Green manure legumes for organic seed production of *Phleum pratense*

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Abstract. Experiments conducted during the period 2003–2008 were designed to study the effects of six legume species (*Trifolium pratensis, Medicago sativa, Trifolium repens, Galega orentalis, Medicago lupulina, Trifolium resupinatum*) ploughed down as green manure on the seed yield of timothy (*Phleum pratense)*. Legumes were undersown into barley. In the first year of production legumes were chopped and ploughed down for green manure in June. The sown timothy was used for seed for three years. *Trifolium resupinatum* grew luxuriantly in the year of sowing, however, it completely disappeared after winter. Depending on the legume species before ploughing down, the content of nitrogen accumulated in the above ground part and roots was 165–272 kg ha^{-1}, phosphorus 16–25 kg ha^{-1}, and potassium 75–165 kg ha^{-1}. According to the data averaged over three years of production, the highest timothy seed yield was obtained having ploughed down a mixture of the three legumes (*Trifolium pratensis + Medicago sativa + Trifolium repens*). Having ploughed down legumes, the positive effect on seed yield of timothy was more substantial in the second and third years of production.

Key words: *Phleum pratense,* green manure legumes, organic seed production

INTRODUCTION

Timothy (*Phleum pratense*) is well adapted to northern conditions, but is slow to establish; the best yields are generally obtained in the second year after sowing. Timothy is winter hardy, and is used in leys in cooler areas of northern Europe where ryegrasses would not survive (Frame, 1992; Sheldrick, 2001). This grass is better suited to cutting than intensive grazing. Timothy is suitable for mixtures with red clover and meadow fescue and therefore it is very suitable for organic farming (Niskanen, 2004). According to the Council Regulation (EEC) seed and vegetative plant material for organic farming shall be organic, however, there is a great shortage of organically grown seed of perennial grasses; for many varieties of legumes there is no organically produced seed available. In Lithuania, both the growing of organic seed and related research is scarce. Scandinavian countries seem to have the most considerable experience of growing perennial grass seed on organic farms. In Norway, comprehensive studies have been done on timothy, white clover, red clover and alsike clover (Aamild, 2002). Thorough research has been done in Denmark and valuable experience of growing perennial ryegrass and white clover has been accrued. The seed yields of perennial ryegrass grown on organic farms differed little from those obtained on conventional farms, however the seed yields of white clover were low (Lund-Kristensen et al., 2000; Boelt, 2003). In Denmark and Norway, there were attempts to grow grass seed together with legumes (Deleuran & Boelt, 2000; Solberg et al., 2007, predominantly using red and white clovers. However, red clover often suppresses grasses, therefore the crops of the first year are generally used for forage, and grass seed is harvested in the second year of use (Deleuran & Boelt, 2002). Slurry and liquid manure are frequently used for fertilization of grass seed crops, whereas in Lithuania this possibility is limited due to the lack of suitable machinery and lack of manure on some organic farms. If manure is unavailable on an organic farm, grasses can be supplied with nitrogen by ploughing in green manure or by intercropping with legumes. The study was designed to identify which legume species are best suited for green manuring when growing timothy for seed on an organic farm.

MATERIALS AND METHODS

Field experiments were set up in the central part of Lithuania (55°23' N, 23°51' E) on a medium-textured *Endocalcari-Endohypogleyic Cambisol* with a plough layer thickness of 25 cm, pH 7.0–7.1, humus content 2.16–2.36%, available P and K 119–136 and 141–153 mg kg^{-1} soil, mineral nitrogen 0.12–0.16%. Two field experiments were set up in 2003 and 2004. The soil samples were collected from the 0–25 cm layer in the four replications of each experimental plot in the spring of the sowing year. Soil pH was analysed by a potentiometric method, available P and K by AL, humus by Tyurin methods. Mineral nitrogen was measured by distillation and the colorimetry method (in 1N KCl extraction). Six legume species were tested for green manuring. Herbs designed for green manure were undersown in spring barley (*Hordeum distichum*). Legumes were not sown in the plots of the check treatment. A full seed rate of legumes, which is recommended for herbage growing for forage with a cover crop, was sown: red clover cv.'Vyliai'(*Trifolium pratensis*) 12 kg ha^{-1}, lucerne cv. 'Birute' (*Medicago sativa*) 12 kg ha^{-1}, white clover cv. 'Atoliai' (*Trifolium repens*) 8 kg ha^{-1}, fodder galega cv. 'Gale' (*Galega orientalis*) 30 kg ha^{-1}, black medic cv. 'Arka'(*Medicago lupulina*) 8 kg ha^{-1}, Persian clover (*Trifolium resupinatum)* 8 kg ha^{-1}. The legume mixture (red, white clover and lucerne) 10.6 kg ha^{-1} was composed of an equal share of seed of each species. In the sowing year, having harvested barley at grain milk-wax maturity stage for whole crop silage, legumes were grown until late autumn. The next year, in the second ten-day period of June, we estimated the dry matter yield of the above-ground part of legumes and root biomass. The dry matter biomass of the above-ground plant part was measured in each plot using a small-plot harvester "Hege 211" by cutting 1.35x13.0m strips. For root biomass determination two 25 x 25 x 25 cm soil cores with roots were dug from each experimental plot. The soil cores were transferred on meshed sieve and the soil was washed from roots with running water. The roots were dried at 60ºC temperature, then dried and ground. In the chemical laboratory, the dry roots were analysed for N by Kjeldahl method, for P by a spectrophotometrical method using "Cary 50", for K and Ca by a flame photometry method. In the third ten-day period of June legumes were chopped and ploughed in. The soil was prepared for grass sowing. Timothy was sown in July with narrow row spacings (12 cm) without a cover crop. The area of record plots was 13 × 2.2 m. A randomized block design with four replications was used for each experiment. In the next year before harvesting, timothy seed samples were taken for the determination of biological yield structure. For this purpose, samples were cut per each plot's 0.25 m^2 area and were later analysed in the laboratory. The sample was analysed for the total biomass weight g m^{-2}, number of shoots m^{-2}, length of shoots and inflorescences. Timothy was thrashed by a combine "Sampo 500". The seed was dried, cleaned and analysed for quality, seed purity, germination and 1000 seed weight. Seed yield data were corrected to 100% purity and 14% moisture content. Grass seed was harvested for three years of production. No fertilization and no pesticides were used.

The data were processed by ANOVA. To evaluate the significance of the differences between means, LSD test with 5% significance level was used.

RESULTS AND DISCUSSION

Legumes undersown in barley influenced the total yield of the barley whole crop silage. The content of legumes in the dry matter yield of whole crop silage was from 5.4% (black medic) to 16.2% (Persian clover). Having undersown pure legumes, the highest yield of whole crop silage was in the treatments where the undersown crop was Persian clover - 7.27 Mg ha^{-1} and a mixture of red clover with lucerne and white clover – 6.88 Mg ha^{-1} (Table 1).

Other legumes, except for red and white clover also significantly increased the dry matter yield of whole crop silage. However, 1 kg of forage dry matter accumulated a similar content of metabolisable energy both when barley had been grown without legumes – 9.28 MJ kg $^{-1}$, and with undersown legumes – 9.29–9.44 MJ kg^{-1}. Due to the droughty weather the autumn aftermath was very small (0.26–0.73 Mg ha^{-1}). In autumn of the sowing year the most luxuriant was the mixture of legumes composed of white clover and lucerne 278–267 plants m^{-2}.

The thinnest crops were those of fodder galega and Persian clover 142–168 plants m^{-2}. Under our climate conditions, Persian clover did not survive the winter. In West Europe, Persian clover was traditionally used as a self-regenerating, autumn-sown cool-season annual, but can be grown as a special-purpose summer forage crop. It displays moderate cold tolerance, and withstands moderate water logging and saline conditions (Frame, 2005; Mueller & Thorup-Kristensen, 2001). In our trials, in autumn of the sowing year, Persian clover accumulated 0.54 Mg ha^{-1} of dry matter. However, the data are not comparable. Other herbs grew longer – until the second ten-day period of June - and the plots of Persian clover were tilled (the stubble was broken) early in spring, having made sure that clover had not survived the winter.

In the first year of production legumes sown for green manure produced different above-ground biomass. The highest dry matter yield 6.32 Mg ha^{-1} was accumulated in the above-ground mass of lucerne, while red clover accumulated 5.13 Mg ha^{-1}, mixture of legumes 4.80 Mg ha^{-1} (Table 2). Due to slower growth and development in the first year of production the above-ground mass of fodder galega and white clover was the lowest. The highest content of nitrogen in the above-ground part was accumulated by lucerne, 194 kg ha^{-1}, markedly less by red clover and the mixture of lucerne, red clover and white clover, 136–119 kg ha^{-1}. The contents of other elements (P, K, Ca) that accumulated in legume biomass were proportional to the accumulated dry above-ground biomass.

Table 1. The yield of cover crop barley with undersown legumes and autumn aftermath of legumes, 2003–2004. Data were averaged over two trials.

Undersown legumes	Barley for whole crop silage				Autumn aftermath of legumes	
	DM Mg ha^{-1}	Metabolisable energy GJ ha^{-1}	M J kg^{-1}	Crude protein kg ha^{-1}	DM t ha^{-1}	M J kg^{-1}
Red clover	6.53	60.95	9.33	751.2	0.44	11.36
Lucerne	6.63	62.21	9.38	762.7	0.66	11.83
White clover	6.40	60.22	9.41	736.0	0.47	11.40
R. clover + Lucerne + W. clover	6.88	63.94	9.29	791.5	0.73	11.31
Fodder galega	6.69	63.20	9.44	769.9	0.33	10.40
Black medic	6.68	63.02	9.43	768.5	0.26	10.19
Persian clover	7.27	67.93	9.34	835.5	0.54	10.78
Without legumes	6.12	56.77	9.28	703.5		
LSD$_{05}$	0.51	4.796		58.9	0.06	

Table 2. Biomass of dry matter and contents of N, P, K, Ca accumulated in the above-ground mass of ploughed-in legumes and in roots in the plough layer in 2004–2005. The data were averaged over two trials.

Ploughed-in legumes	Biomass DM Mg ha^{-1}	kg ha^{-1} N	P	K	Ca
		Above-ground plant part			
Red clover	5.13	136	13	81	81
Lucerne	6.32	194	16	120	113
White clover	2.71	79	8	53	49
R. clover + Lucerne + W. clover	4.80	119	11	82	64
Fodder galega	2.98	87	6	41	34
Black medic	3.58	98	10	74	46
Persian clover**	0.54	17	2	17	6
LSD$_{05}$	0.424	15.4	1.0	7.6	6.2
		Roots *			
Red clover	4.88	99	11	56	35
Lucerne	4.27	78	9	45	23
White clover	5.68	123	13	65	47
R. clover + Lucerne + W. clover	5.08	107	12	58	35
Fodder galega	4.16	81	8	34	19
Black medic	3.54	67	6	47	25
Persian clover**	3.10	74	8	39	37
LSD$_{05}$	1.072	21.4	2.4	13.8	7.8

* roots in the plough-layer **–estimated in the autumn of the sowing year

The largest amount of roots with green stubble 5.68 Mg ha^{-1} was left in the plough layer by white clover, while the least amount was left by black medic and Persian clover 3.10–3.54 Mg ha^{-1}, respectively. Root mass, compared with accumulated above-ground biomass, was relatively high. In our trials the ratio of roots to above-ground part changed from 2.09 for white clover to 0.67 for lucerne. The researchers suggest that the ratio of legume roots to above-ground part is highly dependent on legume species, management and plant age (Kendall et al., 1982). In our research the relatively higher amount of roots can be explained by the fact that big green stubble left near the roots, especially of white clover, was attributed to roots. The roots accumulated relatively less nitrogen, potassium, and calcium than the above-ground plant part, while phosphorus contents were similar.

Depending on the legume species (except Persian clover) before ploughing-in, the content of nitrogen accumulated in the above-ground part and roots was 165–272 kg ha^{-1}, phosphorus 10–23 kg ha^{-1}, and potassium 75–165 kg ha^{-1}. When estimating according to individual elements, lucerne was found to have accumulated the highest contents. As a result, the chopped above-ground part and roots present in the plough layer had the highest dry matter biomass, nitrogen, phosphorus, potassium and calcium when lucerne, red clover and mixture of these legumes with white clover had been grown.

The data averaged over the two trials indicate that none of the legumes ploughed in as green manure gave a significant increase in timothy seed yield in the first year of production (Table 3). This can be explained by the fact that control plots in which no legumes were sown were intensively tilled (the stubble was broken 3–4 times). Intensive tillage released more nutrients in the soil, therefore the effect of ploughed-in legumes in the first year was inappreciable and insignificant.

In the second year of production the seed yield of timothy declined considerably. When grass seed is grown in an intensive farming system with optimal mineral fertilizer rates, the seed yield of grasses in the second year of production is either similar to that in the first year of production or the reduction is small (Kryzeviciene, Slepetys, 2003). In the second year of production the positive effect of the ploughed-in legumes showed on timothy seed yield. Significantly more seed was obtained when the legume mixture or pure red clover, lucerne and black medic had been ploughed in. In the third year of production, the seed yield of timothy not fertilized with green manure declined to 95 kg ha^{-1}. The seed yield of timothy fertilised with green manure (legume mixture), was twice as high, reaching 193 kg ha^{-1}.

Table 3. Seed yield of timothy (kg ha^{-1}) as affected by ploughing-in of legumes as green manure 2005–2008. The data were averaged over two trials.

Ploughed-in legumes	Seed yield kg ha^{-1}			
	1st year of production	2nd year of production	3rd year of production	total
Red clover	553	325	131	989
Lucerne	503	319	151	973
White clover	469	314	163	946
R. clover + Lucerne + W. clover	531	387	193	1111
Fodder galega	457	296	104 235*	857
Black medic	493	339	143	975
Persian clover	519	305	122	946
Without legumes	507	267	95	869
LSD_{05}	73.4	47.5	26.1	91.2

*Seed yield of fodder galega

In the third year of production timothy seed yield was significantly increased by all ploughed-in legumes, except for fodder galega. Re-grown fodder galega and sown timothy ripened the seed yield together. The mixture of re-grown fodder galega and timothy produced 104 kg ha^{-1} of timothy seed and 235 kg ha^{-1} of fodder galega seed. The effect of ploughed-in legumes was more tangible in the second and third years. This can be explained by the fact that, by mineralizing, legume stems, and especially roots slowly released nutrients that secured the timothy yield increase. Moreover, legumes, especially clover, fodder galega and lucerne re-grew and by growing together partly supplied timothy with nitrogen. Perennial legumes re-grow from roots in the second third year despite the fact that they have been well ploughed in. The re-growth is less when the soil is tilled more intensively and row crops or cereals are grown after ploughed-in legumes.

Research data about the effects of legumes on seed grasses is rather scarce and controversial. In Norway, *Trifolium subteraneum* increased timothy yield by 20% (Solberg et al., 2007). In Lithuania white clover was found to exert a positive effect on meadow fescue and smooth-stalked meadow grass (Kryzeviciene, 2001). In Denmark, perennial ryegrass seed yield was found to have been significantly increased by black medic, Persian clover and bird’s foot trefoil (*Lotus corniculatus*), however, no positive effects were found using white, red and alsike clover (Boelt et al., 2002).

Our data averaged over two experiments suggest that during the three years of production the highest significant timothy seed yield increase of 27% was obtained having ploughed in a mixture of lucerne, red clover and white clover. It is likely that ploughed-in legumes grown in a mixture mineralised at different times and supplied timothy with nutrients more evenly. The ploughed-in pure legumes increased timothy seed yield less: red clover -13.8%, black medic - 12.2%, lucerne - 12.0%. During the three years of production only ploughed-in Persian clover, fodder galega, and white clover did not significantly increase timothy seed yield.

The seed purity of timothy of the first and second year of use after pre-cleaning was 91.0 – 99.2%; that of the third year declined and ranged from 76.2–99.0%. Other seeds were not only weed seeds, but also the seed of re-grown red clover, lucerne, and fodder galega. Legumes that re-generated and matured helped to supply timothy with nitrogen, however, they impaired the timothy seed quality (purity). Admixtures of legume seed increase seed cleaning costs and reduce seed output. It is possible to separate lucerne, red clover and fodder galega seed from timothy seed during the main cleaning. The losses incurred during seed cleaning are compensated by a significant seed yield increase resulting from legumes growing together with timothy. Self-regenerated or re-grown legumes in an organic seed timothy crop should be viewed positively, since legumes accumulate nitrogen. Timothy produces a poor seed yield without nitrogen fertilizers.

Ploughed-in legumes did not have any effect on timothy seed germination and 1000 seed weight. These seed quality indicators were more markedly affected by diverse weather conditions during timothy seed ripening and at harvesting.

CONCLUSIONS

Six legume species were tested for green manuring of timothy. *Trifolium resupinatum* grew luxuriantly in the year of sowing, however, it completely disappeared after winter. Depending on the legume species, before ploughing in, the content of nitrogen accumulated in the above-ground part and roots was 165–272 kg ha^{-1}, phosphorus 16–25 kg ha^{-1}, and potassium 75–165 kg ha^{-1}. Having ploughed down legumes, the positive effect on the seed yield of timothy was more substantial in the second and third years of production. Over the three years of production the highest significant timothy seed yield increase of 27% was obtained having ploughed in a mixture of lucerne, red clover and white clover as green manure. Ploughed-in pure legumes (lucerne, red clover, black medic) gave a lower but significant increase in timothy seed yield. Over the three years of production, only ploughed-in Persian clover, fodder galega and white clover did not produce any significant yield increase in timothy seed yield.

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