et al., (2008)* with slightly more favourable ratio determined in the bio-mass deriving from cut in the earlier phase (Table 2).

Table 1. Chemical composition and ensilability of red clover and white clover biomass, g kg^{-1}DM ***(Dinić et al., 1994)***

Parameters	Red clover (a_1)		White clover (a_1)	
	Fresh (b_1)	Wilted (b_1)	Fresh (b_1)	Wilted (b_1)
Dry mass	204	320	161	281
Organic mass	897.4	899.1	880.3	894.0
Ash	102.6	100.9	119.7	106.0
Crude protein	180.4	192.5	257.3	240.8
Crude fibre	257.1	247.3	232.4	212.1
Crude lipid	21.6	28.7	31.1	26.3
NFE	438.3	430.6	359.5	411.4
Ca	14.3	12.9	13.0	12.6
P	3.2	3.3	4.2	3.9
WSC	106	124	87	95
BC	60	58	72	50
Ratio WSC/BK	1.77	2.14	1.21	1.90
Min. dry matter	308	279	353	298

Table 2. Ensilability of bio-mass and chemical composition of French ryegrass silages ***(Dinić et al. 2008)***

Parameters	A_1	A_2	B_1	B_2	LSD $_{0.05}$	LSD $_{0.01}$
WSC, g kg^{-1} SM	187,5a	159,3b	182,3a	164,5b	13.90	21.50
BC, meqv lactic acid 100g^{-1} DM	32.82a	29.83b	34.18a	28.47b	2.36	3.57
WSC/BC	5.79a	5.35b	5.79a	5.36a	0.73	1.10
DM, g kg^{-1}	308.3b	322.5a	233.8b	392.0a	4.37	6.63
CP, g kg^{-1} DM	156.0a	138.2b	144.2b	150.0a	2.91	4.40
Lipids, g kg^{-1} DM	50.05a	48.2a	49.3a	48.9 a	9.2	13.9
NDF, g kg^{-1} DM	690.1a	704.4a	711.7a	691.0a	19.51	29.51
ADF, g kg^{-1} SM	338.7a	340.3a	328.8a	335.2a	13.43	20.30
Hemicelluloses, g kg^{-1} DM	351.3a	364.1a	382.8a	355.8a	13.30	20.20
Ash, g kg^{-1} DM	104.4a	97.6b	102.8a	99.2a	4.69	7.10
Ca, g kg^{-1} DM	5.4a	4.9a	4.7b	5.6a	0.78	1.18
P, g kg^{-1} DM	3.53a	3.05b	3.33a	3.25a	0.27	0.40

Legend : A = development stage (a1 = spike forming and a2 = blossoming; interval of 7 days) and B = degree of dry matter (b1 = mass with natural moisture content and b2 = wilted mass).
[a,b] Values in the same row for the same factor with different letters are statistically different (P<0.05)

In development of ensiling technologies, results of numerous trials were either used successfully in the practice (chemical conservants on the basis of organic acids, carbon hydrate additives, wilting, biological preparations) or were determined to be impractical (mineral acids), *(Dinić et al., 2004)*. Today, in Europe but also throughout the world, biological preparations and organic acids are forced.

Wilting

Amount of dry matter in silo mass is one of the most important factors influencing the loss of dry matter and directing the fermentation during ensiling process, especially in silo mass rich in proteins. Therefore, wilting of silo-mass prior to ensiling is one of the most important ways to improve the quality of silage. Numerous researchers have studied the problem of wilting, and the most complex researches were lead by *Zimmer and Wilkins (1984).* They established that silages were well preserved in two treatments, when chemical preparation based on formic acid was used as the conservant in silages made from non-wilted silo-mass and when wilted silo-mass which was ensiled had between 300 and 400 g kg^{-1} of dry matter. Wilting of silo-mass, i.e. increase of the level of DM in silo mass of 300-400 g kg^{-1} proved to be more important than the chemical conservant – formic acid *(Dinić, 1997)*. Wilting of silo-mass contributed to the ensiling process on whole, to greater production of lactic acid in the silage, and at the same time, to ruction of undesired products of ensiling, such as butyric acid and ammonia nitrogen *(Dinić et al., 1999)* which are indicators of protein degradation. Wilting is certainly most cost effective solution for successful ensiling of leguminous plants. It was stated that for successful ensiling of alfalfa, red and white clover, minimum content of dry matter must be within limits of 400 and 300g kg^{-1} *(Dinić et al., 1994; Dinić, 1997)*. Requirement that at the moment of ensiling bio-mass has high level of DM contributed to the situation that livestock farmers from developed countries decide to prepare haylages.

For the purpose of acceleration of the wilting process, cutting machines are equipped with additional squeezers/presses or different blowers/hammers which crush the plant and speed up the process of wilting by 25-30%. Wilting of bio-masses of perennial grasses and leguminous plants contributes to more favourable lactic acid fermentation, i.e. ensures better quality and evaluation of the silage by one, sometimes even by two classes *(Dinić et al., 2003)*.

Bacterial-enzyme fermentation stimulation

Use of bacterial inoculants. Main purpose of inoculation is to intensify and direct the fermentation by adding selected strains of homo-fermentative lactic acid bacteria, primarily in feeds which do not contain sufficient fermentable carbohydrates. Advantage of biological additives is primarily in the fact that they do not leave residues and have no detrimental effect on animal health and quality of their products, so they are suppressing the chemical conservants, regardless of their efficiency. Process of lactic acid fermentation in bio-mass by natural or spontaneous fermentation takes place through several phases. Essence of the process is transformation of sugars into lactic acid, so that pH of the bio-mass is reduced from 6.0-6.5 to approx. 4.0 when silage is considered to be stable. This course of fermentation is achieved in anaerobic conditions. Depending on the level of dry matter, stability of silage is achieved even with pH 5.00, but than it is haylage containing approx. 500 g kg^{-1} *(Beyer et al., 1982)*.

Inoculation for the purpose of ensuring aerobic stability of silage. Problem in well conserved silages where homo fermentative inoculants had been used is reduced aerobic stability compared to non-inoculated silage. *Merry et al. (1997)* stated that acetic, butyric and especially propionic acids have greater fungicide effect compared to lactic acid, therefore they are even desirable in silages to some extent. Lactic and acetic acid, as well as WSC are main sources of energy for micro-organisms which participate in silage degradation. Their oxidation results in forming of CO_2 and water with simultaneous release of temperature.

Yeasts participate in degradation of nutritive substances of silage, i.e. induce aerobic instability of silage. They are usually from genus *Pichia, Hamsenula, Candida, Saccharomyces,* etc. Yeasts also play important role in aerobic degradation of silage, especially silage made of grasses. In some silage, development of moulds is accompanied by development of yeasts, which is manifested by incidence of two temperature maximums. First temperature maximum occurs 2-3 days subsequent to entry of air, and is cause/induced by yeasts, and the second maximum, for which the yeasts are responsible, in 3 to 4 days. The most frequent yeasts which induce degradation of silage belong to genera *Monascus, Geotrichum, Byssochlamus, Mucor, Aspergillus, Penicillium* and *Fusarium (McDonald, 1985)*.

This is the reason why hetero fermentative lactic acid bacteria are important for aerobic stability of silages. In accordance to this, in ensiling of corn, *Elferink et al. (1997)* used as inoculants different obligatory or facultative hetero fermentative bacteria of lactic acid, in which case the greatest aerobic stability was achieved with inoculants *Lactobacillus buchneri,* slightly lower stability with *L. kefir* and *L. parabuchneri,* whereas other studied obligatory or facultative hetero fermentative lactic acid bacteria did not improve, they even reduced the aerobic stability of the silage (*L. graminis, L. plantarum*).

Use of propionic bacteria. Propionic acid is known as very efficient fungicide preparation and it is used for conserving of non-matured corn grain or corn of increased moisture. This stimulated scientists to come up with the idea to investigate the possibility of use of propionic bacteria as inoculants for the purpose of increasing of the silage stability. So, for instance, *Dawson et al. (1998)* in ensiling of high moisture corn inoculated it with bacteria *Propionibacterium acidipropionici* and established improvement of fermentation and aerobic stability.

Enzymes as fermentation stimulators. As stimulators of lactic acid bacteria fermentation during silage preparation, enzyme additives is used which contain cellulase, hemicellulase, amylase, pectin's and lignins. Action of said enzymes induces degradation to crude fibre, and final products of this action are carbohydrates of lower molecular mass which can be used as substrate of the action of lactic acid bacteria *(Đorđević et al., 1998)*.

Principle of action of cellulase enzymes is especially important in ensiling of leguminous bio-masses, which do not contain sufficient amount of fermentable carbohydrates. The effect of the use of cellulotic enzymes is demonstrated especially during their simultaneous use with inoculants of homo fermentative lactic acid bacteria. Therefore, more commercial biological preparations produced as additives for preparation of silage contain in addition to selected strains of lactic acid bacteria also cellulotic preparations. However, compared to carbon hydrate additives or chemical conservents, the effects of the application of enzyme preparations on the fermentation results are considerably lower.

Carbon hydrate additives

Ground grains/cereals is additive which can be easily be produced or provided on every holding, it is easy to administer and to determine the dosage and distribute over the bio-mass, it has positive effect on total nutritive value of the silage, and also it reduces moisture of the ensiled material and leaching of juiciest. Adding of ground grains/cereals is recommended in the amount of 4 to 10%. In experiments and in the practice, mainly ground corn is used, since this feed is available in great quantities *(Dinić et al., 1996; Đorđević et al., 2000)*. Ground barley is investigated and used in countries where climatic conditions are not favourable for growing of corn for grain *(Jelač et al., 1991)*. In ensiling of alfalfa they used ground barley with and without malt, but no significant differences were established between treatments. In trial by *Dinić et al. (1996)* alfalfa was ensiled deriving from the second cut with addition of ground corn (2, 4 and 6%), molasses (1, 2 and 3%) and formic acid (0,15; 0,30 and 0,45%). All silages with different additives had better quality and more points (according to *Zelter)* compared to control alfalfa silage. In regard to effects, in view of pH value and amount of lactic acid, molasses wasn't lagging behind formic acid.

Dried beat pup are also used as additives in ensiling of leguminous plants, in the amount of 4-10%. In addition to positive effects obtained in regard to ensuring of necessary substrates for the activity of lactic acid bacteria, they increase the nutritive value of silage, improve the energy to protein ratio and absorb surplus of moisture – up to 3 l of water per one kilogram of used beat pup *(Jelač et al., 1991; Pavličević, 1993)*.

Nutritive value of bio-mass

Nutritive value of bio-mass and silages of perennial grasses depends on numerous factors, primarily on the stage in which the plants were cut, share of other plant species in the bio-mass, and also on quality of fermentation. Total nutritive value of ensiled material depends on losses during fermentation process, changes occurring as the result of plant and microbe enzymes during storage time. With the increase of plant share of leaves compared to the stem decreases, and at the same time changes in the content of sugar and buffer capacity occur since leaves contain more nutritive substances important in regard to the quality compared to stem *(Ignjatović et al., 1998)*. By aging of plants their ensilability increases, i.e. the sugar concentration increases, and BC decreases. With maturation of plants yield of DM in the vegetation cycle increases, but at the same time digestibility decreases, which is consequence of intensive lignification processes. *Mejakić et al. (1997)* established in a four year research that the highest yield of DM and the highest yield of crude proteins are realized in the customization stage (Table 3).

Table 3. Yield of dry matter and digestible crude proteins of alfalfa in different utilization phases *(Mejakić et al., 1997)*

Cutting phase	Dry matter		Digestible crude proteins	
	%	t/ha	%	kg/ha
Before budding	19.31	10.72	19.38	2163
At the beginning of budding	19.84	11.83	18.80	2229
Budding	21.34	13.88	16.52	2304
Beginning of blossoming	22.39	13.86	15.34	2100
Blossoming	23.67	13.49	12.97	1744

Concentration of crude proteins in the initial material and alfalfa silages was within limits 150-200 gkg^{-1}DM, even over 200 g kg^{-1}DM, and content of Ca 12-20 g kg^{-1}DM *(Dinić et al., 1997; Đorđević, 2000; Đorđević et al. 2003)*. Typical situation is that our farmers do not cut perennial grasses and leguminous plants on time. Consequence of such cutting is poor quality of forage food, and ultimate outcome is expensive production. Results of research carried out by *Dinić et al. (2002)* confirm this. The chemical composition of bio-mass of Italian and English ryegrass cut at the beginning of plant spike forming (29.04. and 05.05) and beginning of blossoming (18.05 and 19.05). Earlier cutting of Italian ryegrass crop provided relatively higher content of crude protein by 34% and approx. 205 less crude fibre, whereas in biomass of English ryegrass, this difference was even greater in regard to content of crude proteins, less by 41% and content of crude fibre, less by 17%.

Corn is the most important forage plant in Serbia, because of the area it occupies and energy value, and therefore it complements protein feeds. In regard to this, *Dinić et al. (1988-a)* ensiled corn in the stage of wax ripeness (DM = 41,90%) in mixture with alfalfa cut at the beginning of blossoming, using fresh and wilted form (DM = 25,80%; 35,0% and 43,0%). Based on chemical analysis of silage, using Flieg method of quality assessment, it was established that mixtures with corn can include 505 of fresh alfalfa max., without detrimental effect on quality of silage. Contrary to other forage plants, where with the maturation/age their nutritive value and quality decline, in corn they increase, which is demonstrated by data presented in Table 4 *(Pejić, 1994)*.

Table 4. Change of nutritive value of corn green mass in different stages of ripeness *(Pejić, 1994).*

Ripeness stages	in 1 kg of feed		
	Nutritive units, %		Digestible proteins, g
Blossoming	0.176	55.89	13.3
Beginning of grain formation	0.183	58.10	13.6
Milky	0.223	70.80	13.0
Milky-wax	0.297	94.28	13.0
Wax	0.315	100.00	14.0
Full	0.393	124.76	14.6

Based on data presented in table and research results obtained by numerous researchers, it can be concluded that corn should be cut, i.e. ensiled in the phase of wax and full was ripeness of the grain when the nutritive value is the highest.

Sorghum bio-mass can be interesting culture for combinations with alfalfa and red clover, because in good years it gives two cuts. Chemical composition of bio-mass of sorghum is very similar to corn, therefore for obtaining of quality silage mixing in following ratio of green mass is recommended 50 : 50. *Dinić et al. (1995)* ensiled sorghum (II cut) and alfalfa (IV cut) in different mass ratios and established high quality of silages (I class) also in ratio 50 : 50 (tab. 5). However, authors recorded high quality of silages also in cases where alfalfa participated with 75% (II quality class).

Table 5. Quality parameters of sorghum and alfalfa silage *(Dinić et al., 1995).*

Treatments	DM, g/kg	pH	NH_3-/∑N %	Acids			Evaluation by DLG
				Lactic	Acetic	Butyric	
$S_{100}L_0$	267	3.84	3.6	34.1	12.7	0.0	I
$S_{75}L_{25}$	269	4.19	5.4	33.1	11.0	1.1	I
$S_{50}L_{50}$	276	4.74	5.6	37.3	18.5	0.1	I
$S_{25}L_{75}$	281	5.07	6.6	38.8	23.5	1.4	II
S_0L_{100}	284	5.07	10.0	27.5	22.2	3.2	III

S = sorghum; L = alfalfa

Conclusion

Problems pertaining to high buffer capacity and insufficient quantity of fermentable carbohydrates in biomass of perennial and annual leguminous plants can be solved by wilting, use of carbon hydrate additives, mixing of bio-masses of perennial leguminous plants and grasses, either during sowing or ensiling or by inoculation. Cutting of perennial grasses at the beginning of spike forming, leguminous plants in the phase of butonization, corn in the phase of full wax ripeness of grain ensures obtaining of high quality forage food, and economically efficient production of milk and meat. By establishing of joint crops of annual leguminous plants and grains/cereals their ensiling provides good yield and good quality of forage food (silage) and possibility for growing of post-crop. By correct ensiling of the whole corn plant, with application of adequate inoculants for aerobic stability and mixing of bio-masses of leguminous plants the problem of lack of proteins, minerals, and vitamins is solved as well as balance of the diet for ruminant nutrition.

Postupci za poboljšanje kvaliteta procesa fermentacije i povećanje hranljive vrednosti silaža

B. Dinić, J. Radović, G. Jevtić

Rezime

Konzervisanje stočne hrane metodom siliranja je izuzetno značajno za obezbeđivanje kvalitetne stočne hrane dobrog kvaliteta za ishranu životinja. Pre siliranja potrebno je analizirati biomasu u pogledu sadržaja vodorastvorljivih ugljenih hidrata (šećera), pufernog kapaciteta i odnosa šećera i pufernog kapaciteta u cilju obezbeđenja uspešne mlečnokiselinske fermentacije. Povećanje nivoa suve materije u silomasi (provenjavanje) je vrlo značajan postupak za smanjenje gubitaka u hranljivim materijama i obezbeđivanja dobijanja kvalitetne silaže bez prisustva buterne kiseline. Primena inokulanata na bazi homo i heterofermentativnih bakterija obezbeđuje se pospešivanje mlečno-kiselinske fermentacije i povećanje aerobne stabilnosti silaža. Košenje biljaka u odgovarajućoj fazi doprinosi dobijanju kvalitetne kabaste hrane visoke hranljive vrednosti.

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Invited paper

Example 2

THE EFFECT OF PARAGENETIC FACTORS ON REPRODUCTIVE TRAITS OF SIMMENTAL COWS

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