Weed control, protein and forage yield of seven grass species in lucerne-grass associations

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Highlights

- Association of grass species with lucerne is a way to limit weed occurrence
- Lucerne-grass associations give yield and quality close to that of pure lucerne
- Grasses differed for direct and associated effects on association's species
- Small direct and large positive associated grass effects favoured protein yield

ABSTRACT

1. CONTEXT: The reduction of chemical inputs in agriculture is a current challenge. Perennial forage legumes such as lucerne produce protein-rich forage without synthetic nitrogen fertilizers but the crops may be invaded by weeds. Association of legumes with grasses are recognized to lower weed pressure but may alter forage yield and quality.

2. OBJECTIVE: Pure lucerne was compared to lucerne-grass mixtures, with the test of grass species differing for morphological, phenological and quality traits. The grass species were compared in mixture with lucerne for their performance, which was evaluated through weed occurrence, forage yield and quality.

3. METHODS: Seven perennial grass species were evaluated in association with lucerne and compared to pure lucerne and pure grass (one species only), in field plot experiments in two locations, without nitrogen fertilisation. Forage yield, botanical composition (lucerne, grass, weeds), protein and acid detergent fibre were measured in four cuts per year during three years. Treatments

were compared, and mixture model effects such as direct and associated effects of the grass on dry matter yield of the species in the associations were calculated.

4. RESULTS: Lucerne-grass association reduced weed development compared to pure lucerne. The protein content was slightly lower in the associations than in pure lucerne but the association generally produced more protein per hectare than expected if the two species were grown in separated plots (i.e. protein overyielding). Depending on the grass species, the weed control, the forage quality, the proportion of lucerne and the dry matter yield of the association were differently affected. Species, with a small direct effect (on grass production) and a large associated effect (on lucerne production), such as timothy and meadow fescue, favoured lucerne proportion in the associations.

5. CONCLUSIONS: Association of grass species with lucerne is a way to limit weed occurrence while maintaining protein content and forage yield in the association. These traits, as well as the lucerne proportion in the association, varied depending on the grass species.

6. IMPLICATIONS: At a practical level, the application of herbicides on lucerne crops could be significantly lowered, and this with a limited impact on forage yield and quality. From a scientific point of view, the calculation of mixture model effects is of interest to analyse the outcome of species associations.

Keywords: alfalfa, digestibility, herbicide, fibre content, legume, *Medicago sativa*, mixture, nitrogen, quality

1. Introduction

One of the current challenges facing agriculture is to reduce the use of synthetic chemicals. Indeed, pesticides, especially herbicides, and synthetic nitrogen fertilisers must be reduced to improve the economic and environmental outcomes of crop productions. Forage legumes offer great opportunities to reduce the use of synthetic nitrogen fertilizers in grasslands (Martin et al., 2020) while providing a protein-rich forage. In rotations, forage legumes provide nitrogen to the following crops (Justes et al., 2001; Vertes et al., 2015; Grange et al., 2022) and relax the weed pressure by a change in weed botanical composition (Meiss et al., 2010). For lucerne (*Medicago sativa*), which is the legume with the highest protein production per unit area under temperate climates, the successive withdrawals of active substance registrations have reduced the chemical weed control solutions in Europe. In pure lucerne grown without weed control at establishment, weeds can represent a significant part of the biomass harvested during the first cuts (Spandl et al., 1999). With a generally upright growth habit, lucerne does not cover the inter-rows during the establishment phase or during the restart of vegetation at the end of winter (Annicchiarico and Pecetti, 2010). These two key periods are favourable to weed development in the canopy. Weed occurrence leads to losses in forage yield and quality and reduces the persistency of lucerne crop.

In the frame of Integrated Weed Management (Swanton et al., 2008), the control of competition between cropped plant and weeds is a lever towards weed reduction (Petit et al., 2018). In grassland ecosystems, species diversity has been associated to weed invasion resistance (Hector et al., 2001; Finn et al., 2013). Perennial legume - grass mixtures have been shown to resist better than monocultures to weed invasion (Spandl et al., 1999; Sanderson et al., 2012; Belanger et al., 2020; Quinby et al., 2021). Unlike lucerne, grasses, due to their spreading habit and drooping blades, have the ability to quickly cover bare soil between rows.

Another often-highlighted advantage of legume-grass forage associations is the additional forage yield (overyielding) that can be expected compared to monospecific stands (Finn et al., 2013). The concept of overyielding is based on the difference between the biomass of the mixture and the weighted average of biomass values of all species in monoculture (Trenbath, 1974). In a situation of grass-legume mixtures where low nitrogen fertilization is applied, the transgressive overyielding concept, defined as the difference between the biomass of the mixture and the single most productive species (usually the legume species), is particularly relevant. However, according to the synthesis of Chamblee and Collins (1988), the transgressive overyielding of lucerne-grass associations is not systematically observed. Depending on the situation, it can be negative or nil (Sheaffer et al., 1990; Cupina et al., 2017; Aponte et al., 2019; Quinby et al., 2021; Glowacki et al., 2023) and when it is positive, it is limited to a 5 to 10% higher forage yield than pure lucerne (Sleugh et al., 2000; Limbourg et al., 2010; Louarn et al., 2016; Hakl et al.; Belanger et al., 2020). The overyielding is mostly related to the first cut in the spring (Mooso and Wedin, 1990; Spandl and Hesterman, 1997; Sleugh et al., 2000).

Two other traits to be taken into account are the protein content and digestibility of the forage produced by these associations, which determine their feeding value for ruminants. For these two quality traits, the values reported in the literature were highly dependent on the grass species associated with lucerne, the proportion of lucerne in the harvested forage, the cut and the year of harvest (Sollenberger et al., 1984; Spandl and Hesterman, 1997; Sleugh et al., 2000). The protein content of pure lucerne was usually higher than that of lucerne-grass associations (Cupina et al., 2017; Maamouri et al., 2017; Belanger et al., 2020; Quinby et al., 2021). In addition, as forage grasses have a better nutritive value than weeds and although they have a lower protein content than that of lucerne, they can contribute to increasing the digestible energy of the association and to increasing the capacity for silage. Mixing grasses with lucerne is also recognised for its benefits on forage exploitation and use, compared to pure lucerne: improved wilting, higher soluble sugar content ensuring better silage fermentation, better balance between protein and energy content enabling better nitrogen utilization in the rumen (Tremblay et al., 2023).

Perennial grass species differ for several traits: stand establishment, plant height, tillering, growth dynamics along the year, heading date, forage quality. The traits related to growth are involved in the interaction with the companion species. Plants compete for resource capture, among which light and nitrogen are the major ones (Malezieux et al., 2009). Competition for light is mainly related to plant height, the tallest species intercepting most of incident sun radiation. For nitrogen, the mixture of a legume and a non-legume species generates facilitation, with the release of nitrogen-rich compounds by the legume to the soil, but also complementarity (or niche differentiation) with the legume that relies on symbiotic fixation of atmospheric nitrogen while the non-legume absorbs soil nitrogen (Corre-Hellou et al., 2006). Among the perennial grass species grown under temperate climates, perennial ryegrass (*Lolium perenne*) and festulolium (*Festuca* × *Lolium* hybrids) establish rapidly after sowing. Tall fescue (*Festuca arundinacea*) and cocksfoot (*Dactylis glomerata*) are tall and erect species that are able to compete for light with lucerne. Meadow fescue (*Festuca pratensis*), brome species (*Bromus* sp.) and timothy grass (*Phleum pratense*) are short and compete less with lucerne for light. In a binary association, the contribution of a component to the performance of the association can be modelled as the sum of a direct effect (effect due to the component itself), an associated effect (effect due to the other component) and an interaction between the direct and associated effect (Sampoux et al., 2020). The general mixing ability of a component is then defined as the sum of its direct effect on its own contribution and its associated effect on the contribution of the other species (Sampoux et al., 2020).

The objective of our study was to determine whether the association of different forage grass species with lucerne reduced the proportion of weeds in the harvested forage, and in particular during the first cuts when weeds are the most troublesome for the crop, while maintaining high forage yield and quality over the whole production period. To do so, seven contrasting forage grass species were grown with lucerne, and their performances were compared to those of pure lucerne in a three-year field experiment in two locations. The proportion of weeds and lucerne in the forage, the dry matter yield and the quality of the harvested forage were recorded. The seven grass species were also compared for their impact on association performance (direct and associated effects, general mixing ability).

2. Material and methods

2.1. Experimental design

Two field plot trials were set up in two contrasting sites: Lusignan in western central France (00°04'48'' East - 46°24'13'' North) and Somme-Vesle in north eastern France (04°35'26'' East - 48°59'08'' North). Lusignan is characterised by deep sandy-clay loam soils with a pH of 6.5. Somme-Vesle is composed of shallow sandy-silt soils. In the first year, nitrogen residues were evaluated at the end of winter in the two trials from six soil samples, that were collected on a diagonal of the experimental designs. The soil was taken at three depths, 0-20, 20-40 and 40-60 cm. Nitrogen residues were determined by measuring ammonia nitrogen and nitrate nitrogen, using continuous flow colorimetry (ISO 14256-2). In Lusignan, the amount of mineral nitrogen available between 0 and 60 cm was only 8.0 kg N/ha with a homogeneous distribution for the three horizons (3.0; 2.8 and 2.2 kg N/ha). In Somme-Vesle, the nitrogen residues amount to 33.9 kg of mineral nitrogen per ha with values of 14.0, 11.2 and 8.7 kg N/ha for the three horizons 0-20, 0-40 and 0-60 cm.

The Somme-Vesle trial was sown in July 2006 after winter barley and the Lusignan trial was sown in July 2007 after wheat and buckwheat intercrop and both trials were studied for three years. During the winter following sowing, phosphorus-potassium fertilisation was applied at a rate of 600 kg/ha of K_2O and 180 kg/ha of P_2O_5. No nitrogen fertilisation was applied, and no weed control was carried out on the plots. Meteorological data for each location and for the three years are displayed in Appendix – Fig. S1. Temperatures and rainfall were in agreement with inter-annual norms in the two locations.

Table 1 Composition of covers (associations or pure crops) and seeding proportion for each species.

Associations		Seed weight kg/ha			
		Proportion 50/50		Proportion 66/33	
Associated grass	Variety	Lucerne	Grass	Lucerne	Grass
Tall fescue (*Festuca arundinacea*)	Flexy	11.0	13.5	14.5	8.9
Cocksfoot (*Dactylis glomerata*)	Lupré	11.0	11.0	14.5	7.3
Meadow fescue (*Festuca pratensis*)	Préval	11.0	11.0	14.5	7.3
Alaska brome (*Bromus sitchensis*)	Hakari	11.0	20.0	14.5	13.2
Timothy (*Phleum pratense*)	Barfleo	11.0	4.5	14.5	3.0
Festulolium (*Festuca glaucescens* × *Lolium multiflorum*)	Lueur	11.0	13.5	14.5	8.9
Perennial ryegrass (*Lolium perenne*)	Brest	11.0	11.0	14.5	7.3
Pure crops		**Seed weight kg/ha**			
Tall fescue (*Festuca arundinacea*)	Flexy	-	27.0		
Lucerne (*Medicago sativa*)	Comète	22.0	-		

Seven perennial grass species were studied: tall fescue, cocksfoot, meadow fescue, Alaska brome (*B. sitchensis*), timothy, festulolium (*Festuca glaucescens* × *Lolium multiflorum*) and perennial ryegrass, each represented by one variety (Table 1). Seven binary associations of each of the seven grasses with the lucerne variety Comète, as well as two pure crops, lucerne and tall fescue, were sown (Table 1); the term 'cover' is used hereafter to refer to these associations (seven covers) or pure crops (two covers). In Lusignan, two seeding proportions were tested. The first one was calculated on the basis of 50% of the pure sowing rate for lucerne and grass and the second on the basis of 66% of the pure rate for lucerne and 33% for grass. In Somme-Vesle, only the 66/33 seeding proportion was sown.

Both trials were complete block designs with three repetitions in each location and for each seeding proportion in Lusignan. Plot size was 8 m², with 6 rows 7.5 m-long, spaced 17.8 cm apart in Lusignan, 7.2 m² with 8 rows, 6 m-long, spaced 15 cm apart in Somme-Vesle. The cutting frequency of the plots was approximately 49 days of regrowth in Lusignan (typical of a hay exploitation) and 42 days in Somme-Vesle (typical of an exploitation for dehydration, which is very frequent in this region), as further detailed in Appendix - Table S2).

2.2. Measurements

During the three years of the trials, all plots were harvested mechanically with a forage harvester and the fresh forage was weighed. The dry matter (DM) content was determined from a forage sample of about 500 grams of green biomass, which was dried at 60°C for 72 hours and then weighed. The dry matter yield of each cut was calculated. The dry forage samples were ground to pass through a 1 mm grid to determine the biochemical composition as estimates of quality (protein content) and energy content (ADF content). The protein (Dumas method (Hansen, 1989) with an elemental analyser Flash 2000, Thermofisher) and ADF contents (Van Soest and Fox, 1992) of the forage were assessed by near infrared spectrometry from an equation set up in the INRAE-URP3F laboratory for mixtures of grasses and forage legumes (Barotin et al., 2021) and validated internally on 10% of the samples.

Code de champ modifié

A second forage sample of 300 grams of green biomass was collected at each cut to assess the botanical composition of the covers. These assessments were based on manual separation of lucerne, grass and weeds. The fresh biomass of each species was weighed, as well as the dried biomass after drying at 60°C for 72 hours. The proportions of lucerne, grass and weeds were calculated as a percentage of total harvested biomass.

In the Somme-Vesle trial, the weed proportion in the third cut in 2007 could not be measured, and data on DM yield, protein content, ADF content and botanical composition are missing for the fourth cut in 2008.

In each location, the annual dry matter yield was calculated by summing the dry matter yields of all cuts of the year. To calculate the annual protein and ADF contents, the contents of each cut weighed by their respective yield were averaged.

2.3. Data analysis

To test the effect of lucerne seeding proportion on weeds and lucerne proportion, DM yield and quality, an analysis of variance (procedure lm of R) was performed on the Lusignan trial, with data from the lucerne-grass associations only. The model included block, grass species and lucerne seeding proportion effects as well as the grass species x lucerne seeding proportion interaction.

With this design, for each cut and location, it was possible to calculate the protein overyielding effects by using the protein content and dry matter yield of pure lucerne, pure tall fescue and the lucerne-tall fescue association. The overyielding was calculated by the difference between the protein yield in the lucerne-tall fescue association and the mean protein yield of pure lucerne and tall fescue, weighted by their proportions in the association, as if there were no interaction between species for N capture. The transgressive protein overyielding was calculated by the difference between the protein yield (calculated as dry matter yield x protein content) in the lucerne-tall fescue association and that of the most productive pure species (i.e. lucerne).

With the data from both locations (using, in Lusignan, the sub-trial in which the associations were sown with 66% lucerne/33% grass), an analysis of variance was carried out with the effects of cover, location, block within location, year within location, cover x location on forage yield, quality and weed and lucerne proportions. As this analysis showed that the cover x location interaction was significant, the following analyses were performed at each location. These analyses of variance by location were performed on the data collected at each cut and on the annual totals to determine the effect of grass species on all variables measured on the associations only. A Dunnett's comparison of means test was used to compare the results of each grass species with those of pure lucerne (procedure glht of R package multcomp).

To illustrate the advantages and disadvantages of the different grass species on the performance of the associations with lucerne, a radar plot with the main variables collected on the two trial sites was drawn (procedure radarchart of the R package fmsb).

A mixture model (Williams, 1962; Griffing, 1967; Gallais, 1970; Jacquard et al., 1978; Wright, 1985; Sampoux et al., 2020) was applied to the dry matter yield data of association plots, in each location. The grass and the legume dry matter yields were estimated from total dry matter yield and grass/legume proportion and the following ANOVA models were applied:

$$Y_g = \mu + d_g + \varepsilon$$

$$Y_l = \mu' + a_g + \varepsilon'$$

where Y_g and Y_l were the contributions of the grass species (*g*) and lucerne (*l*) to the DM yield of an association plot, respectively, μ and μ' were the average contributions of grass and lucerne across association plots, , respectively, d_g was the direct effect of a given grass species on its contribution to the association, a_g was the associated effect of a given grass species on the contribution of lucerne to the association, and ε and ε' were the residual effects. The General Mixing Ability of a given grass species (GMA_g) was calculated as $GMA_g = d_g + a_g$. and its aggressiveness (AGG_g) was calculated as $AGG_g = d_g - a_g$. In each location, these mixture model effects were calculated at each cut and on the average annual yield over three years. Correlations between direct and associated effects, GMA and aggressiveness were calculated.

3. Results

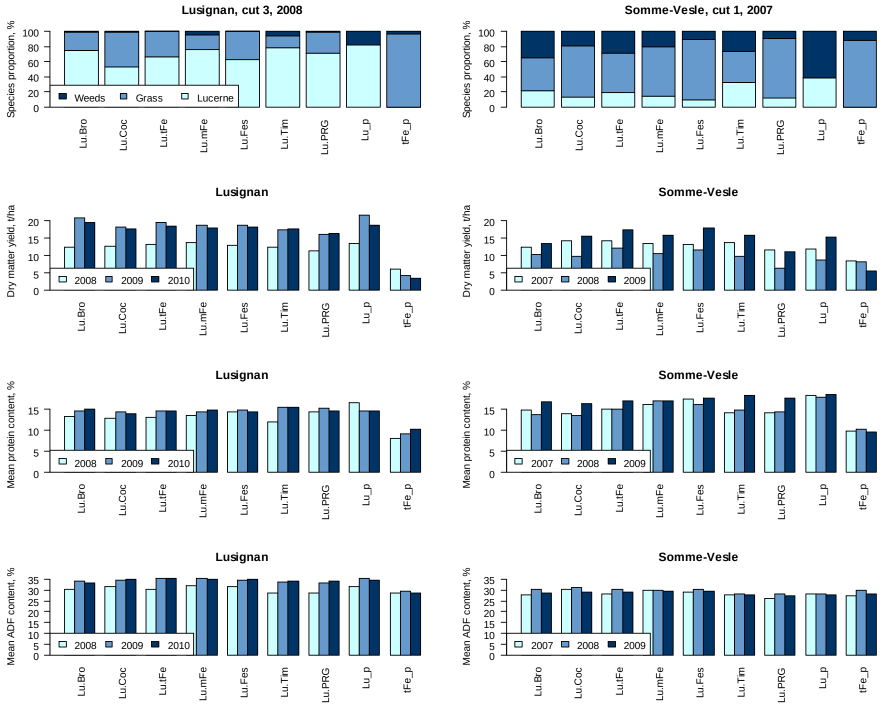
3.1. Effect of seeding proportion

In an analysis of variance on Lusignan trial data for each cut in each year, the interaction between the seeding proportion and the cover was significant ($P < 0.05$) for protein content in cut 3 of 2010, ADF content in cut 4 of 2010, weed proportion in cut 4 of 2008 only. In a second analysis of variance without the interaction of seed proportion x cover, the seed proportion was never significant for species proportions and significant for only eight traits of yield or ADF content. When the seed proportion effect was significant, the forage yield was higher in the 66% lucerne/33% grass than in the 50% lucerne/50% grass seeding proportion (Appendix - Table S1). As the difference between these two seeding proportions is marginal, other reported results only consider the 66% lucerne/33% grass seeding proportion in Lusignan, which is also the seeding proportion in Somme-Vesle.

3.2. Comparison of the annual results obtained in the two locations

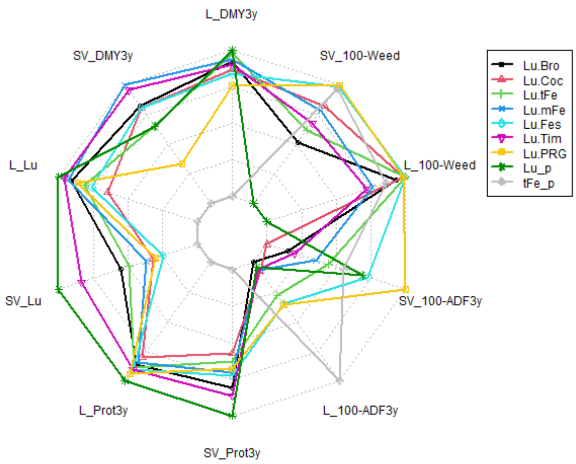
In both locations, the proportion of weeds, especially in the cuts where weeds were the most frequent (cut 3 of 2008 in Lusignan, cut 1 of 2007 in Somme-Vesle), was much higher in pure lucerne than in associations (Fig. 1). The annual yields of the associations were lower or equal to those of pure lucerne in Lusignan, whereas the associations of lucerne with meadow fescue and timothy had a higher yield than pure lucerne in Somme-Vesle (Fig. 1). The protein content of the associations was lower or equal to that of pure lucerne, but the ADF content was very similar for all the covers (except pure tall fescue) in Lusignan. In Somme-Vesle, the protein content of the associations was generally lower than that of pure lucerne and the ADF content was higher. Pure lucerne yielded more protein per hectare than lucerne-tall fescue association in the first six cuts in Lusignan, but the opposite was true for the next six cuts. In Somme-Vesle, apart from cut 4 in year 1 and cut 1 in year 2, protein yield was higher with pure lucerne than with the lucerne-tall fescue association (Fig. 1). Thus, compared to pure lucerne, associations more often exhibited underyielding than transgressive overyielding.

Fig. 1. Species proportion (% of dry matter) in the most weed-invaded cut, annual forage yield, protein and ADF contents in Lusignan and Somme-Vesle in each year for the seven lucerne-grass association, pure lucerne and pure, unfertilized tall fescue (significances in Tables S2 to S5). Species abbreviations: Lu: Lucerne, Bro: Alaska brome, Coc: Cocksfoot, tFe: tall fescue, mFe: meadow fescue, Fes: festulolium, Tim: timothy grass, PRG: perennial ryegrass. "_p" is for pure stands.



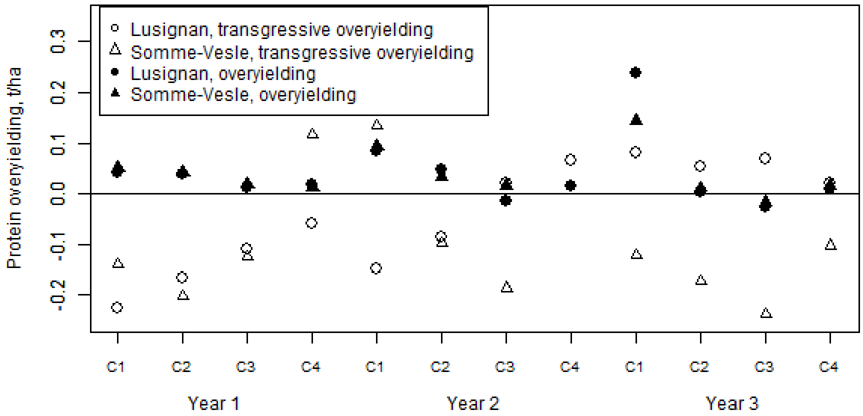
As an overview of weed proportion, lucerne proportion, DM yield, protein and ADF contents in both locations, a radarchart was plotted (Fig. 2). The association of lucerne with timothy, meadow fescue, and to a lesser extent Alaska brome provided a very good DM yield and a high proportion of lucerne without completely suppressing the weeds. Protein content was higher with timothy than with meadow fescue. Cocksfoot penalised the protein content but reduced the occurrence of weeds and produced a forage that was better balanced between lucerne and grass. Perennial ryegrass was very effective at weed control, but penalized DM yield excessively, even if the quality was maintained.

Fig. 2. Comparison of seven grass species for three-year DM yield (DMY3y), protein (Prot3y), 100-ADF (ADF3y) contents, 100-weed (Weed) and lucerne (Lu) proportions in the most weed-invaded cut, in Lusignan (L) and Somme-Vesle (SV). The axes span from the lowest to the highest values observed for each trait. The covers in external position on the radar have "positive" traits (high yield, high protein content, low ADF content, low weed proportion, high lucerne proportion). Species abbreviations: Lu: lucerne, Bro: Alaska brome, Coc: cocksfoot, tFe: tall fescue, mFe: meadow fescue, Fes: Festulolium, Tim: timothy grass, PRG: perennial ryegrass. "_p" is for pure stands.



Focusing on protein yield, the lucerne-tall fescue association produced less than the pure lucerne, with exceptions in some cuts in Lusignan and Somme-Vesle (Fig. 3), which indicates that transgressive overyielding is infrequent. However, in Lusignan, this transgressive overyielding was negative during the first six cuts and positive during the last six ones. When the protein yield of the association was compared to the mean protein yield of the two pure crops (overyielding), the observed values were null or slightly positive (Fig. 3).

Fig. 3. Overyielding calculated by the difference between value of the lucerne-tall fescue association and mean values of pure species (solid symbols) and transgressive overyielding calculated by the difference between values of lucerne-tall fescue association and pure lucerne (empty symbols) for the protein yield (t/ha) over time in Lusignan and Somme-Vesle. Values above 0 indicate protein overyielding.



A strong interaction between grass species and location was found for all measured variables (Table 2). The different soil and climatic conditions and the one-year difference in sowing date in the two locations may explain this interaction. Therefore, all experimental data are analysed in each location separately.

Table 2 Analysis of variance over the two locations for average weed and lucerne proportions, annual DM yield, average protein and ADF contents: F-test value and significance (***: 1‰, **: 1%, *: 5%, ns: non-significant)

	%Weeds		%Lucerne		DM yield		Protein content		ADF content	
Effect	F	Pr>F	F	Pr>F	F	Pr>F	F	Pr>F	F	Pr>F
Location	76.9	***	275.2	***	126.0	***	97.6	***	585.2	***
Block(Location)	1.8	ns	4.1	**	3.5	*	4.2	**	4.7	**
Year(Location)	22.9	***	115.7	***	61.1	***	19.1	***	57.8	***
Cover	16.7	***	23.3	***	52.9	***	65.9	***	20.8	***
Cover * Location	5.6	***	5.1	***	10.9	***	3.1	**	8.2	***

3.1. Proportion of weeds, lucerne and grass

3.1.1. Weeds

In Lusignan, the weed proportion was limited to 10 and 9% in pure lucerne, to be compared to 1 and 2% in associations, on average, for the first cut of the first two years (Appendix - Table S2). In Somme-Vesle, for pure lucerne, the weed proportion reached 61% and 44% in the first cut of the first and second year, respectively. In the associations, this weed proportion was much lower, representing on average only 21.4% and 10.3% in the first cut in the first and second year, respectively. Pure tall fescue generally had weed proportion close to that of lucerne-grass associations and the weed proportion was significantly lower than that of pure lucerne.

Weed proportions were the highest in the first six cuts (Appendix - Table S2), averaging 5.6% compared to 1.4% in the last six cuts. In the first six cuts, the presence of a grass in the associations significantly reduced the proportion of weeds (average 3.9%) compared to pure lucerne (average 18.9%). In the last six cuts, weed proportions measured in associations and pure lucerne were not different, with an average of 1.2% in lucerne and 1.7% in associations.

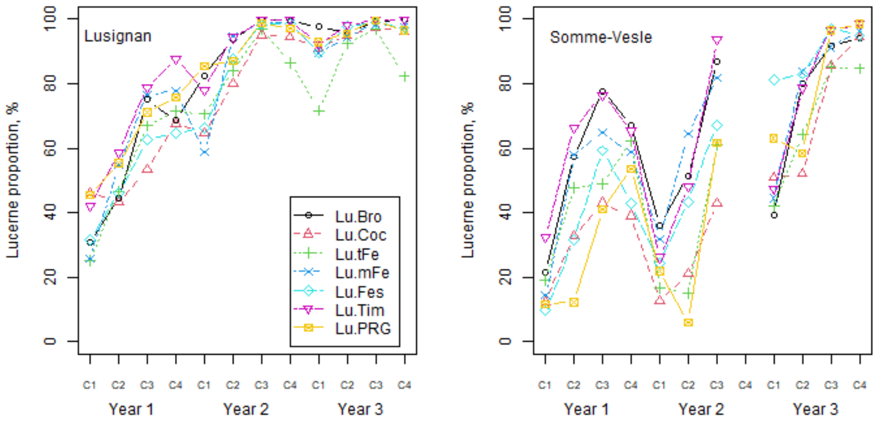
Weed proportions were the lowest in the lucerne-perennial ryegrass and lucerne-festulolium associations and the highest in the lucerne-Alaska brome and lucerne-timothy associations, especially in the first six cuts (Appendix - Table S2).

On average, weed occurrence was therefore lower in the associations than in pure lucerne, with differences depending on the grass species.

3.1.2. Evolution of the proportion of lucerne in the associations

The proportion of lucerne in the associations averaged 57%, 89% and 95% in Lusignan, compared to 44%, 44% and 78% in Somme-Vesle in the first, second and third year, respectively. In both trials, lucerne eventually became dominant. In all associations, the proportion of lucerne increased continuously from the first to the last year of the experiment, reaching 95-100% by the third cut in the second year in Lusignan and by the third cut in the last year in Somme-Vesle (Fig. 4). In each year, the proportion of lucerne in the associations increased from the first to the third (summer) cut, and then stabilised or decreased in the fourth cut in autumn. The Somme-Vesle trial, compared to the Lusignan trial, was characterised by a lower proportion of lucerne in the associations but also by a higher proportion of weeds. The proportion of lucerne in the associations also depended on the grass with which it was associated. At both sites, the highest proportion of lucerne was in association with timothy, with an average of 76.5% lucerne over the three years (85.8% in Lusignan and 65.4% in Somme-Vesle). Conversely, over the three years, the most competitive grass against lucerne was cocksfoot with an average of 61.3% lucerne in the cover (77% in Lusignan and 43% in Somme-Vesle). In Somme-Vesle, the lucerne-perennial ryegrass association had a very low proportion of lucerne in the first three cuts of the first year. Tall fescue tends to be the most persistent grass in association with the lowest proportion of lucerne in the last year of the experiments. In this association, the lucerne proportion represented on average 86% in Lusignan and 69% in Somme-Vesle.

Fig. 4. Evolution of lucerne proportion in the associations of lucerne with different grass species in 12 cuts over three years, in Lusignan and Somme-Vesle. Species abbreviations: Lu: Lucerne, Bro: Alaska brome, Coc: Cocksfoot, tFe: tall fescue, mFe: meadow fescue, Fes: festulolium, Tim: timothy grass, PRG: perennial ryegrass.



3.2. Annual DM yield and distribution of yield over the years

Annual DM yield tended to be higher in Lusignan than in Somme-Vesle (Appendix - Table S3). It ranged from 12.0 to 21.6 t DM/ha for pure lucerne and from 11.2 to 20.9 t DM/ha for the associations, depending on the year and location (Appendix - Table S3). Pure fescue without nitrogen fertilisation produced between 3.5 and 8.4 t DM/ha annually.

In Lusignan, the annual yield of pure lucerne was higher or equal to that of the associations. In the first and third years, there was no difference in DM yield between the two types of covers (associations and pure lucerne) except for the lucerne-perennial ryegrass association, which produced significantly less forage in the third year (16.5 vs. 18.7 t DM/ha). In the second year of experiment, pure lucerne produced more forage than associations containing cocksfoot, Alaska brome, timothy, festulolium and perennial ryegrass. Only the associations containing tall fescue and meadow fescue had a comparable DM yield to pure lucerne.

In Somme-Vesle, the average yield of the associations tended to be higher than that of pure lucerne (13.3 vs. 12.7 t DM/ha) in the first year but this trend was reversed in the second and third years. Associations containing meadow fescue and timothy produced significantly more forage than pure lucerne (+1.3 to 3.3 t DM/ha in each year). The lucerne-cocksfoot and lucerne-festulolium associations had a higher DM yield than pure lucerne in the first year only, and this by 2.2 and 1.7 t DM/ha, respectively. The lucerne-brome association had a higher yield than pure lucerne in the first and second years, by 1.4 and 1.8 t DM/ha, respectively, but this was not the case in the third year. The association containing tall fescue produced more forage than pure lucerne in the second year but significantly less in the third year.

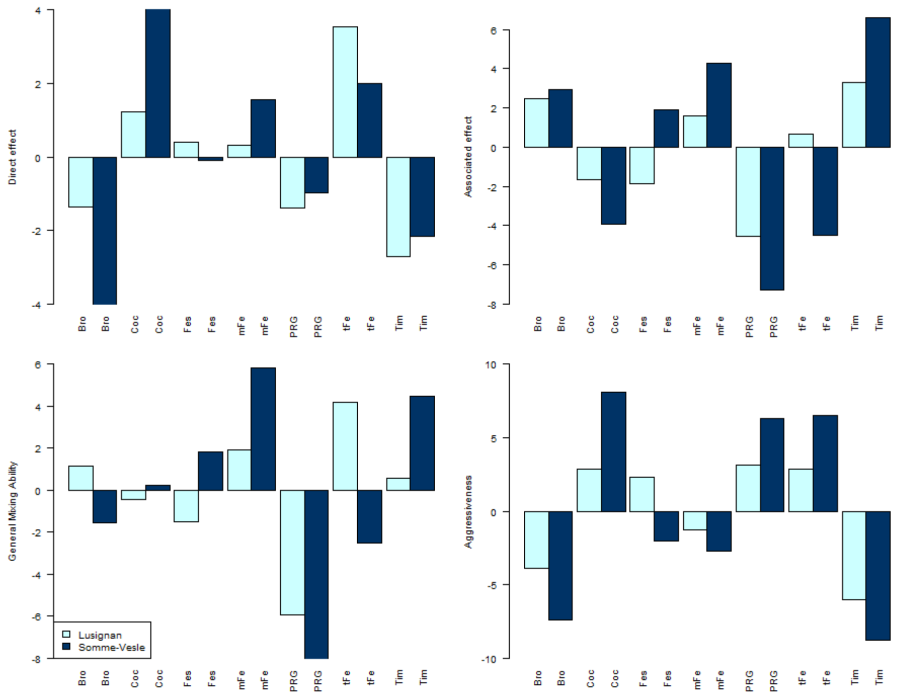
In Lusignan, the distribution of DM yield among the different cuts of the year was not modified by the presence of a grass in the associations (Appendix - Table S3). Contrastingly, in Somme-Vesle, the DM yield of the lucerne-timothy and lucerne-meadow fescue associations during some spring and autumn cuts (C4 in 2007, C1 in 2008, C1 in 2009) was higher than that of pure lucerne. This benefit did not come at the expense of the yield of the other cuts, so the total yield of these associations was higher than that of pure lucerne.

3.3. Quality of biomass

The protein content was higher and the ADF content was generally lower in Somme-Vesle than in Lusignan (Appendix - Tables S3 and S4). These differences can be related to a lower average DM yield in Somme-Vesle than in Lusignan, as described above. The protein content ranged from 12.2 to 23.0% for pure lucerne and from 9.6 to 23.7% for associations, depending on the cut and location (Appendix - Table S4). Associations never produced a forage with a significantly higher protein content than pure lucerne. The differences in protein content between associations were significant in only three out of 12 cuts in Lusignan, two of which were in the first year. This may be related to the very high proportion of lucerne in these associations from the end of the first year until the last cut of the third year. In Somme-Vesle, the differences between associations were significant in eight out of 11 cuts. In each cut, the protein content of the associations was strongly related to the proportion of lucerne (Appendix – Fig. S2), especially when the proportion of lucerne was less than 80%. The lucerne-timothy associations in both locations, the lucerne-perennial ryegrass association in Lusignan and the lucerne-Alaska brome association in Somme-Vesle had protein contents close to those of pure lucerne (Appendix - Table S4).

The ADF content ranged from 22.4 to 41.2% for pure lucerne and from 21.5 to 42.1% for the lucerne-grass associations, depending on the cut and the location (Appendix – Table S5). The ADF content of the associations was not significantly lower than that of pure lucerne except for the perennial ryegrass-lucerne association. The presence of perennial ryegrass in this association significantly reduced the ADF content in four out of 23 cuts (1 in Lusignan and 3 in Somme-Vesle).

Fig. 5. Mixture model effects for seven grass species grown in association with lucerne, evaluated on the means of the 3 years of experiments in t/ha: direct effect, associated effect, general mixing ability and aggressiveness, in Lusignan and Somme-Vesle. Species abbreviations: Bro: Alaska brome, Coc: Cocksfoot, Fes: festulolium, mFe: meadow fescue, PRG: perennial ryegrass, tFe: tall fescue, Tim: timothy grass.



3.4. Comparison of grass species for direct and associated effects

On average over the three years, the cover factor was significant for the direct effect of the grass species in both locations. The indirect effect, the general mixing ability and the aggressiveness were significant in Somme-Vesle only. In Lusignan, even if the F test for the cover factor was not significant, the LSD test showed a difference among covers for the indirect effect and the aggressiveness. The direct effect of grass species on annual grass yield in the associations was positive for cocksfoot, tall fescue and meadow fescue and negative for Alaska brome, timothy and perennial ryegrass in both locations (Fig. 5). The associated effect of grass species on annual lucerne yield in the associations was negative for cocksfoot and perennial ryegrass, and positive for Alaska brome, meadow fescue and timothy in both locations. The associated effect of tall fescue was null in Lusignan and negative in Somme-Vesle, while Festulolium had a negative associated effect in Lusignan and a positive effect in Somme-Vesle. The general mixing ability, as the sum of direct and associated effects, was strongly negative for perennial ryegrass and positive for meadow fescue and timothy in both locations. It was positive for tall fescue in Lusignan but not in Somme-Vesle. The aggressiveness, as the difference between direct and associated effects, was negative for timothy, Alaska brome, meadow fescue and positive for cocksfoot, tall fescue and perennial ryegrass in both locations. Aggressiveness of Festulolium was positive in Lusignan and slightly negative in Somme-Vesle (Fig. 5). Direct and associated effects were not significantly correlated in either location (Table 3, Appendix – Fig. S3). General mixing ability was positively correlated to the associated effect only. Aggressiveness was negatively correlated to the associated effect. These associated effects, calculated for each cut in each location, indicated that the status of grass species in the association with lucerne was established as early as the first two or three cuts in the first year (Appendix - Fig. S4). For example, timothy had negative direct effects and positive associated effects along the three years while cocksfoot had mostly positive direct effects and negative associated effects.

Table 3 Correlation among mixture model effects, estimated on mean annual yield over three years in Lusignan (above diagonal) and Somme-Vesle (below diagonal). ns: P>0.05, *: P < 0.05, **: P<0.01

	Direct effect	Associated effect	General mixing ability	Aggressiveness
Direct effect		-0.171 ns	0.497 ns	0.177 ns
Associated effect	-0.436 ns		0.770 *	-0.841 *
General mixing ability	0.127 ns	0.837 *		-0.302 ns
Aggressiveness	0.739 ns	-0.928 **	-0.574 ns	

4. Discussion

The proportion of weeds was much lower in associations than in pure lucerne. During the first six cuts, the presence of the grass reduced the proportion of weeds by 65 to 97% compared to pure lucerne. Perennial ryegrass and festulolium seemed to be the most competitive grasses against weeds, but they were also the most competitive grasses against lucerne. As suggested by Peters and Linscott (1988), grasses associated with lucerne substitute for weeds and compete with lucerne. Jung et al. (1991) who tested several seeding proportions of perennial ryegrass in association with lucerne, clearly showed that the presence of weeds was negatively correlated with the seeding proportion of the grass. The competition for light between grasses and legumes seemed to be a determining factor. Kruidhof et al. (2008) showed that the competitiveness of a species with respect to weeds was correlated to its light interception during the establishment phase. When the sown species intercepted the light efficiently, fewer weeds were found in the canopy. In the same study, lucerne, compared to radish, rapeseed and

Italian ryegrass, appeared to be the least competitive species with respect to weeds due to its low capacity to intercept light during the establishment phase (Kruidhof et al., 2008). During this phase, lucerne, with its erect growth habit, provided little shade on the inter-rows. Conversely, grasses with a more spread-out growth and drooping blades quickly covered the inter-rows, which significantly reduced the presence of weeds in the cover. As a consequence, the association of lucerne with grass, instead of the application of herbicide on pure lucerne, was found to be a solution to limit weeds (Spandl et al., 1999; Sanderson et al., 2012; Belanger et al., 2020; Quinby et al., 2021).

Code de champ modifié

The protein content of the associations was often lower than that of pure lucerne, as indicated in the literature (Spandl and Hesterman, 1997; Sleugh et al., 2000; Cupina et al., 2017; Belanger et al., 2020; Quinby et al., 2021), especially for the cuts where the grass proportion was the highest. Moreover, the protein content of the harvested forage, in associations, was related to the percentage of lucerne, and this proportion largely depended on the associated grass species. However, as expected, the lucerne-tall fescue association produced more protein than expected, based on the amount of protein produced by the two pure, non N-fertilized species that exhibited protein overyielding. This is consistent with the efficiency of nitrogen uptake from the soil by grasses, which locally depletes the soil of nitrogen and boosts the symbiotic fixation activity of the associated legume (Davies, 2001). Regarding digestibility used as an estimate of energy content, which was inversely correlated to the fibre content (ADF), we observed little differences between the covers. Only the association of perennial ryegrass and lucerne improved the digestibility (or reduced the ADF content) of the harvested forage in some cuts compared to pure lucerne, but DM yield was reduced. As a total, lucerne-grass mixtures produced forages with high protein content and digestibility. This comes in addition to other benefits on forage conservation and nitrogen utilization in the diet, conferred by soluble sugar contents of some grass species (Tremblay et al., 2023).

Mixture model effects, that were conceived a long time ago (Gallais, 1970; Jacquard et al., 1978) and were recently revisited to propose breeding schemes to improve varieties for mixtures (Sampoux et al., 2020), have rarely been evaluated on experimental datasets. In this experiment, they prove their relevance to document the behaviour of grass species in association with lucerne. This characterisation of the species with negative or positive effects is relative to the species included in the study; the species could also be described as producing below average or above average effects. Some grass species such as timothy had a low yield contribution (negative direct effect) but were favourable to lucerne yield (positive associated effect). Conversely, cocksfoot had a relatively high yield contribution (positive direct effect) but was detrimental to lucerne yield (negative associated effect). Perennial ryegrass combined negative direct and associated effects; as a result, it displayed a strongly negative general mixing ability and a positive aggressiveness. Timothy and Alaska brome were not found aggressive while cocksfoot and tall fescue were found to be aggressive. The absence of significant correlation between direct and associated effects offers the possibility of choosing the grass to be mixed to lucerne depending on the objectives assigned to the association. If a dominance of lucerne is required, a grass species that generates a low direct effect and a positive indirect effect will be chosen. If a more balanced lucerne/grass proportion is required, then a grass with a high direct effect and a moderate indirect effect will be preferred. The associated effect positively contributed to the general mixing ability and negatively contributed to aggressiveness. Conversely, the direct effect has no significant effect on these traits. Such a situation could be related to the dominance of lucerne in the trials.

In association with lucerne, some grasses, mainly meadow fescue and timothy, improved the annual DM yield in Somme-Vesle, thereby exhibiting transgressive overyielding. Theoretically, in an association with lucerne, the grass contributes to lengthening the production period, and/or increasing the yield in the first and last cuts. Indeed, the growth of forage grasses is less affected than that of lucerne by low temperatures (Knievel and Smith, 1973; Smith and Struckmeyer, 1974), which gives them an earlier start to vegetation in spring and a later growth in autumn. This was the case in Somme-Vesle where meadow fescue and timothy associations tended to have an improved yield in spring and autumn. However, in Lusignan, where the proportion of lucerne was higher in the associations, no transgressive overyielding was observed, and neither annual yield nor yield at the beginning and end of the year were improved. In both sites, perennial ryegrass limited yield in the second and third years. A possible explanation could be the very early competition of perennial ryegrass on lucerne during establishment, visible on the first two cuts of the first year in Somme-Vesle, where the proportion of lucerne hardly exceeded 10%. In addition to the benefit provided by grasses to extend the production period in spring and autumn, lucerne has the capacity to produce significant biomass in summer under drought conditions when grasses stop their growth. As a total, biomass potential production is increased with grass-lucerne mixtures, even if this expression of this potential depends on climate conditions.

In both trials, lucerne became dominant over grass species. This feature could be related to the seeding proportion (66/33) that favoured lucerne over grasses. However, in the Lusignan trial, the two seeding proportions (50/50 and 66/33) gave a very similar lucerne proportion in the mixtures. Such insensitivity of the contribution of the individual species to an increase in density has already been described (Cavalieri et al., 2022). The validity of our results is thus not restricted to the choice of seeding proportion, provided very unbalanced seeding proportions among species are not used. This may be explained by the high plant mortality during the first months after planting (Rotili et al., 1994). Lower proportions of lucerne in the lucerne-grass associations in Somme-Vesle than in those in Lusignan could be related to higher amounts of mineral nitrogen in Somme-Vesle (33.9 kg N/ha) than in Lusignan soils (8.0 kg N/ha). These nitrogen residues also allowed for substantial DM yield of pure grass (here tall fescue) in the first cut of the first year in both locations, without nitrogen fertilisation. It has been reported that longer cutting intervals tend to favour lucerne in associations with grasses (Comstock and Law, 1948; Sprague et al.; Jung et al., 1996; Belanger et al., 2020). In this experiment, the interval between two cuts was longer (49 days versus 42 days) and the first cut was later in Lusignan than in Somme-Vesle. The higher soil nitrogen residues associated with longer cutting intervals in Somme-Vesle than in Lusignan can contribute to explain the difference in the proportion of lucerne between the two sites, even if other pedoclimatic descriptors, such as temperature or soil, could also be involved.

The species of forage grasses associated with lucerne also has an impact on the proportion of lucerne in the canopy. Associations with cocksfoot and tall fescue were less rich in lucerne than associations with timothy, meadow fescue and Alaska brome. After three years of association trials, Casler and Walgenbach (1990) also noted that cocksfoot and tall fescue had better persistence (soil cover rate) than ryegrass, timothy and brome. These observations were confirmed in our two trials. Even if in the third year, lucerne was very dominant in the associations, the two grasses that maintained themselves best in the associations were tall fescue and cocksfoot. The strong dominance of lucerne on forage grass when the association is subjected to long cutting intervals (> 30 days) has been commonly reported in the literature (Frame and Harkess, 1987; Jung et al., 1991; Sleugh et al., 2000). In these studies, the proportion of lucerne quickly reached 80 to 90% of the harvested biomass. When the cutting frequency of the associations became lower than 30 days, this trend was reversed and grasses became dominant (JUNG et *al.*, 1996). The low cutting frequency in our trials was also favourable to the replenishment of lucerne root reserves between cuts (Ourry et al., 1994), which was favourable to the persistence of the plants. In both locations, timothy was effective for weed control from the first year of production, and the lucerne-timothy association was highly productive, with a high lucerne proportion and protein content.

Conclusion

Lucerne-grass associations are of unquestionable interest for reducing the use of phytosanitary products by limiting the proportion of weeds in the covers. On one of the two sites, the associations that was composed of lucerne and meadow fescue or timothy allowed a gain in forage yield during the three years of experiments. The choice of the grass species to be associated with lucerne should be considered according to the objective assigned to the associations. Tall fescue and, to a lesser extent, cocksfoot are the two species that persisted longer in association with lucerne, producing a forage with a good protein/energy ratio. Perennial ryegrass, an aggressive species with good feeding value, strongly limited weed development in association with lucerne and offered good quality forage, but also highly penalised forage yield. In order to maximise protein yield, an interesting compromise was obtained with meadow fescue and timothy in association with lucerne. These two species provided effective weed control while producing as much as or more than pure lucerne, without penalizing the protein content of the harvested forage. These results benefit from the use of mixture model effects that can therefore be recommended for the analysis of the agronomic performance of any type of species mixtures.

CRediT authorship contribution statement

Fabien Surault: Conceptualization, Methodology, Investigation, Data curation, Visualization, Writing-Original draft preparation, **Christian Huyghe**: Conceptualization, Methodology, Funding acquisition. **Jean-Paul Sampoux**: Investigation, Writing - review & editing. **Damien Larbre**: Methodology, Investigation. **Philippe Barre**: Writing - review & editing, Funding acquisition, **Gaëtan Louarn:** Writing-Reviewing and Editing. **Bernadette Julier:** Conceptualization, Methodology, Data curation, Formal analysis, Visualization, Writing- Original draft preparation, Writing - review & editing.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability

Data will be made available on a data repository when the paper is accepted. https://doi.org/10.57745/K7VLL2

Appendix

Table S1, Table S2, Table S3, Table S4, Table S5, Fig. S1, Fig. S2, Fig. S3, Fig. S4

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