

Establishment and Early Yield Development of Five Possible Alternatives to *Trifolium repens* as a Grassland Legume

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Abstract

The performance of *Trifolium repens* as the main grassland legume in temperate climates may decrease under climate change due to more frequent water shortages. This calls for alternative legumes with agronomic potential. We examined germination rates, establishment, winter tolerance and yield potential of *Medicago lupulina*, *Medicago falcata*, *Lotus corniculatus*, *Lotus uliginosus* and *Onobrychis viciifolia* both in monoculture and in mixture with *Lolium perenne* in a two-year container experiment. Germination and establishment of all alternative legumes were comparable to *T. repens* except of *M. falcata* with a retarded initial development. *L. uliginosus* was the only species with an insufficient winter tolerance. In pure stands *M. lupulina* and *L. corniculatus* showed a yield potential almost as high as of *T. repens*. However, their performance in mixture with *L. perenne* was lower than *T. repens*. This has to be considered with the choice of less competitive grass partner species when designing seed mixtures.

Keywords: *Lotus corniculatus*, *Lotus uliginosus*, *Medicago lupulina*, *Medicago falcata*, *Onobrychis viciifolia*, *Lolium perenne*, winter tolerance, early development

1. Introduction

Legumes are important for grassland productivity, especially in swards with low or no nitrogen (N) fertilisation due to their ability to fix atmospheric N. Nevertheless, in conventional agriculture in Europe the proportion of forage legumes in swards has decreased in the last decades (Peeters, 2009) mainly because of the ready availability of inorganic N-fertilizer (Rochon et al., 2004). However, with increasing energy and N-fertilizer prices along with higher costs for concentrates, the use of grassland legumes becomes more attractive (Jensen & Hauggaard-Nielsen, 2003; Crews & Peoples, 2005).

The main fodder legume in grasslands in Central Europe is *Trifolium repens* (Frame, Charlton, & Laidlaw, 1998). Under appropriate climatic conditions, *T. repens*/grass mixtures can produce high yields and a good fodder quality (Wilman & Williams, 1993; Wilkins, Gibb, Huckle, & Clements, 1994; Topp & Doyle, 2004). However, *T. repens* has been shown to need a good supply of water for growth (Foulds, 1978). This may become challenging in times of climate change, as summer rainfall is predicted to become sparse (Alcamo et al., 2007).

Other legumes may be better adapted to drier conditions or have special feeding values and may therefore have potential as alternatives for *T. repens*. Currently, the agronomic knowledge, including early stages of establishment in monoculture and in mixture with grasses, of other legumes of permanent grasslands is limited (Hopkins, Martyn, Johnson, Sheldrick, & Lavender, 1996; Rochon et al., 2004; Hopkins & Wilkins, 2006; Sölter, Hopkins, Sitzia, Goby, & Greef, 2007) and their cultivation and use insignificant. Early development determines to a great deal the successful establishment and yield contribution of legumes especially when sown in mixture with grasses (Petersen, 1967).

In this study, we therefore tested the agronomic potential in early development of five promising grassland legumes (Table 1) against *T. repens*. A container experiment was conducted in a vegetation hall from 2009 to 2010. All legumes were sown in monoculture and in mixture with *Lolium perenne*. The climatic conditions in the

vegetation hall followed a normal seasonal pattern with frost in winter and higher temperatures in summer. We considered the germination rates, establishment, the response to a winter stress phase and the yield potential in the sowing and first main production year.

Table 1. Used plant species, cultivars, seed weight, ecological strategy types, tolerances (mowing, grazing, trampling) and nutritive values of all species

Plant species	Cultivar	Seed weight [mg]	Strategy type ¹	Tolerance			Nutritive value ²
				Mowing ³	Grazing ²	Trampling ²	
<i>Lotus corniculatus</i>	Bull	1.45	csr	6	4	4	8
<i>Lotus uliginosus</i>	wild seeds	0.74	csr	4	4	4	7
<i>Medicago lupulina</i>	Ekola	1.69	csr	7	4	6	8
<i>Medicago falcata</i>	wild seeds	0.95	cs	5	2	2	7
<i>Onobrychis viciifolia</i>	Matra	21.90	c	6	2	2	8
<i>Trifolium repens</i>	Rivendel	0.62	csr	8	8	8	9
<i>Lolium perenne</i>	Signum	2.75	c	8	8	8	9

¹ according to Klotz, Kühn and Durka (2002); c: competitor. s: stress tolerator. r: ruderal

² according to Dierschke and Briemle (2002); values range from 1 (low) to 9 (high)

³ according to Briemle and Ellenberg (1994); values range from 1 (low) to 9 (high)

2. Material and Methods

The experiment consisted of a germination test and a container experiment with six legumes and the grass *Lolium perenne*. Both, wild flower seeds and cultivars were used depending on the availability (Table 1). The trial was separated into three phases: germination and establishment (establishment phase), effects of lower temperatures and short days (winter stress phase) and the first main production year (initial yield phase).

2.1 Germination Test

For the germination test, 30 seeds of each species were sown on Petri dishes (9 cm diameter, bottom covered with two pieces of moistened filter paper, Schleicher and Schuell Microscience). The lids were replaced and fastened with laboratory film (American National Can). Five replicate dishes for each species were arranged in a randomized block design in a greenhouse (night temperatures: 13 to 16°C, day temperatures 21 to 26°C, no extra lighting, germination between February 8th and March 4th 2009). Every two days, germinated seeds (with visible radicles) were counted and removed. The filter paper was kept moist (tap water).

2.2 Container Experiment

2.2.1 Experimental Setup

The container experiment was set up in 2009, sowing date was July 15th. For this experiment, 30 l containers (diameter 33 cm, height 42 cm) were filled with a homogeneous mixture of 20 kg air-dried sand (sieved to pass a mesh of 5 mm; August Oppermann Kiesgewinnung GmbH), 0.9 kg vermiculite (particle size 8–12 mm; Deutsche Vermiculite GmbH) and 5.5 kg compost (air dried; Bioenergiezentrum Göttingen GmbH) and covered with 1.5 kg compost as a seed bed. The six legumes and *L. perenne* were sown in monoculture (1000 germinable seeds per m² for legumes and 5000 for the grass) and the legumes also in mixture with *L. perenne* (half the amount of seeds of each species sown in monoculture). This resulted in 13 treatments, which were replicated four times, leading to 52 containers that were arranged in a randomized block design in a vegetation hall.

The minimum and maximum air temperatures were recorded daily at three locations distributed over the vegetation hall (Table 2) and temperatures adjusted by venting in summer and heating on frost days in winter (temperature should not fall below 0°C for more than 24 h). Nevertheless, *L. uliginosus* was strongly reduced in all containers during winter and had to be resown at full seed strength in March 2010. There was no extra lighting in the vegetation hall and lighting conditions followed seasonal patterns. No fertilisation took place, but all plots were treated with rhizobium solution (Radicine, Jost GmbH) three times in 2009 and twice in 2010 (per application,

0.015 ml Radicine mixed with 250 ml tap water per m²). The Radicin solution mixture contained all rhizobia strains in same proportions for an effective infection of all legumes. Containers were kept moist during germination of the seeds. Starting two weeks after sowing, all containers were weighed regularly and irrigated when the water content was below 50% of field capacity.

Table 2. Temperatures [°C] in the vegetation hall from July 2009 until October 2010

Year	Month	Average minimum – maximum temperature
2009	July	14–31
	August	14–33
	September	13–32
	October	6–25
	November	6–14
	December	-1–8
2010	January	-1–5
	February	0–14
	March	5–25
	April	6–29
	May	9–26
	June	12–35
	July	16–36
	August	15–33
	September	11–26
	October	6–24

2.2.2 Sampling

The aboveground biomass was harvested two times in 2009 and five times in 2010. Harvesting took place 50 (establishment phase), 104, 272 (winter stress phase), 315, 356, 407, and 462 (yield phase) days after sowing. Shoots were cut 3–4 cm above the soil surface. Biomass of mixtures was sorted into species directly after harvesting. All samples were dried at 60°C for 72 h and weighed.

2.3 Statistical Analysis of Data

Statistical data analysis was carried out using the Genstat 6.1 software package. Analysis of variance (ANOVA) considered one factor. Residuals were used to check the validity of the models. Normality in data was achieved by applying logarithmic or square root transformations, if necessary. Where significant treatment effects ($\alpha < 0.05$) were found by ANOVA, least significant differences (Tukey Test) were used to compare mean values. Relationships between legume dry matter yield in monocultures and mixtures of the first harvest, in monocultures before and after winter and between the accumulated yield of the legume partner and the total mixture yield in the first main harvest year were examined with a linear regression model.

3. Results

3.1 Establishment Phase

The germination rate after 24 days on petri dishes ranged from 34% (*M. falcata*) and 100% (*M. lupulina*, Table 3). There were significant differences between *M. falcata*, the two *Lotus* species, which formed an intermediate group, and the other legumes, which had germination rates between 88 and 100% ($P < 0.001$). The germination rate of the grass *L. perenne* was 93% and similar to that of the latter group of legumes.

The dry matter yield of the legumes in pure stands during the establishment phase (50 days after sowing) ranged from 5.0 g pot⁻¹ to 16.0 g pot⁻¹ for *M. falcata* and *O. viciifolia*, respectively (Table 3). Yields of the other legumes were intermediate, with *L. corniculatus*, *M. lupulina* and *T. repens* producing similar yields to *O. viciifolia*, and *L. uliginosus* being closer to the low yielding *M. falcata*. For comparison *L. perenne* produced in pure stands 27.8 g pot⁻¹ in that first harvest. Mixtures of the single legumes and *L. perenne* did not differ in dry matter yield ($P=0.144$). Yield in mixtures was generally larger than in legume monocultures, but smaller than that of *L. perenne* in pure stand. The contribution of the legume partner to the total yield in mixture varied significantly among species ($P<0.001$), with yields increasing in the order *L. uliginosus*, *M. falcata*, *T. repens*, *M. lupulina*, *L. corniculatus*, and *O. viciifolia*.

Table 3. Germination rate after 24 days [%] on Petri dishes and dry matter yield of all species in pure and mixed stands (total yield and yield of the legume partner in the mixture) of the container experiment at the first harvest (Establishment phase).

Plant species	Germination after 24 days [%]	Dry matter yield [g pot ⁻¹]		
		Pure stand	Mixed stand	
			Total	Legume
<i>L. corniculatus</i>	73±4 ^b	14.3±4.5 ^{ab}	20.8±1.1	2.3±0.3 ^b
<i>L. uliginosus</i>	71±8 ^b	9.1±1.4 ^{bc}	19.6±1.9	0.1±< 0.1 ^f
<i>M. lupulina</i>	100±0 ^a	11.8±2.0 ^{ab}	25.8±3.8	1.6±0.2 ^c
<i>M. falcata</i>	34±10 ^c	5.0±1.2 ^c	22.5±1.4	0.3±0.1 ^c
<i>O. viciifolia</i>	89±7 ^a	16.0±2.1 ^a	22.1±4.9	4.0±0.5 ^a
<i>T. repens</i>	88±5 ^a	11.4±0.7 ^{ab}	21.5±2.2	1.0±0.3 ^d
<i>L. perenne</i>	93±3	27.8±2.4		
<i>P</i> -value	< 0.001	< 0.001	0.144	< 0.001

Shown are means and standard deviations. Different superscript letters indicate significant differences among species (ANOVA with Tukey Test ($\alpha<0.05$) analysis; the last row gives the corresponding *P*-values). *L. perenne* was not included in the statistics.

3.2 Winter Stress Phase

The winter phase lasted from beginning of November 2009 to early March 2010. Low temperatures (Table 2) associated with low radiation and short days limited plant growth similar to field conditions. Frost occurred but temperatures were prevented from falling below 0°C for more than 24 h.

To evaluate the effects of the winter phase the last harvest in 2009 (end of October) and the first harvest in 2010 (mid of April) were considered. For the harvest in October 2009, the legumes growing in pure stands showed two distinctive groups ($P<0.001$): a high-yielding group consisting of *L. uliginosus*, *T. repens* and *M. lupulina*, with yields between 27.1 and 34.4 g pot⁻¹, and a low-yielding group of *M. falcata*, *L. corniculatus*, and *O. viciifolia*, with yields between 7.8 and 15.2 g pot⁻¹ (Table 4). At the harvest in April 2009, the dry matter yields of most legumes in pure stand (apart from *L. uliginosus*) were similar to or larger than before winter. While *L. uliginosus* produced only 0.4 g pot⁻¹, a significantly smaller yield than all other legumes ($P<0.001$; Table 4), *M. falcata*, *O. viciifolia* and *L. corniculatus* showed intermediate yields of 21.6- 29.9 g pot⁻¹ and *M. lupulina* and *T. repens* were again the highest-yielding legumes (34.4 and 37.0 g pot⁻¹, respectively).

Total yields in mixed stands of legume and *L. perenne* before winter were largest in containers containing *M. lupulina*, the only legume besides *T. repens* that managed to produce appreciable amounts of biomass in this phase (Table 4). Containers with *M. falcata* or *O. viciifolia* as legume partner produced significantly less total biomass before winter ($P=0.005$), while yields of mixtures with *L. uliginosus* and *L. corniculatus* were intermediate. The yield contribution of the legume was smallest for *L. uliginosus* and also *M. falcata* and *L. corniculatus*, while only *M. lupulina* and *T. repens* produced considerable amounts of biomass. After winter, differences in biomass

production of the mixtures were not significant ($P=0.678$). At that time only *T. repens* produced an appreciable yield of 3.7 g pot^{-1} , significantly larger than that of any other legume in mixture.

Table 4. Dry matter yield of all species in pure and mixed stands with *L. perenne* (total yield and yield of the legume partner in the mixture) at the last harvest before winter in 2009 and the first harvest after winter in spring 2010 (Winter stress phase)

Plant species	Dry matter yield [g pot^{-1}]					
	Pure stand		Mixed stand			
	Before winter	After winter	Total		Legume	
Before winter			After winter	Before winter	After winter	
<i>L. corniculatus</i>	15.2±2.8 ^b	25.6±4.1 ^{ab}	30.2±2.6 ^{ab}	33.3±5.6	0.4±0.1 ^b	0.5±0.4 ^b
<i>L. uliginosus</i>	27.1±6.6 ^a	0.4±0.4 ^c	32.5±1.1 ^{ab}	34.1±2.4	<0.1±0.1 ^c	<0.1±<0.1 ^b
<i>M. lupulina</i>	34.4±2.7 ^a	34.4±5.6 ^a	35.3±2.0 ^a	32.8±3.2	3.1±1.3 ^a	0.5±0.1 ^b
<i>M. falcata</i>	7.8±5.3 ^b	21.6±4.2 ^b	28.2±2.1 ^b	35.0±3.8	0.1±0.1 ^c	0.2±0.1 ^b
<i>O. viciifolia</i>	12.9±0.5 ^b	29.9±3.1 ^{ab}	27.9±4.0 ^b	33.9±2.5	0.8±0.2 ^{ab}	0.1±<0.1 ^b
<i>T. repens</i>	32.4±5.4 ^a	37.0±10.9 ^a	33.2±1.3 ^{ab}	36.8±3.5	3.5±2.2 ^a	3.7±2.4 ^a
<i>L. perenne</i>	26.1 ± 3.7	35.9 ± 2.4				
P-value	< 0.001	< 0.001	0.005	0.678	< 0.001	< 0.001

Shown are means and standard deviations. Different superscript letters indicate significant differences among species (ANOVA with Tukey Test ($\alpha<0.05$) analysis; the last row gives the corresponding *P*-values). *L. perenne* was not included in the statistics.

3.3 Initial Yield Phase

The yield phase comprises of four harvests in the first main production year following the initial harvest after winter. The accumulated yield of the pure stands over the four main harvests 2010 ranged from 88.1 to 288.6 g pot^{-1} . It was smallest for *O. viciifolia* and *L. uliginosus*, significantly larger for *M. falcata* and *L. corniculatus* and largest for *T. repens* with *M. lupulina* being intermediate ($P<0.001$, Table 5). Accumulated yield of mixed stands was by far largest for mixtures with *T. repens*, followed by those with *M. lupulina* and *L. corniculatus*. In mixed stands *L. uliginosus* and *O. viciifolia* as the legume partner did not produce any biomass. Yield contribution of *T. repens* was largest with 0.59 (149.0 g pot^{-1}), while that of *M. lupulina*, *L. corniculatus* and *M. falcata* amounted to 0.26, 0.16 and 0.08, respectively. In pure stands the coefficient of variation (CV), as a measure of the yield variability among harvests, was largest for *M. falcata*, *L. corniculatus*, and *M. lupulina* and significantly smaller for *O. viciifolia* ($P<0.001$, Table 5). Generally, mixtures had a smaller CV than pure stands of legumes (Table 5). Greatest variability in yields between harvests (CV, $P<0.001$) was observed for the mixture with *T. repens* with a CV of 0.34 which was significantly larger than that of *L. uliginosus*, *M. lupulina*, *M. falcata* and *O. viciifolia* ranging from 0.18–0.21; *L. corniculatus* was intermediate with 0.24. Coefficients of variation considering the yields of the legume partner in mixtures were high and in a range from 0.71–0.95 but differences between legumes not significant ($P=0.395$).

4. Discussion

We compared five legumes as alternatives to *T. repens* in early development as pure stands and in mixture with *L. perenne*. To be agronomically competitive, it is important that legumes have a good establishment, show good winter tolerance, can perform in mixtures with partner species (here *L. perenne*) and thus provide good yields.

4.1 Establishment Phase

Generally, cultivated legumes had larger germination rates than wild seeds (*L. uliginosus* and *M. falcata*, Table 3). Especially *M. falcata*, from wild seeds, had very poor germination rates, with only 34% of seeds germinated after 24 days (Table 3). This was likely due to a higher percentage of hard seed. *Medicago* species may have up to 100% hard seed, depending on the habitat (Young, Evans, & Kay, 1970; Crawford, Lake, & Boyce, 1989). Hard seed coats help to survive unfavourable environmental conditions like long droughts (Kemp, 1989), but also influence both, water uptake and germination rate (Argel & Paton, 1999; Uzun & Aydin, 2004). Mechanical scarification

may have increased germination of *M. falcata*. However, in the present experiment, we accounted for low germination rates by adapting the sowing density. The German Regulation for Seeds (Saatgutverordnung, 2006), requires a good and homogeneous germination for cultivars, consequently resulting in a smaller share of seeds with hard seed coats. This may have led to the larger germination rates of the tested cultivars compared to the wild seeds. While germination is better in cultivars, the ability to survive periods of drought might be reduced and the timing of sowing and weather conditions during germination becomes more important. In the first harvest 50 days after sowing, cultivars also had larger dry matter yields than the wild type legumes both in monoculture and mixture (Table 3). Tauro, Nezomba, Mtambanengwe and Mapfumo (2009) observed similar results in their study. The size of seeds also has an effect on plant establishment; large-seeded species have often been found to have a better seedling establishment than small-seeded ones (Moles & Westoby, 2004), because of larger nutrient reserves in heavier seeds (Baker, 1972). This could only be confirmed in part in our experiment (Table 1 and Table 3): The species with the largest seeds, *O. viciifolia*, produced the largest biomass at the first harvest, both in pure stands and in mixtures. However, the large difference in seed weight between *O. viciifolia* and the next two legumes, *M. lupulina* and *L. corniculatus* (Table 1) was not reflected in large differences in yield (Table 3). Furthermore, *M. lupulina* and *T. repens* produced similar amounts of dry matter (Table 3) despite seed weights differing by a factor of 2.7 (Table 1). The absence of a strong relationship between seed weight and dry matter yield during the establishment might partly be explained by the use of wild seeds and cultivars for different legume species.

In mixtures, *L. perenne* was the main contributor to dry matter yields at the first harvest (Table 3). This was both due to the fast growth of *L. perenne* (Petersen, 1967) and the relatively high seed density of the grass compared to the legumes, deliberately chosen to test the competitive strength of the legumes. There was a positive linear correlation between the dry matter yield of legumes in monoculture and that in mixture ($R^2=0.75$; $P=0.026$). But, neither germination rate nor seed weight were good explanatory factors for the ability of species to compete in mixtures.

To sum up, *O. viciifolia* and *M. lupulina* did compete well with *T. repens* in germination rates. In terms of dry matter yield at the first harvest, *O. viciifolia* and *M. lupulina* again, but also *L. corniculatus*, were similar or even superior to *T. repens*. In mixtures with *L. perenne*, these three legumes produced significantly more biomass than *T. repens* at the first harvest. However, total yields in mixtures were only slightly larger and differences not significant (Table 3).

4.2 Winter Stress Phase

For legumes, the winter period is a crucial and sensitive phase, especially in newly established swards (Brandsæter, Smeby, Tronsmo, & Netland, 2000). All legumes in monoculture in this study, apart from *L. uliginosus*, survived the winter period well (Table 4). This is partly due to the conditions of a vegetation hall where moderate frost occurred, but long-term and sharp frost was prevented (Table 2). Results of only moderate winter tolerance for *L. uliginosus* have been reported before (Hedqvist, Murphy, & Nilsdotter-Linde, 2002). In line with that, the good winter tolerance found for *M. falcata*, *M. lupulina*, *T. repens*, *L. corniculatus* and *O. viciifolia* is in agreement with earlier findings (Frame et al., 1998; Brandsæter et al., 2000; Hedqvist et al., 2002). This was confirmed by a positive linear correlation between the dry matter yield of legumes in monocultures before and after winter ($R^2=0.83$; $P=0.033$, *L. uliginosus* not considered). Two legumes, *M. lupulina* and *T. repens*, had a fast establishment in the sowing year and a corresponding early development in the next spring, which was the basis for good yields in the first main production year. This is consistent with Petersen (1967).

As in the first harvest, *L. perenne* was the dominant plant in all mixtures in the harvests before and after winter (Table 4) - with the exception of *T. repens*, the yield of all legume partners in mixtures was well below one gram in the first harvest after winter (Table 4). This can be explained by a good and fast growth of *L. perenne* (Petersen, 1967) and temperatures that were closer to the optimum for grass than for the legumes (Wilson & Ford, 1971), (Table 2). For *T. repens* there is evidence that the presence of *L. perenne* is beneficial for the development of the legume in younger pastures (Turkington & Jolliffe, 1996).

In summary, only *M. lupulina* in monoculture produced a yield similar to *T. repens* at the harvests before and after winter and in mixture before winter. After winter, only *T. repens* produced a considerable yield in mixtures (Table 4).

4.3 Initial Yield Phase

A good establishment (Finch-Savage, 1995) of legumes associated with sufficient winter tolerance (Brouwer, Duke, & Osborn, 2000) is the basis for a good yield and sufficient yield contribution in a mixture in the first main production year. In our experiments, *T. repens* and *M. lupulina* but also *L. corniculatus* showed a good

establishment and winter tolerance (Tables 3 and 4). These three legumes also produced the largest accumulated yields in monocultures (Table 5). Despite a good establishment and winter tolerance, *O. viciifolia* in monoculture had a small accumulated yield in the main production year (Table 5). This may be due to the low cutting height (3–4 cm) and the high cutting frequency (five times in the main harvest year) in this trial. *Onobrychis viciifolia* is generally known to be susceptible to a low cutting height and in particular a frequent defoliation (Slepetys, 2008), although some authors also consider this species to be moderately tolerant to cutting (Briemle & Ellenberg, 1994; see Table 1). Most likely, in our experiment a reduced cutting frequency of two to three cuttings per year would have increased the yield of *O. viciifolia*. The poor establishment and/or an inadequate or poor winter tolerance (Table 3 and 4) of *M. falcata* and *L. uliginosus* probably caused the only moderate accumulated yields in the first main harvest year (Table 5).

Table 5. Accumulated dry matter yield over the four harvests 2010 (Initial yield phase) of all species in pure and mixed stands with *L. perenne* (total yield and yield of the legume partner in the mixture) and coefficients of variation over these harvests

Plant species	Accumulated dry matter yield [g pot ⁻¹]			Coefficient of Variation		
	Pure stand	Mixed stand		Pure stand	Mixed stand	
		Total	Legume		Total	Legume
<i>L. corniculatus</i>	213.0±15.3 ^b	137.4±16.4 ^{cb}	21.8±15.3 ^b	0.64 ^a	0.24 ^{ab}	0.95
<i>L. uliginosus</i>	125.4±29.0 ^c	118.9±9.2 ^c	n.p. [#]	0.56 ^{ab}	0.18 ^b	n.p.
<i>M. lupulina</i>	239.3±33.3 ^{ab}	165.5±7.6 ^b	42.4±5.7 ^b	0.62 ^a	0.21 ^b	0.76
<i>M. falcata</i>	192.4±18.2 ^b	121.0±9.0 ^c	9.9±2.2 ^b	0.69 ^a	0.20 ^b	0.85
<i>O. viciifolia</i>	88.1±21.0 ^c	119.7±5.3 ^c	n.p.	0.32 ^c	0.21 ^b	n.p.
<i>T. repens</i>	288.6±12.2 ^a	253.3±53.9 ^a	149.0±63.8 ^a	0.39 ^{bc}	0.34 ^a	0.71
<i>L. perenne</i>	115.4 ± 10.1			0.19		
<i>P</i> -value	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	0.395

Shown are means and standard deviations. Different superscript letters indicate significant differences among species (ANOVA with Tukey Test ($\alpha < 0.05$) analysis; the last row gives the corresponding *P*-values). *L. perenne* was not included in the statistics.
[#] n.p.: not present; in these mixtures. The legume partner did not produce any more biomass at these harvests.

In mixtures, there was a positive linear correlation between the accumulated yield of the legume partner and that of the total mixture (grass and legume; $R^2 = 0.99$; $P < 0.001$). *T. repens* was the most productive legume in mixtures with a yield proportion of nearly 60% of the total yield; this illustrates the strong competitive ability of *T. repens* (Petersen, 1967). A relative good competitive ability could be attributed to *M. lupulina* (Rehm & Espig, 1991) with a yield proportion in mixture with *L. perenne* of above 25%, while *L. corniculatus* had 16%. Where the yield of legumes in mixtures was small, as for *M. falcata*, a species with low competitive ability against fast-growing grasses (Petersen, 1967), the total mixture yield was also only slightly increased compared to the grass monoculture, and even smaller than that of the legume monoculture (Table 5). When the legume partner was no longer present, as was the case with *L. uliginosus* and *O. viciifolia*, mixtures produced a similar accumulated yield as *L. perenne* in monoculture (Table 5).

In general, legumes require higher temperatures for optimal growth than *L. perenne* (Wilson & Ford, 1971; Frame et al., 1998). Therefore, the growth of legumes in summer was faster compared to spring and autumn and yields differed between the harvests according to the individual temperature requirements of the respective legumes. This is well displayed in a higher coefficient of variation for the yields of all harvests for all six legumes in the main harvest year compared with the small CV for *L. perenne* (Table 5). In mixtures, the CV was depending on the legume partner and accordingly highest for the mixture with *T. repens*. In pure stands, *O. viciifolia* and *T. repens* showed the most stable yield over the first main production year.

4.4 Outlook and Need for Research

For the alternative legumes, an intensive breeding, like for *T. repens* in the last decades (Abberton & Marshall, 2005), might help to enhance not only the yield potential but also the competitive ability in mixtures with fast

growing grasses. A good and lasting contribution of the legume in grass-clover mixtures is essential for a successful introduction of new species.

This experiment provides some worthwhile information on early development of some legumes as possible alternatives to *T. repens*. Nevertheless, further work is necessary to test these legumes under field conditions with different cutting regimes, soil conditions and fertilizer applications. Of particular interest would be the reactions of these alternative legumes in view of possible future climate change conditions like drought or higher temperatures.

5. Conclusion

General, the yield in the first main productive year relies on a good germination and establishment along with a sufficient winter tolerance. Furthermore, the yield of a mixture strongly depends on the yield contribution of the legume partner. Thus, a good competitive ability of legumes against fast-growing grasses like *L. perenne* (Petersen, 1967) is essential.

In conclusion, *M. lupulina* and - to a somewhat lesser extent - *L. corniculatus* in monoculture showed potential to produce similar yields as *T. repens* in the first main production year, however, yield stability for *M. lupulina* and *L. corniculatus* was not sufficient. In mixtures, *M. lupulina* and less so *L. corniculatus* showed some potential, but only *T. repens* showed a strong competitive ability against *L. perenne*. This has to be considered with the choice of less competitive grass partner species when designing seed mixtures.

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