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Potential in mixed swards and breeding of tall fescue (*Festuca arundinacea* Schreb.)

Mathias Cougnon

Proefschrift voorgedragen tot het bekomen van de graad van Doctor in de Bio-ingenieurswetenschappen: Landbouwkunde

Thesis submitted in fulfillment of the requirements for the degree of Doctor (PhD) in Applied Biological Sciences: Agronomy

“Wie had het in den witten winter kunnen denken dat er in die kale harde aarde en die bloote zwarte bomen zulke macht van hartverscheurend leven zat bijeengekoekt?”

(Felix Timmermans, Pallieter)

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MATHIAS COUGNON

POTENTIAL IN MIXED SWARDS AND BREEDING OF TALL FESCUE (*Festuca arundinacea* Schreb.)

Thesis submitted in fulfillment of the requirements for the degree of Doctor (PhD) in Applied Biological Sciences: Agronomy

Dutch translation of the title:

Rietzwenkgras (*Festuca arundinacea* Schreb.): gebruik in grassenmengsels en veredeling

Illustrations on the cover:

Front: Seed multiplication field of *Festuca arudinacea* ‘Femelle’ in Melle (April, 2012).

Back: Seed multiplication field of *Festuca arudinacea* ‘Femelle’ in Melle (February, 2012).

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Mathias (Melle 19/09/2013)

Summary

The incentives of this thesis are some of the challenges that grassland production in Belgium are exposed to. First, more dry summer spells are predicted due to climate change (IPPC, 2007). Second, more grass is cut and conserved due to current trends in dairy production. Tall fescue (*Festuca arundinacea* Schreb.; **Fa**) is a forage grass species that is expected to cope with these challenges.

This thesis had three objectives:

1. to study the agronomy of Fa under Belgian conditions;
2. to develop methods that could be used in breeding to overcome the main disadvatages of Fa;
3. to breed an Fa Variety adapted to Belgian circumstances.

1. We explored the available literature and (unpublished) trial results in order to gain insight in the main agronomic characteristics of Fa. We compared the yield and the feeding quality and of Fa with that of perennial ryegrass (*Lolium perenne* L.; **Lp**), which is actually the most important forage grass species in Western Europe. We found that Fa was on average 20% higher yielding than Lp under favourable growing conditions. Under drought growing conditions, we found even larger yield benefits for Fa: in years with severe drought periods, the annual yield of Fa was up to 60 % higher compared to Lp. We found that the main disadvantages of Fa were its lower digestibility and animal voluntary intake compared to Lp. Digestibility of Fa is on average over the whole year 5-10 % points lower than that of Lp. The slow establishment of Fa, results in a low yield in the first production season.

   We hypothesized that the high yield and good drought resistance of Fa and the good feeding quality of ryegrasses could be combined by sowing both species in one sward, and that these mixed swards would eventually lead to transgressive overyielding (yield of the mixed sward higher than yields of swards composed of the highest yielding component). To test this hypothesis, we conducted 3 field trials.

   In a first field trial, we compared monospecific swards of Fa and Lp and swards sown with different mixtures of both species with or without white clover. Mixtures differed in the ploidy and the initial proportion of the ryegrass component in the seed mixture. The yield, the feeding quality and the botanical composition of the mixtures was tested over 15 cuts

in three successive years (2010 – 2012). The yields of the monospecific swards, were in line with the results found in literature: averaged over the three years Fa yielded 23 % more than Lp but the digestibility of the organic matter of Fa was at least 5 % points lower compared to Lp. The mixtures of both species did not lead to the expected transgressive overyielding: the yield of the mixtures was intermediate to that of the monospecific swards. Although the swards sown with mixtures of Fa and Lp were initially dominated by Lp, this did not prevent Fa to become dominant in the third year of the trial.

The second trial was analogous to the first trail, comparing monospecific swards of Fa, Italian ryegrass (*Lolium multiflorum* Lam.; **Lm**) and mixtures of both species with or without red clover. Over a three years period (2010 – 2012), Lm overyielded Fa due to the low yield of Fa in the first production year. The highest yields were obtained with mixtures of Fa and Lm, which combined the good yield of Lm in the first cuts of every year and the good yield of Fa later in the season, but the yield of the mixtures was not significantly higher than that of the highest yielding monospecific sward. The digestibility of Fa was 3 to 6 % points lower compared to Lm. The mixtures were dominated by Lm in the first two years, but in the third year, the swards evolved to pure Fa swards. This trial learned us that sowing mixtures of Fa and Lm is a good strategy to overcome the low yield of Fa in the first production year and that Lm can be conceived as a cover crop for Fa.

In a third trial, we compared monospecific swards of Fa and Lp and different mixtures of both species with white clover, **under grazing management**. Apart from yield and botanical composition, we also measured whether there was a difference in weed invasion between the monospecific swards and mixtures. In contrast to a cutting management, we found no higher yield of Fa compared to Lp due to high environmental variation in this trial. Neither did we find transgressive overyielding in the mixtures. The composition of swards sown with seed mixtures containing 1/4 of Lp was approximately evenly distributed between Fa and Lp in the third year of the trial. We found no differences in weed content between the different swards.

The determination of the botanical composition of the species mixtures described above, involved a lot of labour intensive hand sorting of species: clover *versus* grass; Fa *versus* Lp and Lm. We tested different NIRS (near infrared reflectance spectroscopy) calibration strategies to predict the botanical composition of forage samples. We found that a calibration strategy based on hand sorted samples with a lot of environmental variation resulted in the most robust equations. We proposed an alternative calibration strategy based on hand sorted samples with added environmental variation. Applying these findings to the samples of our trials resulted in an equation that predicted the clover content of the forage samples well (root mean square error of prediction (RMSEP) = 4 %), but the prediction of the grass species was unsatisfactory (RMSEP of 8 % and 10 % for Fa and Lp respectively).

The superior drought resistance of Fa compared to Lp is generally explained by the deeper rooting of Fa, but quantitative data on rooting depth are relatively scarce. We determined root biomass of Fa and Lp in different trials on different locations. We found that root biomass of Fa was between 1.5 and 4.2 times higher than that of Lp below 30 cm soil depth. The largest difference between Fa and Lp was found on locations with loam soils, the lowest difference was found on a location with a sandy soil. For both species the root biomass below 30 cm was higher in late summer or early autumn compared to spring, resulting in a more pronounced difference between the species in the former period.

2. The main disadvantages of Fa are its low animal voluntary intake compared to Lp, and its slow early vigour after sowing. Provided good methods are available to select plants with better animal preference or early vigour, breeding is expected to be able to improve these traits.

Firstly, we tested if ruminant preference could be predicted without using ruminants. We tested the correlation between sheep preference and leaf morphological parameters, swards characteristics and rabbit preference for 16 tall fescue clones. We found that sheep preference was negatively correlated with pre-grazing sward height and leaf harshness and positively correlated with rabbit preference. Although we gained more insight in the factors influencing preference, our goal was not achieved: we could not predict the sheep preference in a reliable way using leaf or sward characteristics.

Secondly, we tested whether digital image analysis can be used to quantify ground cover and early vigour of Fa in a repeatable way in the field. Digital image analysis determined the ground cover accurately under field conditions and allowed to distinguish significant differences in ground cover, but we found some shortcoming to use the method efficiently in a selection process for better early vigour of Fa. A high correlation between ground cover and biomass yield was only found at the beginning of the sward development. Moreover, the method was cumbersome to use in the field: not all weather conditions were suited for picture taking, and weeds biased the results. Hence, the method actually has only limited value to select Fa for improved early vigour.

3. In 2008 we started a Fa breeding programme. The breeding goal was to select a soft-leaved variety with a good yield and a good rust resistance that grows well under Belgian conditions. We collected over 600 ecotypes, gene bank material and varieties and established a nursery with over 6000 plants. Four plants resulted in the synthetic variety Femelle in 2012. We immediately applied for plant breeder’s right and for the VCU testing in Belgium.

Samenvatting

De drijfveer van deze doctoraatsthesis zijn twee belangrijke uitdagingen waarmee de graslandproductie in België het jongste decennium geconfronteerd wordt. Ten eerste is er een toename van het aantal droogteperiodes ten gevolge van de klimaatopwarming. Ten tweede neemt het belang van gemaaid grasland ten aanzien van begraasd grasland toe ten gevolge van het steeds groter aantal melkkoeien dat jaarrond op stal gehouden wordt. Rietzwenkgras (*Festuca arundinacea* Schreb., **Fa**) is een grassoort waarvan we verwachten dat ze een aantal eigenschappen heeft om het hoofd te bieden aan deze uitdagingen.

Deze thesis omvat 3 onderzoeksdoelstellingen:

1. onderzoek naar de landbouwkundige waarde van rietzwenkgras;
2. ontwikkelen van methoden die in de veredeling kunnen gebruikt worden om de voornaamste knelpunten van rietzwenkgras te verbeteren;
3. rietzwenkgras veredelen dat aangepast is aan Belgische omstandigheden.

1. Ons baserend op de literatuur en onderzoeksresultaten vergeleken we de landbouwkundige waarde van Fa met die van Engels raaigras (*Lolium perenne* L., **Lp**), actueel de belangrijkste grassoort in West-Europa. We vonden dat de opbrengst van Fa onder gunstige groeiomstandigheden, gemiddeld 20 % hoger was dan die van Lp. Onder ernstige droogteomstandigheden vonden we dat de jaaropbrengst van Fa tot 60 % hoger was dan die van Lp. Fa heeft twee ernstige nadelen ten opzichte van Lp: de lage verteerbaarheid en opname door het vee en de trage vestiging. We vonden dat de verteerbaarheid van Fa gemiddeld 5-10 % eenheden lager is ten opzichte van Lp. De trage jeugdgroei zorgt voor een lage opbrengst in het eerste productieseizoen.

   We onderzochten of het hoge opbrengstpotentieel en de goede droogteresistentie van Fa enerzijds en de goede voederwaarde van raaigrassen anderzijds kan gecombineerd worden in een mengteelt van beide soorten. We verwachtten dat de complementaire eigenschappen van beide soorten zouden leiden tot een opbrengstvoordeel (*m.a.w.* opbrengst van soortenmengsel hoger dan opbrengst van meest productieve soort). We legden hiertoe drie veldproeven aan.

In een eerste veldproef, vergeleken we reinteelten van Fa en Lp en mengteelten ingezaaid met verschillende zaadmengsels van beide grassoorten, al dan niet met witte klaver. De graszaadmengsels verschilden in het aandeel raaigras en de ploidie van het raaigras. De opbrengst, de voederwaarde en de botanische samenstelling van het geoogste gras werd gemeten in 15 sneden in 3 opeenvolgende jaren (2010 - 2012). Voor de reinteelten Fa en Lp, waren de opbrengstresultaten conform met de resultaten uit de literatuur: gemiddeld over drie jaar was de opbrengst van Fa 23 % hoger dan die van Lp, maar de verteerbaarheid van de organische stof van Fa was ieder jaar ten minste 5 % eenheden lager in vergelijking met Lp. De mengsels van Fa en Lp leidden niet tot het verwachte opbrengstvoordeel: de opbrengst van de mengsels lag tussen die van de reinteelten van Fa en Lp. Hoewel mengsels aanvankelijk gedomineerd werden door Lp weerhield dit Fa er niet van langzaam maar zeker toe te nemen in belang om uiteindelijk de mengsels te domineren vanaf het derde productiejaar.

De tweede proef was analoog aan de eerste proef: we vergeleken reinteelten van Fa en Italiaans raaigras (*Lolium multiflorum* L.; **Lm**) en mengsels van beide grassoorten al dan niet met rode klaver. Over een periode van drie jaar (2010 – 2012) was de opbrengst van Fa niet hoger dan die van Lm ten gevolge van de lagere opbrengst van Fa in het eerste jaar na zaai. De hoogste opbrengsten in deze proef werden behaald met mengsels van Fa en Lm, die de hoge opbrengst van Lm in het jaar na zaai combineerden met de hoge opbrengst van Fa aan het einde van ieder groeiseizoen. Hoewel de mengsels gedomineerd werden door Lm in de eerste twee jaar van de proef, evolueerden de mengsels naar Fa reinculturen in het derde jaar van de proef. Hieruit leidden we af dat het nadeel van de trage jeugdgroei van Fa in het eerste productieseizoen kan opgevangen worden door een mengsel te zaaien van Fa met een laag (1/8) aandeel Lm zaden. De verteerbaarheid van Fa was 3 tot 6 % eenheden lager dan die van Lm.

In een derde proef vergeleken we reinteelten van Fa, Lp en mengsels van beide grassoorten met witte klaver **onder begrazing**. Naast opbrengst en botanische samenstelling, onderzochten we of er een verschil was in onkruidkolonisatie in de verschillende grassoorten en mengsels. In tegenstelling tot de maaiomstandigheden, was Fa niet significant productiever dan Lp onder begrazing, wellicht ten gevolge van de hoge omgevingsvariatie. Evenmin vonden we een opbrengstvoordeel van de mengsels. In het derde jaar van deze proef waren Fa en Lp ongeveer gelijk vertegenwoordigd in een mengteelt ingezaaid met mengsels van Fa en 1/4 Lp. We vonden geen verschil in onkruidkolonisatie tussen reinteelten of mengteelten.

Het bepalen van de botanische samenstelling van de hierboven beschreven soortenmengsels vereiste veel arbeidsintensief sorteerwerk: klaver scheiden van gras en Fa scheiden van Lp en Lm. We onderzochten de mogelijkheid om dit werk te verlichten via NIRS (near infrared refelctance spectroscopy). We vergeleken verschillende strategieën om vergelijkingen te calibreren die toelieten om de botanische samenstelling van de mengsels te voorspellen. Vergelijkingen gebaseerd op met de hand gesorteerde stalen die veel omgevingsvariatie bevatten, leverden het beste resultaat. Werd deze strategie toegepast op stalen uit onze proeven, dan werd het klavergehalte goed (root mean square error of prediction (RMSEP) = 4%) voorspeld. De resultaten voor Fa en Lp waren onbevredigend (RMSEP of 8 % en 10 % voor Fa en Lp respectievelijk).

Ten slotte gingen we op zoek naar een verklaring voor de betere droogteresistentie van Fa ten opzichte van Lp. In de literatuur wordt dit verschil veelal verklaard door de diepere beworteling van Fa ten opzichte van Lp, maar kwantitatieve gegevens hieromtrent zijn schaars. Om deze leemte in de kennis op te vullen, bemonsterden we de wortelbiomassa van Fa en Lp in verschillende proeven op verschillende locaties tot op 90 cm diepte. Op een diepte beneden 30 cm vonden we dat de wortelbiomassa van Fa tussen 1.5 en 4.2 keer hoger was dan die van Lp. Het grootste voordeel voor Fa ten opzichte van Lp werd gevonden op locaties met leembodems; het kleinste voordeel werd gevonden op een locatie met een zandbodem. Wanneer bemonsterd werd in de late zomer of aan het begin van de herfst was de wortelbiomassa hoger en waren de verschillen tussen de soorten meer uitgesproken.

2. De nadelen van Fa zijn in de eerste plaats de relatief lage opname van Fa en in de tweede plaats de trage jeugdgoei. Op voorwaarde dat goede methoden voorhanden zijn om planten te selecteren met een betere opname of een snellere jeugdgroei, kunnen deze eigenschappen door veredeling verbeterd worden.

   Ten eerste, testten of we de voorkeur van herkauwers voor Fa kon voorspeld worden zonder gebruik te maken van herkauwers. We onderzochten de correlatie tussen de voorkeur van schapen enerzijds en bladmorfologie, zode eigenschappen en voorkeur van konijnen anderzijds voor 16 Fa klonen. We vonden dat de voorkeur van schapen negatief gecorreleerd was met de grashoogte vóór begrazen en de ruwheid van de bladeren en positief gecorreleerd was met de voorkeur van konijnen. Hoewel we meer inzicht verwierven in de factoren die de voorkeur beïnvloeden, bereikten we ons doel niet: we konden de voorkeur van schapen niet op een betrouwbare manier voorspellen aan de hand van de gemeten parameters.

Ten tweede testten we of digitale beeldanalyse toelaat om de bodembedekking en jeugdgroei van Fa op een herhaalbare manier te bepalen onder veldomstandigheden. Beeldanalyse liet toe de bodembedekking accuraat te bepalen, maar we vonden een aantal tekortkomingen om de methode te gebruiken in de selectie van Fa planten met een betere jeugdgroei. Bodembedekking en opbrengst waren slechts tot een beperkt groeistadium met elkaar gecorreleerd. Bovendien was de methode niet eenvoudig in gebruik in het veld: niet alle weersomstandigheden lieten toe om bruikbare foto's te maken en de aanwezigheid van onkruiden beïnvloedde de resultaten. Bijgevolg vonden we dat de methode actueel slechts een beperkte waarde heeft om te selecteren voor jeugdgroei.

3. In 2008 startten we een Fa veredelingsprogramma. Het veredelingsdoel was om een zachtbladig ras te selecteren met een goed opbrengstpotentieel en een goede roestresistentie onder Belgische omstandigheden. We verzamelden 600 ecotypen, genenbankmateriaal en rassen, en legden een selectieveld aan met meer dan 6000 individuele planten. Vier van deze planten, met een excellente roestresistentie, gaven aanleiding tot het synthetisch ras 'Femelle'. In 2012 hebben we het ras aangemeld in de Belgische rassenproeven en hebben we kwekersrecht aangevraagd.

List of abbreviations

AIC	Akaike’s information criterion
B	Brightness
CP	Crude protein
CPC	Crude protein content
Dg	*Dactilys glomerata* L.
DM	Dry matter
DMC	Dry matter content
DMD	Dry matter digestibility
DMI	Dry matter intake
DMY	Dry matter yield
DOM	Digestibility of the organic matter
DOY	Day of the year
DUS	Distinctness, Uniformity and Stability
EU	European Union
Fa	*Festuca arundinacea* Schreb.
FaC	*Festuca arundinacea* content in dry matter yield
Fl	*Festulolium*
Fp	*Festuca pratensis* Huds.
H	Hue
H	Hypothesis
ILVO	Institute of agriculture and fisheries research
K	Potassium
Lh	*Lolium x boucheanum* Kunth.
Lm	*Lolium multiflorum* Lam.
Lm2	Diploid *Lolium multiflorum* Lam.
Lm4	Tetraploid *Lolium multiflorum* Lam.
Lp	*Lolium perenne* L.
Lp2	Diploid *Lolium perenne* L.
Lp4	Tetraploid *Lolium perenne* L.
MP	Milk production
N	Nitrogen
NDF	Neutral detergent fibre

NH	Neighbourhood H value
NIR	Near infrared
NIRS	Near infrared reflectance spectroscopy
NY	Nitrogen yield
P	Phosphorous
PBR	Plant breeder’s rights
PC	Principal component
PCA	Principal component analysis
Pp	*Phleum pratense* L.
PLS	Partial least square regression
RDOM	Apparently rumen degraded organic matter
RMSEP	Root mean square error of prediction
RPD	ratio of prediction to deviation
RQ	Research question
RSQ	R square value
S	Saturation
SD	Standard deviation
SEC	Standard error of calibration
SECV	Standard error of cross-validation
SEP	standard error of prediction error
Tp	*Trifolium pratense* L.
Tr	*Trifolium repens* L.
TrC	*Trifolium repens* content in dry matter yield
UK	United Kingdom
USA	United States of America
VCU	Value for Cultivation and Use
WSC	Water soluble carbohydrates

Chapter 1

Introduction and objectives

1.1 Taxonomy, Morphology and Geographical distribution of *Festuca arundinacea* Schreb.

In the vegetative stage, tall fescue (*Festuca arundinacea* Schreb.; **Fa**) is described as (Gibson and Newman, 2001): "*A perennial grass producing large, loose, but sometimes dense, tussocks, normally without short rhizomes, and without stolons. The lower sheaths are not fused, and are smooth and rounded on the back. Ligule, < 2 mm, membranous with ciliate auricles (cilia often few, and wearing off with age). Leaves, dark-green, stiff, usually flat, 10–60(105) cm long, (1)3–12 mm wide, distinctly ribbed above, rough or smooth below only, scabrous on margin, and tapering to a fine tip.*" In the vegetative stage, the ciliate auricles (**Figure 1.1**) are the most straightforward character to distinguish Fa from related grass species.

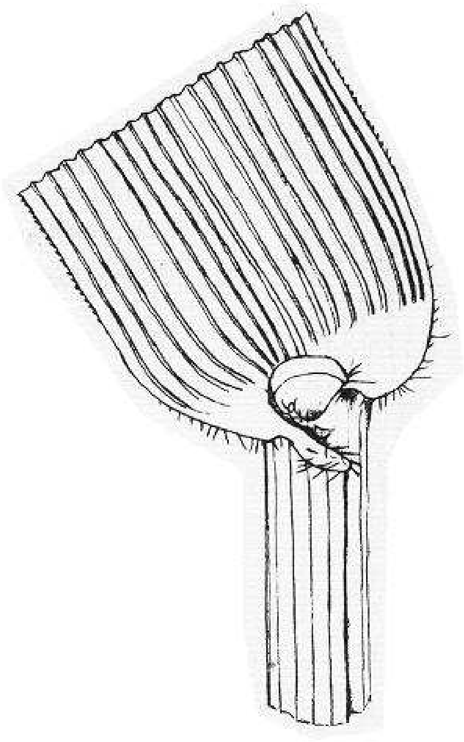


Figure 1.1 Ciliate auricle of *Festuca arundinacea* (source: van der Meijden, 2005)

Fa is native to Europe, and has a wide geographical distribution (Gibson and Newman, 2001): "*Festuca arundinacea is widely distributed as a native grass in temperate and cool climates throughout Europe (north to 62°in Scandinavia), North Africa, west and central Asia, and Siberia, but is absent from most of north, central and eastern Russia. It occurs throughout these areas except in the Faeroes, Iceland, northern Russia and Spitsbergen. In North-West*

Europe, it is mainly limited to coastal areas around the Baltic, with some inland locations in southern Sweden."

The further geographical distribution of Fa is limited by climatic and topographical limitations (Gibson and Newman, 2001): "*The range of Festuca arundinacea in Europe corresponds in the north in Scandinavia to a January mean of –5° C and altitudes < 200 m, and in the south to a mean July temperature of 25°C. Within this area, average yearly precipitation ranges from 2000 mm in the Alps to < 500 mm in central Spain. F. arundinacea occurs principally at sea level but it has been recorded at 2760 m in the High Atlas mountains of Morocco."*

Fa has many habitats. In the UK, Fa is found on sloping, wet meadows and pastures, on coastal cliffs, roadside verges, railway embankments and field margins. When present in salt marshes it seems to be limited to an uppermost zone that is effectively freshwater and only rarely subjected to tidal flooding (Gibson and Newman, 2001). In Belgium, Fa is present frequently in most regions, but its presence is lower in the region called 'Kempen' in the north of Flanders and in the centre of Flanders (**Figure 1.2**) (Van Landuyt, 2006); both regions are characterised by a sandy soil (**Figure 1.3**). The habitats of Fa in Belgium are mainly (in order of importance) roadsides, riverbanks and pastures (own observations).

Fa is a polyploid species (Gibson and Newman, 2001): *"Fa is a (bivalent forming) allohexaploid (2n = 6x = 42 chromosomes) (Berg et al. 1979). The progenitor species are meadow fescue F. pratensis Huds. (2n = 2x = 14) and F. glaucescens Hegetschw. & Heer. (2n = 4x = 28). The identification of these progenitors has been confirmed using DNA restriction fragment length polymorphisms (Xu et al. 1992)."*

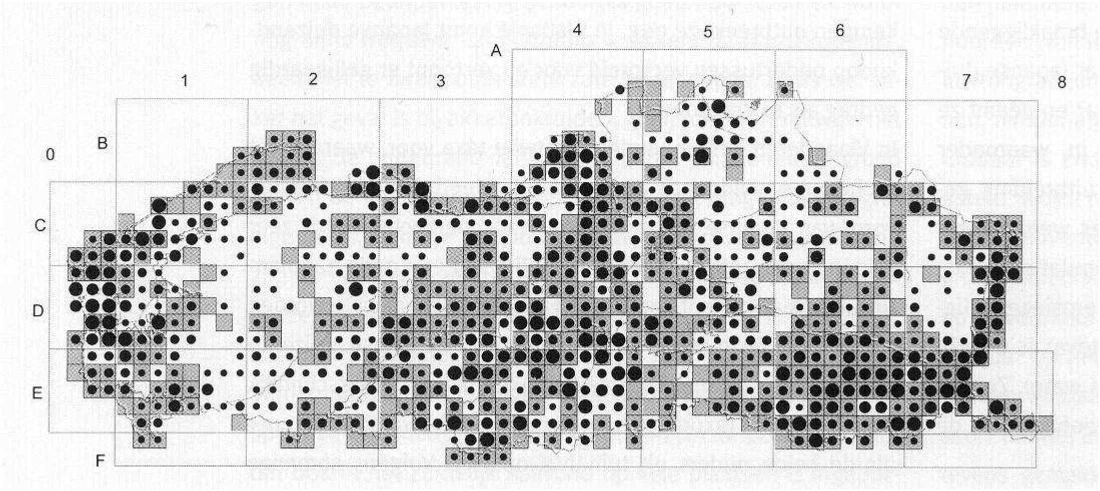


Figure 1.2 Distribution map of *Festuca arundinacea* (Fa) in Flanders (Belgium). Squares represent an area of 4 x 4 km². The dots indicate the frequency of presence of Fa in the 16 kilometer-squares (1 x 1 km²) within these squares. Dots have 4 sizes: smallest size: Fa present in 1-25 % of the monitored kilometer-squares, largest dots: Fa present in 76-100 % of the monitored kilometer-squares (source: Van Landuyt, 2006).

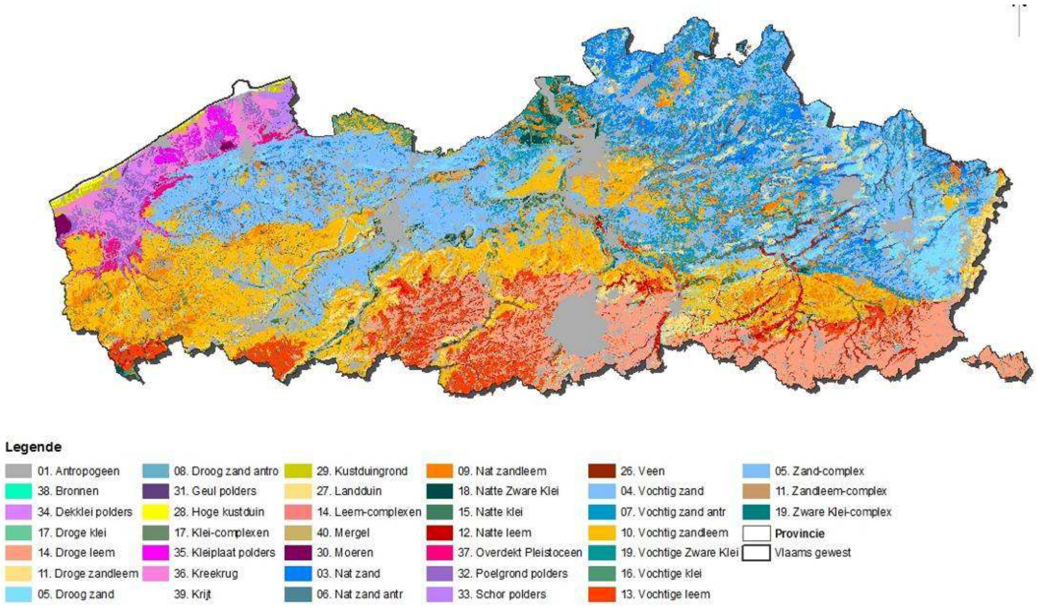


Figure 1.3 Soil map of Flanders: 1. Antropogenic soils, 3-8 sandy soil types, 9-11 sandy loam soil types, 12-14 loamy soil types, 15-19 silty soil types, 28-29 coastal dunes, 30-37 polders. (source: Agiv, 2013)

1.2 Tall fescue as a forage grass species

Grassland in North-West Europe is dominated by ryegrasses (*Lolium* sp.). In France, the EU member state with the largest agricultural area, 23714 t of forage grass seed was sold in 2011. Italian ryegrass (*Lolium multiflorum* Lam.; **Lm**), perennial ryegrass (*Lolium perenne* L.; **Lp**) and orchardgrass (*Dactylis glommerata* L.; **Dg**) being the most important species with approximately 11108 t, 5995 t, and 2498 t sold (Haquin, 2012). In Northern Ireland, UK with its mild maritime climate, ryegrasses are even more predominant. In the period 1980-2004 for example 78-86% of the forage grass seed sold in Northern Ireland, UK was Lp, with the majority of the remainder being Lm and hybrid ryegrass (*Lolium x boucheanum* Kunth.) (Gilliland *et al.*, 2007). Perennial ryegrass can be used both for cutting (early varieties) or grazing (intermediate and late varieties) whereas Lm and hybrid ryegrass are mainly used for cutting only management (Frame, 1992).

Fa is regarded as a secondary forage species in North-West Europe, it can be used both for cutting as for grazing (Frame, 1992). Only 1800 t of the *circa* 20000 t of forage grass seed sold annually in France is Fa (average 2007-2009) (GNIS, 2013). In 2011, 23714 t of forage grass seed was sold in France of which 2087 t was Fa seed (Haquin, 2012). *Anno* 2013, 203 varieties of Lp, 106 varieties of Lm against 39 varieties of Fa were on the French national list (GEVES, 2013); no Fa varieties are on the national lists of Belgium, the Netherlands or the UK. (Pannecoucque, 2013; CSAR, 2013; NIAB, 2013).

In the United-States on the other hand Fa is one of the predominant cool season perennial grass species, occupying 12 to 14 million ha by 1973 and (Buckner and Bush, 1979). More recently, 19 million ha of Fa pastures in the USA were reported (Bouton, 2007). Also in Australia, Fa is an important forage species (Easton *et al.*, 1994).

Climate change and the decreasing importance of grazing in dairy farming, however might increase the importance of Fa in Europe (Reheul *et al.*, 2012).

1.3 Tall fescue and climate change

According to the predictions made by the intergovernmental panel on climate change (IPCC), more dry summer spells are expected in North-West Europe due to climate change. Models disagree on the magnitude and geographical details of precipitation change, but annual precipitation is very likely to increase in most of northern Europe and to decrease in most of the Mediterranean area. In central Europe, precipitation is likely to increase in winter but decrease in summer. The risk of summer drought is likely to increase in central Europe and in the Mediterranean area (IPCC, 2007). As ryegrasses are relatively sensitive to drought stress (Norris, 1982; Frame, 1992), interest in species Dg, Fa and *Festulolium* which are known to have a relatively good drought resistance, is increasing (Gilliland *et al.*, 2010; Graiss *et al.*, 2011; Mosimann *et al.*, 2010; Reheul *et al.*, 2011; Surrault *et al.*, 2007). Fa is generally regarded as the most drought resistant and highest yielding of these species.

1.4 Drought resistance of tall fescue

Wilman *et al.* (1998) tested eight forage grass species under severe drought in Wales, UK. Drought was imposed by restricting rain water access with transparent covers over the field plots. Lm died out after 15 months, meadow fescue and Lp after 2 years of rain exclusion, but Fa was still alive after four years of rain exclusion. The better drought resistance of Fa was explained by its greater number and biomass of roots compared to the other species in the 50–100 cm soil layer. The deeper rooting of Fa compared to ryegrasses was confirmed by other research (Van Eekeren et al, 2012; Durand and Ghesquière, 2002; Deru *et al.* , 2012). Research results of Turner *et al.* (2012) suggest that drought resistance of Fa is not only due to its deeper rooting capacity. They compared the morphological and physiological responses of Lp, Fa and Dg under optimal and suboptimal irrigation regimes in a pot trial (root depth limited to 28 cm) under controlled environment. Fa appeared to have the greatest scope under moisture stress in terms of maintaining productivity and persistence. Leaf DMY was consistently in the order Fa>Lp>Dg (Turner *et al.*, 2012).

Apart from its good drought resistance, Fa resists well to other kinds of abiotic stress: it is believed to be very winter hardy and tolerant to flooding (Gilbert and Chamblee, 1965). In addition, Fa was found to have a wider adaptability to adverse soil conditions like poorly drained soils, acid or alkaline soil compared to other cool season grasses in the USA (Burns and Chamblee, 1979). Buckner *et al.* (1979) noted in the USA that Fa was not well adapted to grow on sandy soils having long periods of drought. Altough natural populations of Fa are less abundant on sandy soils, there are no indications that Fa does not grow well in the sandy soils of Europe, where most of the dairy industry is concentrated. In Jutland, Denmark, that is characterised by a sandy soil, Fa is grown successfully on large dairy farms (own observations).

1.5 Advantages and disadvantages of tall fescue

Fa is praised for its good drought resistance, robustness and high yield potential (see above), but it has two main disadvantages that hamper the success of the species.

Firstly, Fa has a lower voluntary intake and a lower digestibility compared to *Lolium sp.* Consequentially, the potentially higher herbage yield is not necessarily translated into a higher animal production (Easton *et al.*, 1994). The low voluntary intake of Fa is generally brought in relation to its leaf roughness (Gillet and Jadas-Hecart, 1965). In a Dutch study (Luten and Remmelink, 1984) the voluntary intake and milk production of cows fed with cut Fa, Lp and

Lm was compared. When fed fresh, mostly a significantly lower intake and lower milk production was found for Fa compared to the ryegrasses. When ensiled or hayed, the harshness and intake difference with *Lolium sp.* decreased (Peratoner *et al.*, 2011; Luten and Remmelink, 1984).

In addition to the lower voluntary intake, the digestibility of Fa is lower compared to Lm or Lp in a comparable developmental stage (INRA, 1987, Luten and Remmelink, 1984). These properties of Fa hamper an effective feeding of the high genetic merit cows raised on most of the North-West European dairy farms (Peyraud *et al.*, 2010).

Secondly, the establishment of a Fa sward is slower and more delicate compared to ryegrass swards (Jadas-Hecart et Gillet, 1975), resulting in a lower yield in the first production year. Wilman and Gao (1996) compared the establishment of swards of different grass species after spring sowing in Wales. In the year of establishment, the yield of Fa was 16% lower compared to Lp. Some varietal differences do exist, but even with the fastest establishing varieties, the disadvantage continues to be present (Jadas-Hecart et Gillet, 1975).

An ideal grass species or grass sward combines the excellent forage quality of Lp or *Lolium* sp. in general and the resilience against abiotic stress of Fa or *Festuca* sp. in general. This idea led to the creation of intergeneric *Festuca* x *Lolium* hybrids or *Festulolium* (Thomas *et al.*, 2003).

1.6 Festulolium ≠ Festuca + Lolium

Festulolium genetically combines the advantages of *Festuca* and *Lolium* in a single species (Thomas *et al.*, 2003; Humphreys *et al.*, 2012). *Festulolium* varieties are characterised by the specific *Lolium* and *Festuca* species that are combined, and in the degree of hybridisation: 2 complete genomes in amphiploidy *versus* introgression of some genes from one species in the genome of another species (Ghesquière *et al.*, 2010). So far, the commercial success of *Festulolium* was limited, mainly due to problems in seed production and stability of the varieties (Ghesquière *et al.*, 2010).

Another option is to combine the advantages of both species by sowing a seed mixture of both species. While in *Festulolium* a monospecific sward is created, the strategy of mixing *Festuca* and *Lolium* species results in a bispecies sward which may have both advantages and disadvantages. A potential shifting in composition in time may be disadvantageous while interspecific interactions may have positive effects leading to transgressive overyielding (Nyfeler *et al.*, 2009; Huyghe *et al.*, 2012).

‘Overyielding’ is a concept borrowed from the theory of agricultural intercropping. It occurs when the yield of a mixed plot of two or more species exceeds the average yield that would have been obtained by growing species in monospecific swards. ‘Transgressive overyielding’ occurs when a mixture yields more than any monospecific sward of the component species (Drake, 2003). According to Drake (2003), overyielding can only result from niche differentiation (complementarity) of species.

There are several reasons to presume a niche differentiation between Fa and *Lolium sp.* Indeed, literature supports the idea that Fa and Lp or Lm may be complementary species. Firstly, Gilliland *et al.* (2011) compared growth of five grass species during four years on three locations in Ireland. Lp had the lowest and Fa the highest yield per degree centigrade and per unit of photosynthetic active radiation in the spring period under low temperature and light intensity. The situation was inversed in the summer, under higher temperature and photosynthetic active radiation: Lp had the highest yield, that of Fa was significantly lower. The yield of Fa was again higher in the autumn. In autumn, Fa keeps on growing later than any other cool season grass species (Jadas-Hecart et Gillet, 1975). So it can be concluded that Fa and Lp have a complementary growth pattern.

Secondly, Fa has a higher root biomass in the deeper soil layers compared to Lp and Lm (Wilman *et al.*, 1998; Van Eekeren *et al.*, 2010; Deru *et al.*, 2012; Durand and Ghesquière, 2002). The complementary rooting depth between Lp and Fa may be advantageous under drought conditions and may limit or prevent loss of nutrients by leaching, which may result in a better nitrogen use efficiency of a mixed sward.

Thirdly, the positive interactions between the species may extend into the animal. Baert *et al.*, (2012) found a significantly higher amount of rumen escape protein in Fa compared to Lp. In this way, mixing Fa and Lp may lead to a more efficient N utilization by ruminants.

There is some evidence that mixtures of Fa and Lp or Fa and Lm outperform swards consisting of the single species. Wilman and Gao (1996) compared the yield of Fa, Lp and Lm and mixtures of both Fa + Lm or Fa + Lp during five years in Wales. Over the whole experimental period, the yields of Fa, Lp and Lm were 77.0 t DM ha^{-1}, 80.3 t DM ha^{-1} and 75.1 t DM ha^{-1} respectively. Very modest transgressive overyielding occurred in the mixtures: Fa + Lp and Fa + Lm yielded 80.7 t DM ha^{-1} and 78.1 t DM ha^{-1} respectively.The uneven distribution of both species in the mixture might have precluded a further yield advantage of the mixtures compared to the monospecific swards. Both species in the mixture were sown at the same density, which favoured Lm and Lp as these species developed much faster than Fa after sowing.

In a grazing trial in Switzerland, lasting for three years, two grass-clover mixtures either dominated by Lp or by Lp plus Fa were compared (Mosimann *et al.*, 2010). No differences in grass growth nor in organic matter digestibility between the types of sward could be measured in the first two years characterised by normal rainfall. Under dry conditions, the mixture with Fa showed the best performance.

1.7 Breeding of tall fescue

Breeding offers a potential strategy to tackle the disadvantages of Fa. Varieties with softer leaves and an increased animal preference have been bred in France (Rognli *et al.*, 2010, Emile *et al.*, 1992). Owing to the high heritabilities for nutritive quality factors found by De Santis and Chiaravalle (2001), a further progress of the digestibility and intake preference of Fa is expected.

Progress in early vigour through breeding is expected to be possible owing to the genetic variance present in varieties of Fa (Suter *et al.*, 2009; Jadas-Hecart and Gillet, 1975).

The better the plants with the desired traits can be identified in a breeding programme, the faster the progress for that character will be. Therefore, it is important to develop methods that allow phenotyping for a good early vigour and a good animal preference.

1.8 Thesis outline and research questions

This doctoral thesis can be divided in three main research topics:

1. Agronomy of Fa under Belgian conditions
2. Phenotyping methods that are useful for Fa breeding
3. The breeding of FEMELLE, the first Belgian variety of Fa

Hypotheses (H) and research questions (RQ) are explained below:

1.8.1 Agronomy of tall fescue under NW European conditions

The research started from hypothesis 1 (**H1**): *"Fa is more productive, particularly under drought stress, but has a lower digestibility and animal preference than Lp"*. To check **H1**,

Chapter 2 investigates the available literature and unpublished trial results with the aim to answer following research questions

RQ1.1 How large is the yield difference between Fa and Lp under favourable growing conditions?
RQ1.2 How large is the yield difference between Fa and Lp under drought conditions?
RQ1.3 How large is the initial yield loss due to the slow establishment of Fa compared to Lp?
RQ1.4 How large is the difference in digestibility and intake between Fa and ryegrasses?

Owing to the complementary characteritics of Fa and Lp and Fa and Lm, we hypothesize that growing mixtures of both grass species may lead to overyielding. **Chapter 3, 4** and **Chapter 5** verify **H2**: *"There is transgressive overyielding with mixtures of Fa and Lp or Fa and Lm: mixtures can yield more than the monospecific swards of these species"*. Both mixtures and monospecific swards of Fa and Lp as well as mixtures and monospecific swards of Fa and Lm (**Chapter 3** and **Chapter 4** respectively) were studied. Five research questions were put forward:

RQ2.1 Does transgressive overyielding occur in mixtures of Fa and Lp or Lm and if so at what magnitude?
RQ2.2 What proportion of Fa and Lp or Lm in mixtures is highest yielding and does it constitute a transgressive overyield?
RQ2.3 What is the effect of the ploidy of the ryegrass component in the mixtures?
RQ2.4 How large is the difference in feed quality of the mixtures compared to the monospecific swards?
RQ2.5 Does adding clover to the mixtures alter the answer of the RQ2.1, RQ2.2, RQ2.3 and RQ2.4?

The trials in **Chapter 3** and **Chapter 4** were conducted under a cutting only regime. In practice however, Fa and Lp swards are often grazed. Grazing involves factors that influence grass growth like trampling by cows, deposition of urine and cow pads,... Therefore, Fa, Lp and mixtures of Fa + Lp were compared in a grazing trial (**Chapter 5**).

Three additional research questions were put forward:
RQ2.6 How large is the yield gain owing to transgressive overyielding in mixtures of Fa and Lp under grazing management?
RQ2.7 Which proportion of Fa and Lp in the mixtures is leading to the highest transgressive overyielding under grazing management?

RQ2.8 What is the effect of the ploidy of the ryegrass component in the mixtures under grazing management?

As in the trials studied in **Chapter 3** and **Chapter 4**, also monospecific swards of Fa, Lp and Lm were included, the results of these trials complete the answer to **RQ1.1**, **RQ1.3** and **RQ1.4** given in **Chapter 2**.

In **Chapter 5**, we further verify **H3**: “Weed invasion is lower in Fa compared to Lp2 or Lp4, owing to the better persistence of Fa”.

The research on species mixtures in **Chapter 3** to **Chapter 5**, involved a lot of labour intensive sorting of grass and grass-clover samples. In literature, several equations were presented to predict the botanical composition of grass-clover samples using Near Infrared Reflectance Spectroscopy (NIRS). In **Chapter 6** we tested **H4** that: *“NIRS calibration can be used to develop an equation to predict the botanical composition of mixtures of Fa + Lp with white clover”*. In literature different strategies were used to obtain the calibrations. Our research questions were:

RQ4.1 Which calibration strategy leads to the smallest prediction error? **RQ4.2** How large is the prediction error when the best calibration strategy found in **RQ4.1** is applied to the samples of the trials described in **Chapter 3**?

The deeper rooting of Fa is often cited to explain the better drought resistance of Fa compared to Lp. Quantitative data however are scarce and the effect of soil, management and season on the root biomass of both species is ignored. **Chapter 7** focuses on the difference in root biomass between Fa and Lp with **H5***: “Fa has a higher root biomass than Lp below 30 cm but the difference is soil and season dependent”*. Corresponding research questions were: How large is the effect of location (different soil, management and fertilisation) (**RQ 5.1**) and season (spring or autumn) (**RQ 5.2**) on root biomass of Fa and Lp below 30 cm.

1.8.2 Phenotyping methods useful for tall fescue breeding

As mentioned earlier, the two main disadvantages of Fa are its low animal preference and its slow early vigour after sowing. Provided good methods are available to select plants with better preference or early growth, breeding is expected to be able to improve these traits.

We studied methods to ease the selection for animal preference and early vigour of Fa in a repeatable way in **Chapter 8** and **Chapter 9** respectively.

Chapter 8 deals with **H6**: *"Ruminant preference of Fa genotypes can be predicted without using ruminants"*.

Corresponding research questions question the magnitude of the correlation between sheep preference and leaf morphological parameters (**RQ6.1**), sward characteristics (**RQ6.2**), rabbit preference (**RQ6.3**).

In **Chapter 9**, we tested **H7**: *"Digital image analysis can be used to quantify ground cover and early vigour in a repeatable way in the field"*

We studied whether the method allowed detecting significant differences in ground cover between grass species and varieties of the same species (**RQ7.1**). **RQ7.2** provided an answer to the question in which developmental stage the differences between the varieties were maximal.

Additionally we were interested in (**H8): *"There is a good correlation between ground cover measured by digital image analysis and biomass production"*.**

1.8.3 The breeding of FEMELLE, the first Belgian tall fescue variety

We hypothesized that there were enough arguments to believe that breeding is able to produce improved varieties adapted to the Belgian environment and grassland management. **H9** was that: *"Recurrent selection offers opportunities to breed new Fa varieties with soft leaves and a good rust resistance under Belgian conditions"*. 'Femelle' is the first variety that was created in this programme. **Chapter 10** explains in detail how 'Femelle' was bred.

Finally, **Chapter 11** gives a general conclusion and recommendations for further research. Results from **Chapter 2** to **10** are summarized and discussed by answering the research questions and validating the hypotheses listed in **table 1.1.**

Table 1.1 Thesis outline in relation to the defined hypotheses (H) and research questions (RQ)

		Chapter
Thesis incentive	There is a need for a forage grass species with interesting properties to respond to the future challenges of grassland production in Belgium; Fa might be a candidate.	
Thesis objectives	1. To study the agronomy of Fa under Belgian conditions.	2 - 7
	2. To develop phenotyping methods useful for Fa breeding.	8,9
	3. To breed a Fa variety adapted to Belgian circumstances.	10
H1	**Fa is more productive, particularly under drought stress, but has a lower digestibility and animal preference than Lp.** **RQ1.1** How large is the yield difference between Fa and Lp under favourable growing conditions? **RQ1.2** How large is the yield difference between Fa and Lp under drought conditions? **RQ1.3** How large is the initial yield loss due to the slow establishment of Fa compared to Lp? **RQ1.4** How large is the difference in digestibility and intake between Fa and ryegrasses?	2
H2	**There is transgressive overyielding with mixtures of Fa and Lp or Fa and Lm: mixtures can yield more than the monospecific swards of these species.** **RQ2.1** Does transgressive overyielding occur in mixtures of Fa and Lp or Lm and if so at what magnitude? **RQ2.2** What proportion of Fa and Lp or Lm in mixtures is highest yielding and does it constitute a transgressive overyield? **RQ2.3** What is the effect of the ploidy of the ryegrass component in the mixtures under cutting management? **RQ2.4** How large is the difference in feed quality of the mixtures compared to the monospecific swards? **RQ2.5** Does adding clover to the mixtures alter the answer of RQ2.1, RQ2.2, RQ2.3 and RQ2.4? **RQ2.6** How large is the yield gain owing to transgressive overyielding in mixtures of Fa and Lp under grazing management? **RQ2.7** Which proportion of Fa and Lp in the mixtures is leading to the highest transgressive overyielding under grazing management? **RQ2.8** What is the effect of the ploidy of the ryegrass component in	3, 4, 5

	the mixtures under grazing management?	
H3	**Weed invasion is lower in Fa compared to Lp2 or Lp4, owing to the better persistence of Fa.**	5
H4	**NIRS calibration can be used to develop an equation to predict the botanical composition of mixtures of Fa + Lp with white clover.** **RQ4.1** Which calibration strategy leads to the smallest prediction error? **RQ4.2** How large is the prediction error when the best calibration strategy found in RQ4.1 is applied to the samples of the trials described in chapter 3?	6
H5	**Fa has a higher root biomass than Lp below 30 cm but the difference is soil and season dependent.** How large is the effect of location (**RQ 5.1**), and season (spring or autumn) (**RQ 5.2**)?	7
H6	**Ruminant preference of Fa genotypes can be predicted without using ruminants.** How is the correlation between sheep preference and: leaf morphological parameters (**RQ6.1**)? sward characteristics (**RQ6.2**)? rabbit preference (**RQ6.3**)?	8
H7	**Digital image analysis can be used to quantify ground cover and early vigour in a repeatable way in the field.** Does the method allow detecting of significant differences in ground cover between grass species and varieties of the same species (**RQ7.1**)? In which developmental stage differences between the varieties are maximal (**RQ7.2**)?	9
H8	**There is a good correlation between ground cover measured by digital image analysis and biomass production.**	9
H9	**Recurrent selection offers opportunities to breed new Fa varieties with soft leaves and a good rust resistance under Belgian conditions.**	10

Chapter 2

Agronomic performance of tall fescue compared to perennial ryegrass and other cool season grass species

2.1 Introduction

As climate change is predicted to result in a higher frequency of extreme weather conditions as hot and dry summers and wet and cold winters in large parts of Europe (IPCC, 2007), interest in tall fescue is growing. Tall fescue (*Festuca arundinacea* Schreb., **Fa**) is known to be a very adaptable species growing well both in dry as in wet conditions (**Chapter 1**). In addition, it is very winter hardy and has a very high yield potential and can withstand high mowing frequencies. Major drawbacks of this species are however slow establishment and its poor acceptability to grazing animals and its lower digestibility (Frame, 1992).

We compared agronomic performance of Fa with that of other cool season grasses in North-West Europe. As perennial ryegrass (*Lolium perenne* L.; **Lp**) is the most important grass species in Europe (**Chapter 1**), we focused on the comparison of Fa with Lp. Where possible, we also compare with other cool season forage grass species: Orchardgrass (*Dactylis glomerata* L.; **Dg**), Timothy grass (*Phleum pratense* L.; **Pp**), Meadow fescue (*Festuca pratensis* Huds., **Fp**), Italian ryegrass (*Lolium multiflorum* Lam.; **Lm**), *Festulolium* (**Fl**) and hybrid ryegrass (*Lolium x boucheanum* Kunth.; **Lh**). The questions we wanted to answer were:

1. How large is the yield difference between Fa and Lp under favourable growing conditions?
2. How large is the yield difference between Fa and Lp under drought conditions?
3. How large is the initial yield loss due to the slow establishment of Fa compared to Lp?
4. What is the difference in digestibility and intake between Fa and ryegrasses?

2.2 Method

The comparison was conducted based on European data obtained via a search in the principal European journals and proceedings specialized in grassland research and analysis of (unpublished) results of trials. Literature comparing the performance Fa and other forage grasses is relatively scarce. We used publications listed in **table 2.1** to compare the agronomic performance of Fa and Lp:

Table 2.1 Published studies that allow comparison of performance between *Festuca arundinacea* (Fa) and other cool season grass species: Lp: *Lolium perenne*, Lp2: diploid Lp, Lp4: tetraploid Lp, Dg: *Dactylis glomerata*, Fp: *Festuca pratensis*, Pp: *Phleum pratense*, Lm: *Lolium multiflorum*, Fl: *Festulolium*, Lh: *Lolium x boucheanum*, Tr: *Trifolium repens*; DMY: dry matter yield, DMC: dry matter content, CPC: crude protein content, DOM: digestibility of the organic matter, DMI: dry matter intake, MP: milk production, NY: nitrogen yield. NA: data not available.

					Management	
Authors, Location(s) of study	Period	Species	Measurement	Soil type	N fertilisation (kg ha^{-1} yr^{-1})	Nr. cuts (yr^{-1})
Baert *et al.* (2012)Merelbeke, Belgium	2007$^{\circ s}$ 2009-2010	Fa, Lp, Pp, Dg	DMY, CPC, DOM	Sandy loam	250 and 150	3 3
Van Eekeren *et al.* (2010) Helvoirt, The Netherlands	2006$^{\circ s}$ 2007-2008	Fa, Lp, Dg	DMY, NY	Sandy	250 mineral + 232 slurry	4 (2007), 5 (2008)
Gilliland *et al.* (2010) Crossnacreevy, UK Backweston, Ireland Moorepark, Ireland	2006$^{\circ u}$ 2007-2009	Fa +Tr,Lp2 +Tr, Lp4+Tr, Pp+Tr, Fp+Tr, Dg+Tr	DMY	Medium loam Clay loam Medium loam	Each location: 420, 210 and 105	*circa* 10 *circa* 10 *circa* 10
Pontes *et al.* (2007) Theix, France	2001$^{\circ u}$ 2003-2004	Ecotypes of : Lp, Fa, Dg, Pp	DMY, CPC, DOM, Flowering date	Granitic sand	120 and 360	3 and 6 3 and 6
Surault *et al.* (2007) Lusignan, France	2003$^{\circ u}$ 2004-2006	Fa, Lp, Dg, Fp	DMY	NA	180	every 25d every 45 d
Wilman and Gao (1996) Aberystwyth, UK	1989$^{\circ s}$ 1990-1993	Fa, Lp, Fl, Lh, Fp	DMY, tiller density	Silty clay	450	5 (1989), 7 (1990-1992), 4 (1993)
Luten and Remmelink, (1984) Lelystad, The Netherlands	1973$^{\circ u}$ 1974-1978	Fa, Lp, Lm	DOM, CPC, DMC, DMI, MP	NA	NA	NA

° sowing year a: autumn s: spring u: season unknown

Baert *et al.* (2012) compared the dry matter yield (DMY) and quality of perennial fodder grasses for biogas production. Tall fescue, perennial ryegrass, timothy and cocksfoot were sown in 2007 and assessed in 2008, 2009 and 2010. The trial was conducted on a sandy loam soil in Belgium. The trial was a split plot design with three replicates, with the species as main plot factor and high N or low N (250 and 150 kg N ha^{-1} y^{-1}) fertilisation as subplot factor.

In Van Eekeren *et al.* (2010) root biomass and DMY of individual grass species and grass species mixtures were studied on a sandy soil in the Netherlands. Tall fescue, perennial ryegrass, and cocksfoot and mixtures of these grass species were sown in 2006 and compared in 2007 and 2008. The grass received 250 kg N ha^{-1} yr^{-1} from mineral fertilisation and 232 kg N $ha^{-1}yr^{-1}$ from cattle slurry. Only results for 2008 were reported.

Gilliland *et al.* (2010) studied the effect of nitrogen on DMY and composition of grass clover swards of six commonly grown grass species in Ireland. The experiment was conducted on three sites in the north, centre and south of the island. The grass species involved in the study were diploid and tetraploid Lp, Fa, Fp, Pp and Dg in combination with white clover. The trials were randomised complete block designs with three replicates and three N levels: high medium and low (420, 210, 105 kg N $ha^{-1}yr^{-1}$). Each treatment was managed to maximize DMY with a target of 10 cuts per season. Clover content was determined in two cuts per year.

Whereas all previous studies compared cultivars of different species, Pontes *et al.* (2007) compared the productivity and nutritive value of ecotypes of temperate grasses found in semi-natural pastures. Seeds of thirteen grass species including Fa, Lp, Pp, Dg were collected in their native habitat and sown in pure stands in 2001. In addition, one cultivar of Lp was included. From 2002, a factorial design with two levels of cutting frequency (3 and 6 cuts $year^{-1}$) and two levels of N fertilisation (120 and 360 kg N $ha^{-1}yr^{-1}$) was imposed; measurements were obtained in 2003 and 2004. In order to eliminate differences in drought resistance between the species, irrigation was applied below a certain threshold value of soil volumetric water content.

The study of Surault *et al.* (2007), focused on the drought resistance of Fa, Lp, Fp and Dg and multi-species mixtures in the very dry summer of 2005. The (non-irrigated) trial was sown in 2003 and measurements from 2004 till 2006 were reported. Two cutting regimes were imposed: a simulated cutting regime and simulated grazing regime, with cuts every 45 or 25 days respectively. For both cutting regimes, N fertilisation was 180 kg N $ha^{-1}yr^{-1}$.

Wilman and Gao (1996) studied herbage production and tiller density in five related grass species, their hybrids and their mixtures on a silty clay soil in the south of Great Britain. Fa, Lp, Fp, Lm, Lh and Fl were sown in the spring of 1989 in three replicates. The yield was measured from 1989 till 1993 with a fertilisation of 450 kg N $ha^{-1}yr^{-1}$ and a cutting interval of *circa* 5 weeks. In 1993, the trial was stopped after the second cut.

Luten and Remmelink (1984) focused on the animal performance of animals fed with Fa, Lp and Lm. Different intake experiments, in which cut grass was presented to dairy cows, were conducted from 1974 till 1978. The quality of the grass, the grass DM intake and the milk production of the cows fed with the different grass species were measured. In all years except 1977 grass of the different species presented to the cows was harvested at the same height. This was possible by altering cutting and fertilisation regime so that grass in different growth stages for each species was available at any moment. In 1977, the intake of Fa cut at either 20 cm or 30 cm height was compared with that of Lp cut at 25 cm height. The experiments followed a switch back scheme: at the beginning of the experimental period, 24 lactating cows were randomly attributed to one of the two grass species. In the middle of the experimental period the grass species were switched, so that in each period, each cow was eating both grass species. Individual grass intake and milk production were recorded. Quality parameters of the feed were measured on regular intervals.

In 1977 and 1978, also animal trials with conserved grass were conducted. In 1977 Fa and Lp were mown when grass height had reached approximately 30 cm during spring or 20 cm during summer. The grass was prewilted to a dry matter content of *circa* 50 %. Intake trials followed again a switch back scheme with 24 cows. The trials lasted 3 successive weeks; silage intake and milk production were measured per cow. In 1978 the trial was repeated, the grass was cut at approximately 40 cm in the first cut, and at approximately 25 cm in the 2 summer cuts.

Other, unpublished, results were obtained both from public and private trials. Some of these trials were designed to compare different species in the same trial (**Table 2.2**), whereas other trials were (adjacent) variety trials of different species (**Table 2.3**). In the former type of trials statistical comparison between Fa and other species was possible; whereas in the latter type of trials comparison between the species was not possible from a statistical point of view. Only if the management for the different species was equal and if both trials were on the same field, the results of these variety trials were taken into account.

Table 2.2 Unpublished studies that allow comparison of the performance between *Festuca arundinacea* (Fa) and other cool season grass species: Lp: *Lolium perenne*, Lp2: diploid Lp, Lp4: tetraploid Lp, Dg: *Dactylis glomerata*, Fp: *Festuca pratensis*, Pp: *Phleum pratense*, Lm: *Lolium multiflorum*, Fl: *Festulolium*, Lh: *Lolium x boucheanum*, Tr: *Trifolium repens*, Tp: *Trifolium pratense*; DMY: dry matter yield, DMC: dry matter content, DOM: digestibility of the organic matter; DMI: dry matter intake.

					Management	
Name of trial Location(s) of study	Period	Species (number of varieties)	Parameters	Soil type	N fertilisation (kg ha^{-1} yr^{-1})	Nr. cuts (yr^{-1})
ILVO_1 Merelbeke, Belgium	2011°^{a} 2012	Fa (4), Lp (4), Fl(4), Fp (2), Lh (2)	DMY	Sandy loam	300 and 150	5 5
ILVO_2 Merelbeke, Belgium	2011°^{s} 2012	Fa (2), Lp (1), Fl (2) with and without Tr+Tp	DMY, DMC	Sandy loam	Grass : 300 Grass-clover : 179	5 5
RvP Merelbeke, Belgium	1980°^{a} 1981-1984	Fa, Lp, Fp, Pp	DMY, DOM, DMI	Sandy loam	400	3-4

° sowing year a: autumn s: spring

Table 2.3 Variety trials in which *Festuca arundinacea* (Fa) varieties were grown adjacent to varieties of other cool season grass species: Lp: *Lolium perenne*, Lp2: diploid Lp, Lp4: tetraploid Lp, Dg: *Dactylis glomerata*, Fp: *Festuca pratensis*; DMY: dry matter yield, DMC: dry matter content.

					Management	
Name of trial Location(s) of study	Period	Species (number of varieties)	Parameters	Soil type	N fertilisation ($kg\ ha^{-1}\ yr^{-1}$)	Nr. cuts (yr^{-1})
FR	$2008^{\circ s}$	Fa (11), Lp (11), Dg (11), Fp (11)	DMY, DMC	Loam	250	4
Orchies, France	2009					
FM	Fa :$2008^{\circ s}$	Fa (11)	DMY	Stony loam	180	3
Michamps, Belgium	2010-2011	Lp2 (9)				4 cuts for Lp in 2011
	Lp : $2009^{\circ s}$	Lp4 (8)				
	2010-2011					
VCU trial	$2009^{\circ s\ a}$ and	Fa (4)	DMY		Each location:	Each location:
Poperinge, Belgium	$2010^{\circ s\ b}$	Lp4 early (3)		Loam	300 in 2011;	5 cuts in 2011;
Merelbeke Belgium	2011-2012	Lp4 intermediate(3)		Sandy loam	350 in 2012	4 cuts in 2012
		Lp4 late (3)				
		Lp2 late (5)				

° sowing year a: autumn s: spring; a: cycle 1; b: cycle 2 (see Figure 2.1 for clarification)

In the autumn of 2011, a trial comparing the DMY of different varieties of Fa, Lp, Fp, Fl, Lh was established at the institute for agricultural and fisheries research (ILVO) in Belgium. This trial will be named “ILVO_1”. The trial was a split plot design with three replications. N fertilisation was the main plot factor (High N or Low N: 300 and 190 kg ha^{-1}yr^{-1} respectively), varieties of the different species were the subplot factor. The trial included four varieties per species for Fa, Fl, and Lp and two varieties per species for Fp and Lh, the trial included also nine breeding populations of Fa, results of this material are not reported here. Five cuts were harvested in 2012.

In the spring of 2011 a trial comparing the yield of single grass species and the same species in combination with clover was established at ILVO in Belgium. This trial will be identified as “ILVO_2”. The grass species in the trial were 2 Fa varieties, one Lp variety and 2 Fl varieties sown as pure grass or sown in combination with a mixture of white and red clover. The trial design was a split plot design with the presence of the clover as main plot factor, and the varieties of the different grass species as subplot factor. Five cuts were harvested in 2012. N fertilisation was 300 kg ha^{-1}yr^{-1} for the pure grass plots and 179 kg ha^{-1}yr^{-1} for the grass clover plots.

In the autumn of 1980 a yield trial was established at the former “RvP” (now ILVO) to compare the yield of Fa, Lp, Pp and Fp under a cutting regime from 1981 till 1984. This trial will be identified as “RvP”. The summer of the year 1982 was dry. The species were not cut at the same dates, but when they reached a certain yield level. Fp and Pp were not followed any more after 1982 as the swards of both species were decimated after two harvest years. N fertilisation was high: 400 kg N ha^{-1}yr^{-1}. In 1981 the harvested forage was hayed, DOM and DMI of the hays by young bulls were compared.

Results from variety trials of a private breeding company in the North of France were available for the species Fa, Lp, Dg and Fp. This trial will be identified as “FR”. The variety trials were established on the same field in the spring of 2008, in randomized complete blocks with three replicates per species. Four cuts were harvested in 2009. In this chapter, the results of eleven randomly chosen varieties per species are compared. This number corresponded to the number of registered Fa and Lp varieties in the trials.

Results of variety trials from “fourrages mieux”, a non governmental organisation bringing forage science into practise in the south of Belgium, were available for Fa and Lp. This trial will be identified as “FM”. The Fa variety trial included 11 varieties, the Lp variety trial included 9 diploid varieties and 8 tetraploid varieties. The former trial was sown in the spring of 2008, whereas the latter was sown in the spring of 2009. In order to tackle these different sowing dates, only the yields for the years 2010 and 2011 were taken into consideration.

Additional results were obtained from the official VCU (value for cultivation and use) trials for Lp and Fa in Belgium. In these VCU trials two identical trials are sown in the spring of two successive years. These two trials are called cycle 1 and cycle 2 respectively. In both cycles, no observations are done in the sowing year. In cycle 1, yield is measured for three years after the sowing year, and in cycle 2, yield is measured for two years after the sowing year (**Figure 2.1**). Both trials are stopped at the same moment.

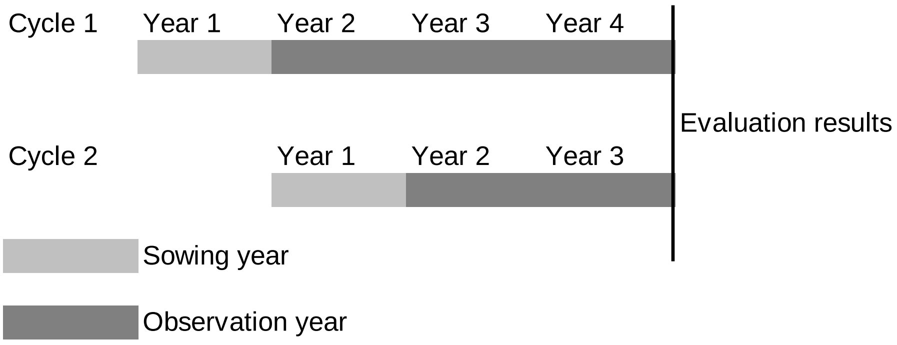


Figure 2.1 Scheme representing VCU trials for forage grasses in Belgium.

Results for both Fa and Lp were available for 2009-2012. Cycle 1 was sown in the spring of 2009, cycle 2 was sown in the spring of 2010. Both trials were conducted on four locations, but only the yield from two locations (Merelbeke and Poperinge) was taken into consideration since in the trials on these locations Fa and Lp were adjacent on the same field and had the same management (same cutting dates and fertilisation). The Fa variety trial included three reference varieties and one candidate variety. The Lp VCU trial included one candidate variety and two reference varieties in the categories early, intermediate and late tetraploid Lp and three reference varieties and two candidate varieties in the category late diploid Lp (Pannecoucque, 2012; Pannecoucque, 2013).

The DMY results from established swards growing under favourable climatic conditions reported in the above mentioned studies, were compared. To eliminate the effect of the differences in establishment speed between Fa and Lp, results from the sowing year in case of spring sowing or first year after sowing in case of autumn sowing are commented in a separate section. To eliminate the difference in drought resistance between the species, results from year in which “dry” periods were reported, are commented in a separate section.

To eliminate the effect of seasonal differences in growth rate, only yield results for entire years were taken into account.

In trials where data for all replicates were available, analysis of variance was performed to study the effect of grass species on the DMY. Full data were available for the trials ILVO_1 and ILVO_2. Analyses of variance were performed in R (R core development team, 2011) using the *aov()* function.

2.3 Results and Discussion

2.3.1 DMY of established swards under favourable conditions

Table 2.4 gives an overview of results from studies where conditions were favourable for grass growth *i.e.* no reported drought stress, high N input.

In the high N level of the study of Baert *et al.* (2012), tall fescue yielded 20 % more relative to the average yield of an intermediate and an early Lp variety. The highest yield averaged over three years was obtained with Pp. No significant differences between the grass species were found.

In the study of Van Eekeren *et al.* (2010) on a sandy soil, both Fa as Dg yielded significantly more than Lp. The yield of Fa was 11 % higher than that of Lp.

In the study of Gilliland *et al.* (2010) at the highest N input and averaged over three years, Lp4 was the highest yielding species in the central and North site and Dg was the highest yielding species in the South site. Averaged over the three sites however, Fa was the highest yielding species. The yield of Fa (13.2 t ha^{-1}) was significantly higher than that of Fp (11.8 t ha^{-1}), Dg (12.3 t ha^{-1}) and Lp2 (11.8 t ha^{-1}). The yield of Pp (12.7 t ha^{-1}) and Lp4 (12.9 t ha^{-1}) were on the same level as Fa. The clover content in the harvested material was very low (< 8 %) as a result of the high N fertilisation, so the presence of the clover could only have had a minor influence on the total yield.

The study of Pontes *et al.* (2007) presented DM yields averaged over two years, two N levels and two cutting frequencies. Fa and Dg were the two most productive species, with yields that were *circa* 40 % higher than those of Lp. Pontes *et al.* (2007) pointed that the effect of a high N fertilisation was highest for Fa and Dg, but that these species had a significant reduction in DMY when cutting frequency was increased from three to six cuts per year, whereas that of Lp increased slightly under high cutting frequency.

In Wilmann and Gao (1996), Fa was yielding less than Lp (17480 *versus* 19370 kg DM ha^{-1} respectively) in 1990, the first year after sowing. In the second year, the yields were on the same level (19970 *versus* 19260 kg DM ha^{-1}) and in the third year, Fa was yielding more than Fa (18310 *versus* 17970 kg DM ha^{-1}). Averaged over the three years, Lp was the highest yielding species, Fa the second highest yielding species. No statistics were performed on the data of this study.

In the ILVO_2 trial, Fa was overyielding Lp with 20 %, (p = 0.008). There was an important yield difference between the two Fl varieties. ‘Hykor’ had a yield close to that of Fa whereas the yield of ‘Lifema’ was below that of Lp. ‘Hykor’ is genetically very close to Fa, as it was created by introgression of Lm genes into the Fa genome. ‘Lifema’ on the other hand is an amphiploid *Festulolium* cultivar (Lm x Fp): the complete genomes of the two species were incorporated into a single genotype (Ghesquière *et al.*, 2010, Thomas *et al.*, 2003).

In the FR trial, Fa was yielding 30 % more than Lp, the yield of Dg and Fp was similar and between that of Fa and Lp. Similar results were found in the FM trial: a 34 % higher yield for Fa compared to Lp averaged over 2 years.

In the RvP trial, in 1981 and 1983, Fa was on average 14 % more productive than Lp.

In the VCU trial, in both cycles and on both locations, Fa was overyielding Lp. The smallest difference between Fa and Lp was found in Merelbeke in the cycle 1 trial in 2011, where yield of Fa (18946 kg DM ha^{-1} yr^{-1}) was only 2 % higher than that of Lp (18541 kg DM ha^{-1} yr^{-1}). The largest difference was found in Poperinge in the cycle 1 trial in 2012, where the yield of Fa (20987 kg DM ha^{-1} yr^{-1}) was 62 % higher than that of Lp (12987 kg DM ha^{-1} yr^{-1}). Averaged over all varieties, cycles, locations and years, Fa had a yield of 19469 kg DM ha^{-1} yr^{-1}and Lp a yield of 14715 kg DM ha^{-1}, resulting in a 32 % higher yield of Fa compared to Lp.

Table 2.4 Dry matter yields of Fa: *Festuca arundinacea*, Lp: *Lolium perenne*, Lp2: diploid Lp, Lp4: tetraploid Lp, Dg: *Dactylis glomerata*, Fp: *Festuca pratensis*, Pp: *Phleum pratense*, Lm: *Lolium multiflorum*, Fl: *Festulolium*, Lh: *Lolium x boucheanum* in different North-West European studies or trials.

Author/Trial	Yields (kg DM ha^{-1}yr^{-1})	DMY Fa/Lp (x 100)
Baert *et al.* (2012) Average 2008-2010 3 cuts yr^{-1}; 250 kg N ha^{-1}yr^{-1}	Fa: 16283 Lp:13540 Pp:17853 Dg:16995	120.3 (NS)[a]
Van Eekeren *et al.* (2010) Average 2007-2008 4-5 cuts yr^{-1} 280 kg N ha^{-1}yr^{-1} (mineral + organic)	Fa: 15340 Lp: 13830 Dg: 15690	110.9 (S)
Gilliland *et al.* (2010) Average 2007-2009 and over three sites 10 cuts yr^{-1}; 420 kg N ha^{-1}yr^{-1}	Fa: 13200 Lp2: 11800 Lp4: 12900 Dg: 12300 Fp: 11800 Pp: 12700	111.9 (Fa/Lp2) (S)
Pontes *et al.* (2007) Average 2003-2004 and over N-levels and cutting frequency	Fa ecotype: 12820 Lp ecotype: 8330 Pp ecotype: 8820 Dg ecotype: 12830 Lp variety: 9040	153.9 (Fa/Lp ecoptype) (NA) 141.8 (Fa/Lp variety) (NA)
Wilmann *et al.* (1996) Average 1990-1992 450 kg N ha^{-1}yr^{-1} 7 cuts yr^{-1}	Fa: 18350 Lp: 19103 Fp: 16857 Lh: 17803 Fl: 17610	96.1 (NA)
ILVO_2 Average varieties Year 2012 300 N ha^{-1}yr^{-1}; 5 cuts yr^{-1}	Fa: 16113 Lp: 13301 Fl: 13962	120.5 (S)

Table 2.4 continued.

Author/Trial	Yields (kg DM $ha^{-1}yr^{-1}$)	DMY Fa/Lp (x 100)
FR Average varieties Year 2009 250 N $ha^{-1}yr^{-1}$; 4 cuts yr^{-1}	Fa: 12948 Lp: 9932 Fp: 11902 Dg: 11645	130.4 (NA)
FM Average varieties Average 2010-2011 180 N $ha^{-1}yr^{-1}$; 3 cuts yr^{-1}	Fa: 10069 Lp: 7509	134.1 (NA)
RvP Average 1981 and 1983 400 N $ha^{-1}yr^{-1}$; 4 cuts yr^{-1}	Fa: 13545 Lp: 11855	114.3 (NA)
VCU Average varieties Average locations Average 2011 and 2012 Average cycle 1 and 2	Fa: 19470 Lp: 14715	132.3 (NA)
MEAN		122.5 (S)[b]

[a] NS: no significant difference between Fa and Lp; S: significant difference between Fa and Lp; NA: no statistics available

[b] One sample t-test (*alpha* = 5 %)

Over all the trials, Fa was on average 23 % higher yielding than Lp2. The ratio of the DMY of Fa and Lp (x 100) proved to be significantly higher than 100 (p <0.001). The largest difference between Fa and Lp was found in Pontes *et al.* (2007) in France, the smallest in Wilman and Gao (1996) in the UK, where yield of Lp was slightly higher than that of Fa. The climate on both sites probably accounts for most of this difference. In contrast to the semi-continental climate in the Auvergne region where the former experiment took place, Wales, where the latter experiment took place, has a climate that is considered as ideal for the growth of cool season grasses. Average annual rainfall in Wales is over 1000 mm (Metoffice, 2013) compared to 760 mm in the Auvergne (Pontes *et al.*, 2007). Moreover the temperatures are milder in Wales throughout the season. Apparently Fa is intrinsically higher yielding in most growing circumstances present in North-West Europe, but Lp can yield as much as Fa under very favourable circumstances for grass growth. The yield potential of Dg was on the same level of that of Fa.

2.3.2 DMY of young, establishing swards

Table 2.5 gives an overview of the yield of Fa and Lp during the establishment period. Yield is given for the sowing year (y_0) and for the first year after sowing (y_{1s}) in the case of spring sowing and for the first year after sowing in the case of autumn sowing (y_{1a}).

In the case of autumn sowing the DMY of Fa in the first year after sowing was around 8 % lower than that of Lp in the ILVO_1 trial (p = 0.008). In the RvP trial on the other hand, Fa was overyielding Lp with 15 % in the first year. The difference between both trials may be attributed to sowing date: the sowing date of the ILVO_1 trial was 15 September, which is seen as a limit date for the successful establishment of Fa swards, whereas the RvP trial was sown in August 1980 (exact date unknown).

In the case of spring sowing the DMY of Fa was lower than that of Lp in the sowing year, but the difference was not significant (p = 0.47). Fa produced 2 % and 14 % less in the sowing year in the ILVO_2 trial and in Wilman and Gao (1996) respectively. In the first year after the sowing year (y_{1s}), Fa was yielding 20 % more than Lp in the ILVO_2 trial (p = 0.008), which is close to what we found as average difference between established swards Fa and Lp2. In Wilmann and Gao (1996) DMY of Fa remained lower than that of Lp in y_1, but the yield difference between both species was lower in y_1 compared to y_0. Reasons for the low yield of Fa compared to Lp in this trial were discussed above.

It is important to note that it is difficult to quantify the annual yield in y_0 after spring sowing, as the yield of the first cut(s) is generally biased by the presence of weeds, and thus not recorded. This was certainly the case in the ILVO_2 trial. Whether the complete DMY is included in the trial of Wilmann and Gao (1996) is not clear. In both trials, yield of Fa was below that of Lp in y_0.

Table 2.5 Dry matter yields (DMY) of establishing swards of Fa: *Festuca arundinacea*, Lp: *Lolium perenne*, Lp2: diploid Lp, Lp4: tetraploid Lp, Dg: *Dactylis glomerata*, Fp: *Festuca pratensis*, Pp: *Phleum pratense*, Lm: *Lolium multiflorum*, Fl: *Festulolium*, Lh: *Lolium x boucheanum* in different North-West European studies or trials in the sowing year (y_0) or in the first year after sowing (y_1).

Author/Trial	Yield (kg DM $ha^{-1}yr^{-1}$)		DMY Fa/Lp (x 100)	
	y_0	y_1	y_0	y_1
Autumn Sowing				
ILVO_1 Average varieties 5 cuts yr^{-1}; 300 kg N $ha^{-1}yr^{-1}$	/	Fa: 14884 Lp: 16255 Fl: 17042 Fp: 12728 Lh: 18060		91.6 (S)[a]
RvP 4 cuts yr^{-1}; 400 kg N $ha^{-1}yr^{-1}$	/	Fa: 13880 Lp: 12070		115.0 (NA)
Spring Sowing				
ILVO_2 Average varieties 3 cuts yr^{-1} (y_0) 5 cuts yr^{-1} (y_1) 300 kg N/ha	Fa: 8101 Lp: 8242 Fl: 7778	Fa: 16113 Lp: 13301 Fl: 13962	98.3 (NS)	120.5 (S)
Wilman and Gao (1996) 450 kg N $ha^{-1}yr^{-1}$ 7 cuts yr^{-1}	Fa: 10390 Lp: 12030 Fp: 10310 Lh: 11630 Fl: 11120	Fa: 17480 Lp: 19370 Fp: 16200 Lh: 17250 Fl: 16830	86.4 (NA)	90.2 (NA)

[a] NS: no significant difference between Fa and Lp; S: significant difference between Fa and Lp ($p < 0.05$); NA: no statistics available

Figure 2.2 presents the distribution of the DMY after autumn (ILVO_1 trial) and spring sowing (ILVO_2 trial) in detail. Both trials took place at the same institute (same soil type) and had a similar management (cutting frequency and N-fertilisation). For both trials, the first year after sowing (y_1) was 2012. In the first three cuts of the autumn sown trial (**Figure 2.2a**), Fa was clearly overyielded by Lp, but the difference between Fa and Lp decreased with every cut and from the fourth cut on, Fa was more productive than Lp. Differences were significant in each cut ($p < 0.01$).

In y_0 of the spring sown trial (**Figure 2.2b**), the first cut was not measured due to the presence of weeds. In the second cut, Fa was largely overyielded by Lp ($p = 0.003$), but from the third cut on, Fa overyielded Lp (non significantly). In the first year after sowing (y_1), Fa overyielded Lp in every cut except the second. Differences between Fa and Lp were significant in cut 4 ($p = 0.001$), cut 5 ($p = 0.005$), cut 6 ($p = 0.015$), cut 8 ($p = 0.003$) and cut 9 ($p = 0.010$).

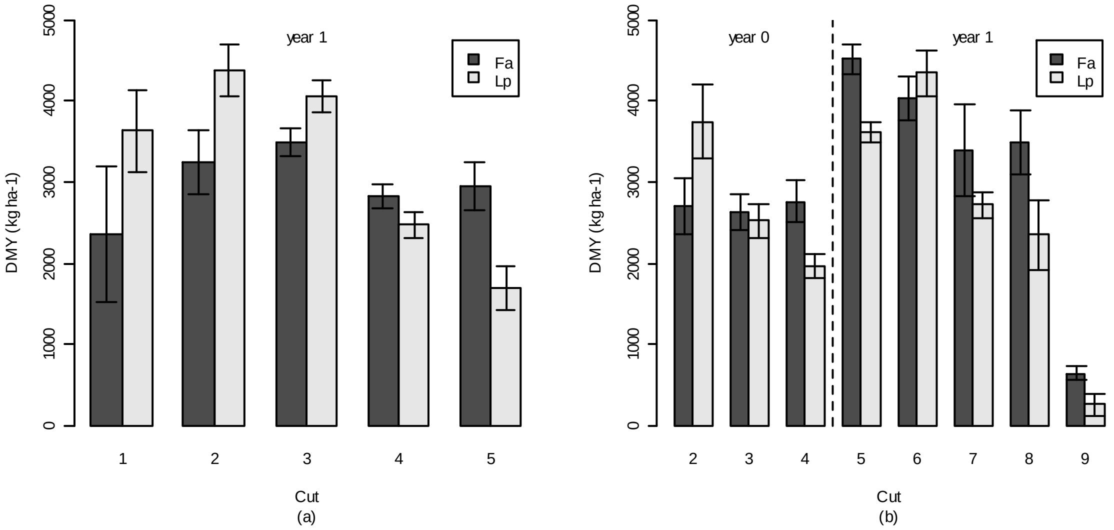


Figure 2.2 Dry matter yield (DMY) of *Festuca arundinacea* (Fa) and *Lolium perenne* (Lp) in the first year after autumn sowing in the ILVO_1 trial (a) and in the sowing year (year 0; cuts 2-4) and the first year after spring sowing (year 1; cuts 5-9) in the ILVO_2 trial (b). Error bars: ± standard deviation.

With the few available results, it was difficult to quantify the exact yield of Fa compared to other species during establishment. ILVO trial 1 and 2 allowed the best comparison of the yield of establishing Fa and Lp swards under spring and autumn sowing: both trials were conducted in the same time period, on the same soil type under a similar management. We can conclude that under the conditions of these trials the yield of Fa is 5-10 % below that of Lp in the first production year, both after spring as after autumn sowing. The year after the sowing year in the case of spring sowing, Fa has its full yield potential.

2.3.3 DMY under conditions of drought

Table 2.6 gives an overview of the DMY of Fa and other grass species in studies were drought stress occurred.

In Lusignan, France where the study of Surrault *et al.* (2006) was conducted, drought occurred in the year 2005 with only 427 mm of rain in the whole year instead of 812 mm in a normal year. Between February and June 2005, the deficit in rainfall amounted to 100 mm. With only 87 mm of rainfall over the months August, September and October, no more grass could be harvested after July 2005, resulting in only 3 cuts in the plots with the slow cutting regime (every 45 days). Fa and Dg had a comparable yield under the drought conditions; both species yielded *circa* 65 % more than Lp. The negative effect of the drought, seemed to persist in the following year for Lp: yield of Lp in 2006 was close to that of 2005 and far below the yield in 2004, while the growing conditions in 2006 were better than those in 2004. For Fa and Dg the yield in 2006 was on the same level as in 2004, the year before the drought.

In Merelbeke, Belgium, were the RvP trial took place, drought occurred in July 1982: with only 13 mm of rainfall instead of 76 mm normally. Due to this drought period, only three Lp cuts could be harvested, whereas four Fa cuts were harvested. This resulted in an annual yield for Fa that was 28 % higher than that of Lp, whereas in 1981 and 1983 four cuts were harvested for Lp, resulting in 10-15 % higher yield for Fa compared to Lp.

Wilman *et al.* (1998) tested eight forage grass species under severe drought in Wales, UK. Drought was imposed by restricting rain water access with transparent covers over the field plots. Italian ryegrass died out after 15 months, meadow fescue and perennial ryegrass after 2 years of rain exclusion, but tall fescue was still alive after four years of rain exclusion. The better drought resistance of Fa was explained by its greater number and biomass of roots compared to the other species in the 50–100 cm soil layer. Two months after the rain exclusion was installed, the number of roots was counted in a 1 m deep and 30cm wide trench dug along the grass swards. Fa had twice as much roots in the 50-100 cm profile compared to Lp. The higher root biomass and deeper rooting of Fa was confirmed by other authors (Van Eekeren, 2010).

Table 2.6 Dry matter yields (DMY) of swards of Fa: *Festuca arundinacea*, Lp: *Lolium perenne* and Dg: *Dactylis glomerata* under drought stress in different North-West European studies. $y_{drought}$: year in which drought occurred.

Author/Trial	Yield (kg DM $ha^{-1}yr^{-1}$)			DMY Fa/Lp (x 100)		
	$y_{drought}$-1	$y_{drought}$	$y_{drought}$+1	$y_{drought}$-1	$y_{drought}$	$y_{drought}$+1
Surrault *et al.* 2007	Fa:12100	Fa:10700	Fa:13000	106.1	164.6	156.6
180 kg N $ha^{-1}yr^{-1}$	Lp:11400	Lp:7500	Lp:8300	(NA)	(S)	(NA)
3 cuts $year^{-1}$	Dg:12300	Dg:11100	Dg:12400			
RvP	Fa:13880	Fa:11600	Fa:13210	114.9	127.9	113.5
400 N $ha^{-1}yr^{-1}$	Lp:12070	Lp:9070	Lp:11640	(NA)	(NA)	(NA)
4 cuts yr^{-1}						

[a] NS: no significant difference between Fa and Lp; S: significant difference between Fa and Lp ($p < 0.05$); NA: no statistics available

Although many of the important dairy regions in Europe are on sandy soils, data form trials on sandy soil are lacking. Sandy soils are very susceptible to drought, but it is not clear whether Fa has a good drought resistance on sandy soils and whether Fa can exploit the advantage of its deeper root system on sandy soils. Ecotypes of Fa are found on all types of soil, but are scarce on sandy soils (Gibson and Newman, 2001; own observations) which might be another indication that Fa might lose its competitive advantages on sandy soils.

Research results of Turner *et al.* (2012) however suggest that drought resistance of tall fescue is not only due its deeper rooting capacity. They compared the morphological and physiological responses of Lp, Fa and Dg under optimal and suboptimal irrigation regimes in a pot trial (root depth limited to 28 cm) under controlled environment. The irrigation treatments involved applications of 133, 100, 66 and 33 % of the replacement water required to maintain soil water potential at -10kPa. Tall fescue appeared to have the greatest scope under moisture stress in terms of maintaining productivity and persistence. Leaf DMY was consistently in the order Fa > Lp > Dg. The relative reduction in leaf DMY compared to the 100 % water treatment was 78 % and 35 % for Lp in the 66 % and 33 % irrigation treatments respectively. For Fa, the reduction was 66 and 32 % in the 66 % and 33 % irrigation treatments respectively. Dg showed the smallest relative reduction in leaf DMY because of moisture stress: 52 % and 22 % in the 66 % and 33 % irrigation treatments respectively, but Dg had a leaf DMY that was consistently lower than that of Fa. In a recovery period after the trial, with sufficient water supply to allow optimal growth, relative reduction of leaf DMY of plants that were previously growing under water stress compared to plants that were fully watered throughout the experiment was 58 % and 50 % for Lp in the 66 % and 33 % irrigation treatments respectively. For Fa and Dg, reduction was from the same order of magnitude for both species and smaller compared to Lp: 33 % and 32 % for Fa and 35 % and 29 % for Dg in the 66 % and 33 % irrigation treatments respectively. The low DMY of Lp in the recovery period was a result of the higher relative loss of daughter tillers in the plants subjected to drought in Lp compared to Fa and Dg (Turner *et al.*, 2012).

We can conclude that the yield advantage of Fa compared to Lp under drought depended on the severity of the drought. Under severe drought, like in the trial of Surrault *et al.* (2006), Fa yielded 65 % more than Lp on a yearly basis.

2.3.4 Digestibility and intake by animals

Table 2.7 gives an overview of studies in which the digestibility of the organic matter (DOM) of Fa and other grass species is compared. The method used to determine the DOM differed between the studies (**Table 2.7**).

In Baert *et al.* (2012), averaged over three years, Fa had a digestibility that was 7 % points lower than that of Lp. This lower digestibility was largely compensated by a higher DMY of Fa compared to Lp, resulting in a significantly higher digestible organic matter yield for Fa compared to Lp. Also in Pontes *et al.* (2007), Fa is among the species with the highest digestible organic matter yield. This higher digestible organic matter yield makes Fa an interesting species for biogas production, it has however only limited value for animal production as animal intake and performance is largely influenced by intrinsic digestibility.

In Pontes *et al.* (2007) the digestibility of a Fa ecotype relative to that of a Lp ecotype varied between 83 % in spring and 93 % autumn; obviously the difference between the Fa ecotype and the single Lp variety was larger (**Table 2.7**).

In the experiments of Luten and Remmelink (1984), the digestibility of the grass was determined only in the years 1977 and 1978. In 1978, grass of a similar height was harvested for both species for every intake trial. This was achieved by applying different cutting frequencies and N fertilisations on both species. Averaged over the 20 harvest dates in 1978, the DOM of Fa was 3.6 % point lower than that of Lp.

In 1977 Luten and Remmelink (1984) compared the digestibility and intake of Fa and Lp with a different growth stage. Long Fa (cut at approximately 30 cm) or short Fa (approximately 20 cm height) were compared with Lp cut at approximately 25 cm height (**Table 2.8**). The short Fa had a digestibility that was slightly higher than that of Lp, whereas that of the long Fa was lower. This indicates that an appropriate cutting management can minimize the difference in digestibility between Fa and Lp, but this could decrease the DMY advantage of Fa (Pontes *et al.*, 2007).

Table 2.7 Digestibility of the organic matter (DOM) of cut Fa: *Festuca arundinacea*, Lp: *Lolium perenne*, Lp2: diploid Lp, Lp4: tetraploid Lp, Dg: *Dactylis glomerata*, Fp: *Festuca pratensis* and Pp: *Phleum pratense* in different North-West European studies or trials.

Author/Trial/Method	DOM (%)	DOM Fa/Lp (x 100)
Baert *et al.* (2012)	Fa: 65.6	
Average 2008-2010	Lp: 72.8	90.0
3 cuts yr^{-1}; 250 kg N ha^{-1}yr^{-1}	Pp: 61.7	
in vitro pepsin-cellulase	Dg: 63.8	
Pontes *et al.* (2007)	Fa ecotype^{a}: 62.6, 65.7, 67.5	
Average 2003-2004 and over N-levels and cutting frequencies	Lp ecotype^{a}: 75.7 , 70.7, 76.3	82.7, 92.9, 88.5
	Pp ecotype^{a}: 76.3, 74.9, 77.1	
spring, summer, autumn	Dg ecotype^{a}: 67.3, 67.4, 70.2	
in vitro pepsin-cellulase	Lp variety^{a}: 80.4, 70.7, 79.0^{c}	77.9, 92.9, 85.4
Luten and Remmelink (1982)	Fa: 73.7	
Results of 1978	Lp: 77.3	95.3
Average over 20 harvest dates		
Cut at same length		
method not specified		
RvP	Fa^{b}: 71.2, 70.7, 67.1	
Year 1981; Hay	Lp^{b}: 63.5, 72.1, 72.3	112.1, 98.1, 92.8
400 N ha^{-1}yr^{-1}	Fp^{b}: 63.5, 72.1, 72.3	
4 cuts yr^{-1}, cut 1-3		
in vivo with fistulated sheep		
Range		77.9 - 112.1

a: values for spring, summer and autumn respectively
b: values for first, second and third cut respectively

Although the management in the RvP trial was similar to that of the other trials, the digestibility of Fa hay in this trial was very close to that of the Lp hay. The hay making however biased the results. The first cut hay of both species was not made at the same moment, resulting in a quality difference in the hay of both species. The field period to reach a sufficiently high DMC was longer for Lp compared to Fa, which had a negative impact on the digestibility of the Lp hay.

The DMI (dry matter intake) and milk production measured in the trials with cut fresh grass performed by Luten and Remmelink (1984) are summarized in **table 2.9**. Averaged over the different trials in the different years, DMI of Fa compared to Lp was 1 kg lower when cut at the same height, resulting in a milk production that was 1.4 kg lower for Fa. The differences in intake and milk production were generally decreasing through the season.

In 1977 long Fa (cut at approximately 40 cm) or short Fa (approximately 20 cm height) were compared with Lp cut at approximately 25 cm height (**Table 2.8**). The DMI of Lp in 1977 was lower than both the short as the long Fa. The milk production with Lp was in between that of the short Fa and the long Fa. The relatively small differences in intake and DOM reflects the longevity of the Fa leaves as indicated by Lemaire *et al.* (2009): leaves of Fa had an average life span of 38 d, whereas Lp leaves had an average life span of only 22 days at an average daily air temperature of 15°C.

Table 2.8 Average dry matter intake (DMI), milk production and digestibility of the organic matter (DOM) of cows fed with *Festuca arundinacea* (Fa) either cut at approximately 40 cm (long) or cut at approximately 20 cm (short) or with *Lolium perenne* (Lp) cut at approximately 25 cm in the trials of Luten and Remmelink (1984) in 1978.

	Fa short	Fa long	Lp
Average length (cm)	28	47	30
DOM (%)	75.3	73.9	75.0
DMI (kg day^{-1} cow^{-1})	13.2	13.5	12.8
Milk production (kg day^{-1} cow^{-1})	13.0	10.2	12.6

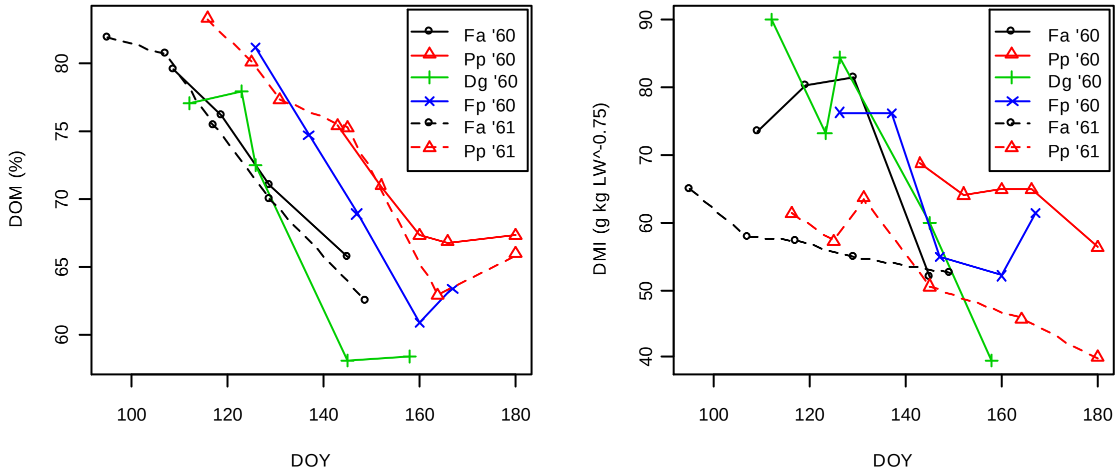
Table 2.9 Dry matter intake (DMI) and milk productions of cows fed with *Festuca arundinacea* (Fa), or *Lolium perenne* (Lp) in the trials of Luten and Remmelink (1984) averaged over the years 1975, 1976 and 1978.

	DMI (kg day^{-1} cow^{-1})			Milk production (kg day^{-1} cow^{-1})		
	Fa	Lp	Fa/Lp (x 100)	Fa	Lp	Fa/Lp (x 100)
Average 1975, 1976, 1978	13.7	14.7	0.93	17.3	18.7	0.93

The evolution through the time of the digestibility and the DMI of different grass species was tested by Minson *et al.* (1961) in Hurley, UK. Fa, Pp, Dg, and Fp were sown in the spring of

1959. In 1960 and 1961, grass was harvested at regular intervals between the start of the growth and ear emergence and fed freshly to sheep. In 1961, only Fa and Pp were harvested, as the plots of the remaining species contained too much unsown species. The apparent digestibility of the dry and organic matter in each cut was calculated from the weights of the consumed DM or OM and the corresponding faecal output. The evolution of the DOM and the corresponding DMI are shown in **figure 2.3a** and **figure 2.3b** respectively.

Growth of Fa and Dg started earlier than that of Pp and Fp. The digestibility decreased with approximately around 0.4 units day^{-1} for Fa and Pp and around 0.5 units day^{-1} for Dg and Fp from the first harvest date on. Both in 1960 and 1961, the DOM was around 8 % points lower for Fa compared to Pp at the same moment. The digestibility of Dg fell very fast after the emergence of the first ears (on day 114). In 1960, intake differences between Fa, Dg and Fp were small. The DMI for Fa and Pp were much lower in 1961 compared to 1960. No explanation could be found for this difference by the authors.



(a) (b)

Figure 2.3 Evolution of the digestibility of the organic matter (DOM) (left) and the dry matter intake (DMI) of cut *Festuca arundinacea* (Fa), *Festuca pratensis* (Fp), *Phleum pratense* (Pp), *Dactylis glomerata* (Dg) over time (DOY: day of the year) (Redrafted after Minson *et al.* (1961)).

Intake and milk production of cows fed with either Fa or Lp differed less when the grass was ensiled (**Table 2.10**). Luten and Remmelink (1984) found a higher intake and corresponding milk production with Fa compared to Lp in 1977. In 1978, intake was higher for Lp. It was not clear why the milk production with the long Fa was higher than that of Lp, given that the intake and the quality of the Lp were higher compared to Fa.

Also when hayed, the intake of Fa seems to be relatively good. In the RvP trial, Fa and Lp hay from the first cut or from the second + third cut were presented to young bulls. Intake and growth was better for Fa compared to Lp with the first cut hay (**Table 2.11**). In the second and third cut intake and growth was better with the Lp hay. It is however important to note that in the first cut, the drying period of Lp was longer compared to Fa, which might have had a negative influence on the quality of Lp.

Table 2.10 Average dry matter intake (DMI) and milk production of dairy cows fed with silage of *Festuca arundinacea* (Fa) or *Lolium perenne* (Lp) cut at different heights in the trials of Luten and Remmelink (1984).

Year		Cutting height (cm)	DMI (kg day^{-1} cow^{-1})	Milk production (kg day^{-1} cow^{-1})
1977	Lp	20	11.8	10.6
	Fa	20	12.3	10.7
	Fa/Lp (x 100)		104.2	101.0
	Lp	30	10.8	8.4
	Fa	30	11.5	9.1
	Fa/Lp (x 100)		106.5	108.3
1978	Lp	25	12.7	8.8
	Fa	25	11.4	8.1
	Fa/Lp (x 100)		89.8	92.0
	Lp	40	12.4	9.0
	Fa	40	11.1	9.4
	Fa/Lp (x 100)		89.5	104.4

Table 2.11 Average dry matter intake (DMI) and growth of young bulls fed with hay of *Festuca arundinacea* (Fa) or *Lolium perenne* (Lp) made from different cuts in the year 1981 in the RvP trial.

		DMI (g DM day^{-1} kgLW$^{-0.75}$)	Growth (kg day^{-1} bull^{-1})
1st cut hay	Lp	43.2	0.75
	Fa	57.4	1.04
	Fa/Lp (x 100)	133.0	139.2
2nd + 3rd cut hay	Lp	47.2	0.90
	Fa	44.8	0.80
	Fa/Lp (x 100)	94.9	88.6

From the studies of Luten en Remmelink (1984), we can conclude that the intake of freshly cut Fa is generally lower than that of Lp in the same growing stage. Averaged over the trials, both the intake and the milk production of the cows fed with Fa was 7 % lower compared to Lp (**Table 2.9**). Fa harvested in an earlier stage than Lp, compensated these lower intake and milk production, but it was not clear which influence this had on the DMY of Fa compared to Lp. The intake difference between the species seemed to be smaller when the grass was hayed or ensiled. This is in accordance with the findings of Peratoner *et al.* (2012), who did research on the effect of forage conservation on the leaf texture of different varieties of tall fescue. Both drying and ensiling of tall fescue reduced the roughness of Fa leaves significantly: on a scale of 0-9, drying reduced the roughness between 1.1 and 1.7 points and ensiling between 2.1 and 2.4 points. Results of experiments where intake and animal production of grazed Fa and Lp were directly compared were not found, but it can be expected that the difference in intake between grazed Fa and Lp is at least equal to the difference found in fresh cut grass. Emile *et al.* (1992) found a significantly higher animal production when cattle grazed a Fa variety selected for high palatability compared to a reference Fa variety.

2.3.5 Dry matter content

In the French reference tables for the feeding value of forages, the DMC at ear emergence for Fa was 19.5 % on average, whereas DMC of Lp was 16.4 % (INRA, 1978).

In the FR trial, the weighed average DMC for the year 2009 and averaged over all the varieties was 25.2 % for Fa, 25.1 % for Dg 23.2 % for Lp and 21.7 % for Fp.

In the ILVO_2 trial on the other hand, Lp had a higher DMC than Fa. The weighed average DMC for the year 2012, averaged over the two Fa varieties was 16.4 % whereas that of the single Lp variety was 17.7 %. In the first and the last cut, the DMC of both species was almost equal, whereas in the 2nd, 3rd and 4th cut the DMC of Lp was superior to that of Fa (**Figure 2.4**). The DMC of the grass harvested in the FR trial was higher than that in the ILVO_2 trial, the different management and the different harvest year may explain this difference. Moreover only one Lp variety was included in the ILVO_2 trial, whereas in the FR trial 11 Lp varieties were included.

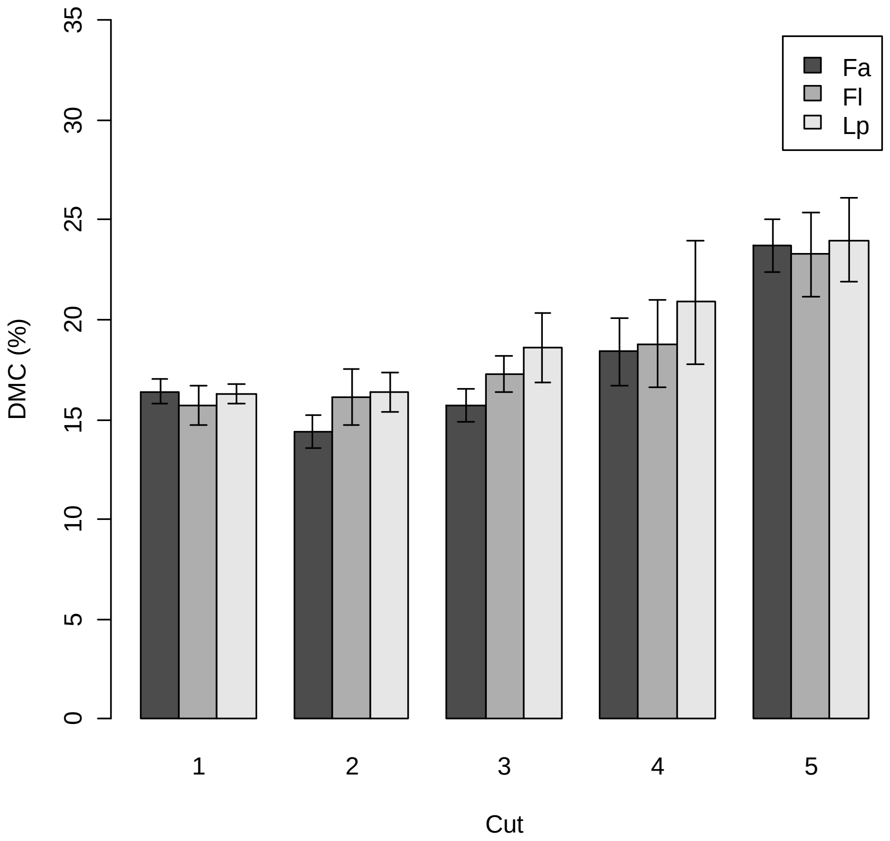


Figure 2.4 DMC of *Festuca arundinacea* (Fa), *Festulolium* (Fl) and *Lolium perenne* (Lp) in 2012 in the ILVO_2 trial. Error bars: ± standard deviation.

In addition to the higher DMC, Jones and Pricket (1981) found a higher rate of water loss of cut Fa compared to Lp, Pp and Lm when dried in a controlled-environment room and exposed to a horizontal flow of air. Comparisons between the species were made on 6 occasions in the period Mid-April till Mid-June. Fa dried faster than the other species on every occasion, and the time to reach 33 % DMC varied little between the six harvests for Fa. In contrast, the drying time for Lp and Pp increased to maximal values in mid-May; by that time drying times to reach a DMC of 33 % were greater by a factor three or four than those for Fa. With increasing maturity however, the drying time of the other species declined to values close to that of Fa. It is very unlikely that this large difference in drying time between Fa and Lp found by Pricket and Jones (1981) would hold if the grass would have been dried to a higher DMC.

The water soluble carbohydrate content on the other hand was found to be lower in Fa and Dg than in ryegrasses (Vignau-Loustau and Huyghe, 2008; Pricket and Jones, 1981), which makes it recommendable to ensile Fa and Dg with a higher DMC than ryegrasses in order to obtain good grass silage.

2.4 Conclusion

The literature we consulted and the trial results we analysed support our research hypothesis: generally Fa was found to be more productive than Lp, but digestibility of Fa was lower than that of Lp. Generally, intake of Fa was lower compared to Lp, leading to a lower animal performance.

Regarding our research questions, we can answer that:

1. Fa is on average 20 % more productive than Lp under North-West European conditions. Under very favourable conditions for grass growth however, Lp can yield as much as Fa.
2. Under drought conditions, the yield advantage of Fa compared to Lp was dependent of the severity of the drought. Under severe drought, Fa yielded up to 60 % more than Lp on a yearly basis.
3. Due to its slow establishment, Fa yields up to 10 % less than Lp in the first production year, regardless of sowing season (spring or autumn).
4. DOM of Fa was found to be up to 23 % lower than that of Lp. Based on the data of Luten and Remellink (1984), DM intake of Fa by dairy cows was found to be 5 – 10 % lower compared to Lp when fed fresh. Harvesting Fa in an earlier stage however could remediate these disadvantages of Fa. When ensiled or hayed, the differences in intake and animal production between Fa and Lp decreased and in some cases Fa intake was found to be higher than Lp intake.

Chapter 3

Performance and quality of tall fescue and perennial ryegrass and mixtures of both species grown with or without white clover under cutting management

Redrafted after: Cougnon M., Van Waes C., Baert J. and Reheul D., 2013. Performance and quality of tall fescue and perennial ryegrass and mixtures of both species grown with or without white clover under cutting management. In revision after submission to *Grass and Forage Science* in January 2013.

Partially based on: Reheul D., De Cauwer B., Cougnon M. and Aper J., 2012. What global and/or European agriculture will need from grassland and grassland breeding over the next 10-15 years for a sustainable agriculture. In Barth S. and Milbourne D. (eds) *Breeding Strategies for sustainable forage and turf grass improvement.* Springer, Dordrecht, The Netherlands.

3.1 Introduction

More dry summer spells are expected in North-West Europe due to climate change (IPCC, 2007). As perennial ryegrass (*Lolium perenne* L.; **Lp**), currently by far the most important forage grass species in North-West Europe both for cutting and grazing management, is relatively sensitive to drought stress, interest in tall fescue (*Festuca arundinacea* Schreb.; Fa) is increasing (**Chapter 1**)

As pointed in **Chapter 1**, an ideal grass species or grass sward combines the excellent forage quality of perennial ryegrass or *Lolium* sp. and the drought resistance and persistence of tall fescue or *Festuca* sp. *Festulolium* genetically combines the advantages of *Festuca* and *Lolium* in a single species (Thomas *et al.*, 2003; Ghesquière et al., 2010; Humphreys *et al.*, 2012*).*

There is some evidence that mixtures of Fa and Lp outperform swards consisting of the single species. Wilman and Gao (1996) compared the yield of Fa and Lp and a mixture of both species during five years in Wales, UK. The seed mixture contained equal amounts of seed of both components. Over the whole experimental period, the yields of Fa, Lp and the mixture of both species were 77.0 t DM ha^{-1}, 80.3 t DM ha^{-1} and 80.7 t DM ha^{-1} respectively (differences were not significant). In the sowing year, the yield of Fa was 16 % lower than that of Lp, whereas yields of Lp and the mixture were almost equal. The proportion of the Fa tillers in the mixture increased from 4.8 % in the year of sowing to 7.8 % in the third year of the experiment: the initial dominance of Lp, owing to its faster early development, did not prevent the Fa component to increase in time although Lp remained by far the most abundant species.

In a grazing trial in Switzerland, lasting for three years, two grass-clover mixtures either dominated by perennial ryegrass (16 kg ha^{-1} Lp + 10 kg ha^{-1} *Poa pratensis* + 4 kg ha^{-1} *Festuca pratensis* + 3 kg ha^{-1} *Trifolium repens*) or by tall fescue (3 kg ha^{-1} Lp + 10 kg ha^{-1} *Poa pratensis* + 15 kg ha^{-1} Fa + 4 kg ha^{-1} *Trifolium repens*) were compared (Mosimann *et al.*, 2010). No differences in grass growth nor in organic matter digestibility between the types of sward could be measured in the first two years characterised by normal rainfall. Under dry conditions, the mixture with tall fescue showed the best performance.

In our study, several mixtures of Fa and Lp were compared with monospecific swards of both species. The mixtures differed in the ploidy of the Lp variety, and the proportion of Lp in the seed mixture. Moreover, two management types were applied: plots were either fertilised with a high mineral N dose or with a low mineral N dose. In the latter case white clover (*Trifolium* *repens* L.; **Tr**) was added to the seed mixture. Yield, botanical composition and quality parameters were followed for three successive years after the sowing year.

Our research hypotheses were that:

1. Mixtures of Lp and Fa are combining the advantages of both species, resulting in a higher and more stable yield compared to the monospecific swards.
2. A small Lp fraction in the seed mixture offers good opportunities to obtain a sward with an even species composition after three years.
3. A tetraploid Lp variety is less competitive and allows a quicker progression of Fa in the mixture.
4. The management influences the interaction between Fa and Lp.

3.2 Materials and Methods

3.2.1 Trial design

The trial was sown in April 2009 on a very homogeneous sandy loam soil in Merelbeke, Belgium. The land had been used for three years as arable land before the trial was established. The soil was analysed in 2007: the pH (KCl) of the soil was 5.5, the soil organic matter content was 1.4 % and P and K content were 14 and 8 mg (100 g dry soil)$^{-1}$ respectively. The trial consisted of two adjacent subtrials; a “**pure grass trial**” and a “**grass-clover**” trial. Both trials were randomized complete block designs with three replicates; plot size was 7.8 m² and the gross area of each trial was 252 m². In the pure grass trial, monospecific swards of Fa *cv.* ‘Castagne’ (Fa), diploid Lp (Lp2) *cv.* ‘Plenty’ and tetraploid perennial ryegrass (Lp4) *cv.* ‘Roy’ were compared with mixtures of Fa and Lp. Mixtures were created by substituting 1/4 or 1/8 of the Fa seeds by Lp2 or Lp4 seeds (**Table 3.1**). Sowing densities were 1500 germinating seeds m^{-2} in all cases. Annual fertilisation was 300 kg N ha^{-1}, 11kg P ha^{-1} and 270 kg K ha^{-1}. The concept of the grass-clover subtrial was in line with the concept of the pure grass trial, but all seeding densities were supplemented with 700 germinating seeds m^{-2} (approximately 5 kg ha^{-1}) of ‘Merwi’ a medium leafed white clover variety. Annual fertilisation was 165 kg N ha^{-1}, 32 P ha^{-1} and 258 kg K ha^{-1}. Throughout the text the abbreviations indicated in **table 3.1** will be used to identify the different sward compositions in the trials.

Table 3.1 Sowing density (number of germinating seeds m^{-2}) for *Festuca arundinacea* (Fa), diploid and tetraploid *Lolium perenne* (Lp2, Lp4) and mixtures of both grass species with or without *Trifolium repens* (Tr).

Sward compositions	Tall fescue	Diploid perennial ryegrass	Tetraploid perennial ryegrass	White clover
Pure grass trial:				
Fa	1500	0	0	0
Lp2	0	1500	0	0
Lp4	0	0	1500	0
1/8Lp2	1312	188	0	0
1/8Lp4	1312	0	188	0
1/4Lp2	1125	375	0	0
1/4Lp4	1125	0	375	0
Grass-clover trial:				
Fa+Tr	1500	0	0	700
Lp2+Tr	0	1500	0	700
Lp4+Tr	0	0	1500	700
1/8Lp2+Tr	1312	188	0	700
1/8Lp4+Tr	1312	0	188	700
1/4Lp2+Tr	1125	375	0	700
1/4Lp4+Tr	1125	0	375	700

3.2.2 Measurements

Five cuts were harvested in 2010, 2011 and 2012 (**Table 3.2**). Plots were mown and weighed with a Haldrup (Haldrup, Logstor, Denmark) plot harvester. At each cut, samples of ± 150 g were taken on the harvester for determination of the dry matter content (DMC), crude protein content (CPC) and the digestibility of the organic matter (DOM). Samples were dried for 24 h at 60 °C and ground (Brabender shear mill) to pass a 1mm sieve. The near infrared reflectance spectroscopy (NIRS) spectra of the ground samples were collected with a Foss NIRSystems 5000 (FOSS-NIRSystems, Silver Springs, MD, USA) and ISIscan 2.85.1 software (Infrasoft international, Port Mathilda, PA, USA). The NIRS equation for CPC was based on 396 grass and grass-clover samples that had been analysed by the Kjeldahl method. The NIRS equation for DOM was based on 396 grass and grass-clover samples analysed according to Tilley and Terry (1963). In 2010 and 2011, the 20 samples that were spectrally most distant from the calibration samples were analysed using the reference methods for CPC and DOM in order to expand the calibration library. Ten supplementary samples were chosen ad random, and analysed using the reference methods for validation of the equations for CPC and DOM. The root mean square error of prediction (RMSEP) was below 0.38 % for CPC and below 1.72 % for DOM; the bias (the mean difference between the wet chemistry lab value and the NIRS value) was -0.02 % for CPC and -0.28 % for DOM.

A second sample was taken for the determination of the botanical composition of the harvested material. At least 1 % of the fresh yield of each plot (corresponding to weights between 150 and 300 g) was separated by hand into the different species and the fresh and dry weights were recorded.

Table 3.2 Harvesting dates in 2010-2012

Cut	2010	2011	2012
1	27 April	2 May	11 May
2	2 June	14 June	18 June
3	19 July	18 July	25 July
4	24 August	22 August	5 September
5	4 October	10 October	15 October

The N-fixation by the white clover was estimated according to the total N difference method (Carlsson and Huss-Danel, 2003). This method is based on the assumption that the difference in nitrogen yield (NY) in plots with and without clover with the same mineral N fertilisation is explained by N fixation. Since the N application differed in the pure grass trial and the grass clover trial we applied a modification and calculated N fixation as follows:

$N_{\text{fixation}} = NY_{\text{grass-clover}} - NY_{\text{pure grass}} + (\text{Nfertilisation}_{\text{pure grass}} - \text{Nfertilisation}_{\text{grass-clover}})$

NY was calculated using the CPC and DMY of each plot.

After the final cut in 2012, sward tiller density was determined on the pure grass trial. Two random sward samples of 20 cm x 20 cm were dug out per plot. The sward samples were decomposed into individual tillers, tillers were sorted per grass species and counted.

Meteorological data were obtained from an official meteorological station at approximately 3 km from the experimental site (**Appendix 1**). Dry spells occurred in the early summer of 2010 (83 mm of rainfall instead of 157 mm normally from April 1st till June 30th), in the spring of 2011 (33.8 mm of rainfall instead of 135.3 mm normally from March 1st till May 20th) and in August-September 2012 (49.4 mm instead of 96.6 mm normally from August 1st till September 10th). The summers of 2010, 2011 and 2012 were relatively wet, so the occurring dry spells did not really hamper grass growth dramatically. Two cold winter periods occurred: December 2010 was the coldest December month since 1950, with freezing temperatures below -10 °C; and in February 2012, it was freezing for 15 successive days, with minima until -15°C. No excessive heat periods occurred during the experiment. Nevertheless, the year 2011 was the warmest since the start of the Belgian registration of meteorological data in 1833.

3.2.3 Data analysis

Analyses of variance were computed using the *aov()* function in R (R Development Core Team, 2011). Although the two subtrials, were adjacent to each other and covered a small area on a very homogeneous soil, the experimental design did not allow statistical comparison between the results of the subtrials: the treatments of both trials did not appear in the same blocks. Data with and without clover were analysed separately, using a model for a randomized complete block design (Crawley, 2007). In each trial the different sward compositions were considered as a fixed factor with seven levels, replications as a random factor with three levels. Multiple comparison of means between the mixtures was done using the *TukeyHSD()* function. For the anova of the proportion of Fa in the grass DMY, there were only four levels as the swards with only one species contained either 0 % or 100 % Fa. A supplementary anova was performed to model the effect of the initial proportion of Lp seeds in the seed mixture and the ploidy of the Lp on the Fa proportion in the DMY. Analysis of covariance (ANCOVA) was performed to model whether the effect of the Fa content (FaC) on total DMY and DOM of the mixtures was purely linear or quadratic. The analysis was done with the *lm()* function, and the minimum adequate model (i.e. the model that minimised the Akaike’s information criterion) was selected with the *step()* function (Crawley, 2007).

3.3 Results

3.3.1 Pure grass trial

3.3.1.1 DM yield

Both the monospecific swards and the mixtures established well in 2009. In 2010, Fa significantly outyielded Lp2 and Lp4 in the first and the third cut (**Table 3.3**). Lp2 yielded significantly more than Fa in the fifth cut. Over the five cuts, the yield of Fa was significantly higher than that of Lp2 and Lp4. The yield of the mixtures was between that of the monospecific swards.

In 2011 Fa significantly outyielded Lp2 and Lp4 in the first two cuts. In the fourth and the fifth cuts there were no significant yield differences. The yield of the mixtures was mostly between that of the monospecific swards. Over the five cuts Fa and all mixtures except 1/4Lp2 yielded significantly more than Lp2 and Lp4.

Table 3.3 Dry matter yield (kg ha^{-1}) for *Festuca arundinacea* (Fa), diploid and tetraploid *Lolium perenne* (Lp2, Lp4) and mixtures of both species (indicated in Table 3.1) in 15 successive cuts (C1-C15) in three successive years (2010-2012).

	1/4Lp2	1/4Lp4	1/8Lp2	1/8Lp4	Fa	Lp2	Lp4	Sign.^{A}
2010								
C1	3574^{ab}	3783^{ab}	3897^{ab}	4042^{a}	3981^{a}	3388^{ab}	3113^{b}	*
C2	4920	4819	5188	4556	4746	5104	4806	NS
C3	1903^{c}	2075^{c}	2501^{b}	2278^{bc}	2953^{a}	1170^{d}	1447^{d}	***
C4	1653	1574	1595	1665	1658	1557	1315	NS
C5	2267^{ab}	2350^{ab}	2189^{ab}	2393^{ab}	2149^{b}	2527^{a}	2365^{ab}	**
Subtotal	14319^{ab}	14600^{ab}	15363^{a}	14934^{ab}	15487^{a}	13746^{bc}	13046^{c}	***
Relative (Fa = 100)	92.5	94.3	99.2	96.4	100.0	88.8	84.2	
2011								
C6	4303^{ab}	4646^{ab}	4922^{a}	4399^{ab}	4991^{a}	3345^{c}	3952^{bc}	***
C7	1907^{bc}	1939^{bc}	2385^{a}	1965^{b}	2549^{a}	1623^{cd}	1352^{d}	***
C8	2220^{ab}	2080^{ab}	2395^{a}	2333^{a}	2255^{ab}	2052^{ab}	1844^{b}	**
C9	2309	2138	2217	2517	2084	2403	2206	NS
C10	2346	2367	2408	2503	2478	2376	2200	NS
Subtotal	13085^{ab}	13170^{a}	14321^{a}	13716^{a}	14357^{a}	11798^{bc}	11555^{c}	***
Relative (Fa = 100)	91.1	91.7	99.7	95.5	100.0	82.2	80.5	
2012								
C11	4446^{a}	4737^{a}	5076^{a}	4654^{a}	4984^{a}	2975^{b}	2983^{b}	***
C12	3244	3401	3778	3174	3936	3680	3241	NS
C13	4165^{ab}	4086^{ab}	4613^{a}	4206^{ab}	4435^{ab}	3649^{bc}	3170^{c}	***
C14	2556^{b}	2491^{b}	2828^{ab}	2495^{b}	2944^{a}	1704^{c}	1623^{c}	***
C15	564^{abc}	587^{abc}	664^{ab}	602^{abc}	690^{a}	543^{bc}	481^{c}	**
Subtotal	14974^{a}	15301^{a}	16958^{a}	15131^{a}	16988^{a}	12550^{b}	11499^{b}	***
Relative (Fa = 100)	88.1	90.1	99.8	89.1	100.0	73.9	67.7	
Total	42379^{c}	43070^{bc}	46643^{ab}	43781^{bc}	46832^{a}	38094^{d}	36099^{d}	***
Relative (Fa = 100)	90.5	92.0	99.6	93.5	100.0	81.3	77.1	

A: Significance of the anova model with the seven sward compositions as fixed factor and the three replicates as random factor. (Significance codes: ***: $p < 0.001$, **: $p < 0.01$, *: $p < 0.05$, NS: $p > 0.05$). Values in the same row not sharing a same superscript are significantly different from each other ($p = 0.05$, Tukey test).

In 2012, Fa significantly outyielded Lp2 and Lp4 in all cuts except the second. The yield of the mixtures was close to that of Fa or slightly higher than it; mixtures overyielded Lp2 and Lp4. Over the five cuts, Fa had again the highest yield, and 1/8Lp2 had a yield that was closely to that of Fa.

The yield gap between Fa and Lp grew every year: Fa yielded 13 %, 22 % and 35 % more than Lp2 in first, second and third year after the year of establishment respectively. Every year Lp2 overyielded Lp4 slightly, but this difference was in none of the years significant. Over the 15 cuts in the three growing seasons, Fa and the mixtures were yielding significantly more than Lp2 and Lp4.

The minimal adequate model describing the effect of Fa content (FaC) and year on total DMY was a linear model for 2010 and 2011 with a different intercept but a common slope for both years. The DMY of the mixtures increased with 28.5 kg DM ha^{-1} for each extra % point in FaC. For 2012, the quadratic term was significant. The model explained 83 % (R^2 value) of the total variability (**Table 3.4, Figure 3.1**)

Table 3.4 Regression coefficients for minimal adequate Ancova models for the dry matter yield (DMY), digestibility of the organic matter (DOM) and crude protein content (CPC) of mixtures of *Festuca arundinacea* (Fa) and *Lolium perenne* (Lp) as a function of the Fa content in the DMY (FaC). (Y= I+a.(FaC)+b.(FaC)^2) (Significance codes: ***: $p < 0.001$, **: $p < 0.01$, *: $p < 0.05$, NS: $p > 0.05$).

	Year	I	a	b	R²
DMY	2010	13460 ***	28.5 ***	-0.071 NS	
	2011	11624 ***	28.5 ***	-0.001 NS	0.83
	2012	12023 ***	28.5 ***	0.197 ***	
DOM[B]	2010	81.0 ***	-0.084 ***	/[A]	
	2011	77.1 ***	-0.065 **	/[A]	0.95
	2012	78.3 ***	-0.084 ***	/[A]	
CPC[B]	2010	12.9***	-2.48*10^{-3}*	/[A]	
	2011	13.5***	-2.48*10^{-3}*	/[A]	0.73
	2012	12.1***	-2.48*10^{-3}*	/[A]	

A: / : the quadratic term was not included in the minimal adequate model
B: regression based on weighted average over all cuts.

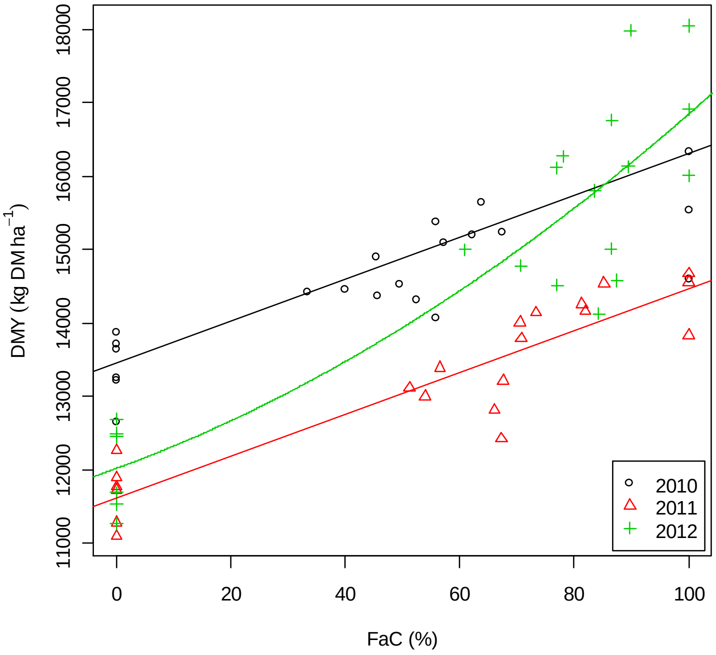


Figure 3.1 Regression of the dry matter yield (DMY) on the *Festuca arundinacea* content (FaC) in mixtures of Fa and *Lolium perenne.*

Three of the fifteen cuts were preceded by a drought period where Fa was clearly superior to Lp. A first drought period preceded the 3rd cut of 2010, with only 19.3 mm instead of 67.4 mm of rain from the 11th of June till the 9th of July resulting in a 2.5 times higher yield for Fa compared to Lp2. The yields of the mixtures were between 1.6 and 2.1 times higher than that of Lp2. In the spring of 2011, the first cut was preceded by six weeks of very dry weather, but the yields were very high for both Fa and Lp. After the first cut, it remained dry for another 18 days, resulting in a big yield gap between Fa and Lp in the second cut: Fa yielded 57% more than Lp. A third drought period occurred in the fifth cut of 2012: the DMY of Fa was higher than that of Lp2, but this difference was not larger than the difference found in the fifth cut in the years 2010 and 2011.

3.3.1.2 Botanical composition

In the first year after sowing the proportion of Fa in all mixtures was lower than the sown proportion (**Table 3.5**). FaC was increasing over the years: over all cuts FaC was between 40 % and 64 % in 2010, between 54 % and 83 % in 2011 and between 69 % and 89 % in 2012. Within each year it mostly decreased after the first cut (**Figure 3.2**). FaC was significantly higher when the initial proportion of Lp in the seeds mixture was 1/8 compared to 1/4 and when Lp2 was the companion species compared to Lp4 (**Table 3.6**).

Table 3.5 Content of *Festuca arundinacea* (FaC) in the dry matter yield, dry matter content (DMC), digestibility of the organic matter (DOM), crude protein content (CPC) and N yield (NY) for *Festuca arundinacea* (Fa), diploid and tetraploid *Lolium perenne* (Lp2, Lp4) and mixtures of both grass species (indicated in table 3.1) in three successive years (2010-2012). Data are weighted averages over all cuts.

		1/4Lp2	1/4Lp4	1/8Lp2	1/8Lp4	Fa	Lp2	Lp4	Sign.^{A}
2010									
FaC	(%)	50.4^{b}	39.6^{c}	64.4^{a}	55.1^{ab}	$100^{\#}$	$0^{\#}$	$0^{\#}$	***
DMC	(%)	22.0^{ab}	21.6^{ab}	22.1^{ab}	21.6^{ab}	22.3^{a}	22.3^{a}	20.5^{b}	*
DOM	(%)	77.2^{bc}	78.1^{b}	74.8^{c}	75.9^{c}	72.7^{d}	80.5^{a}	81.4^{a}	***
CPC	(%)	12.8	12.6	12.7	13.0	12.5	12.9	12.9	NS
NY	(kg N ha^{-1})	293.4^{ab}	294.8^{ab}	313.4^{a}	311.7^{a}	309.9^{a}	283.6^{ab}	270.1^{b}	*
2011									
FaC	(%)	67.9^{b}	53.9^{c}	82.8^{a}	70.6^{b}	$100^{\#}$	$0^{\#}$	$0^{\#}$	***
DMC	(%)	21.4^{a}	20.9^{a}	21.4^{a}	20.5^{ab}	21.1^{a}	21.7^{a}	19.5^{b}	***
DOM	(%)	72.6^{e}	74.1^{c}	71.3^{ef}	72.7^{d}	70.4^{f}	76.0^{b}	77.9^{a}	***
CPC	(%)	13.4	13.0	13.2	13.3	13.3	13.7	13.5	NS
NY	(kg N ha^{-1})	281.1^{abc}	274.6^{abc}	303.4^{a}	291.3^{ab}	305.6^{a}	258.3^{bc}	250.7^{c}	***
2012									
FaC	(%)	79.8^{ab}	69.4^{b}	88.6^{a}	85.8^{a}	$100^{\#}$	$0^{\#}$	$0^{\#}$	**
DMC	(%)	20.8^{abc}	20.4^{bc}	21.2^{ab}	20.5^{bc}	20.8^{abc}	21.9^{a}	19.9^{c}	*
DOM	(%)	71.1^{bc}	72.5^{b}	70.8b^{c}	70.5^{bc}	69.8^{c}	77.6^{a}	79.1^{a}	***
CPC	(%)	12.1	11.8	11.7	12.2	11.9	12.1	12.0	NS
NY	(kg N ha^{-1})	288.4^{c}	288.5^{c}	317.0^{ab}	293.9^{bc}	324.3^{a}	242.4^{d}	220.9^{d}	***

A: Significance of the anova model with the seven sward compositions as fixed factor and the three replicates as random factor. (Significance codes: ***: $p < 0.001$, **: $p < 0.01$, *: $p < 0.05$, NS: $p > 0.05$.). Values in the same row not sharing a same superscript are significantly different from each other ($p = 0.05$, Tukey test).
The botanical composition of the monospecific swards was not taken into account in the statistical analyses.

Table 3.6 Effect of ploidy and proportion of *Lolium perenne* (Lp) in the initial seed mixture and the interaction of both factors on the *Festuca arundinacea* (Fa) content in the total DMY of swards with mixtures of both species (indicated in table 3.1). (Significance codes: ***: $p < 0.001$, **: $p < 0.01$, *: $p < 0.05$, NS: $p > 0.05$).

	Pure grass trial			Grass-clover trial		
Year	Ploidy	Proportion	Interaction	Ploidy	Proportion	Interaction
2010	***	***	NS	NS	***	NS
2011	***	***	NS	NS	**	NS
2012	*	***	NS	NS	**	NS
2010-2012	***	***	NS	NS	***	NS

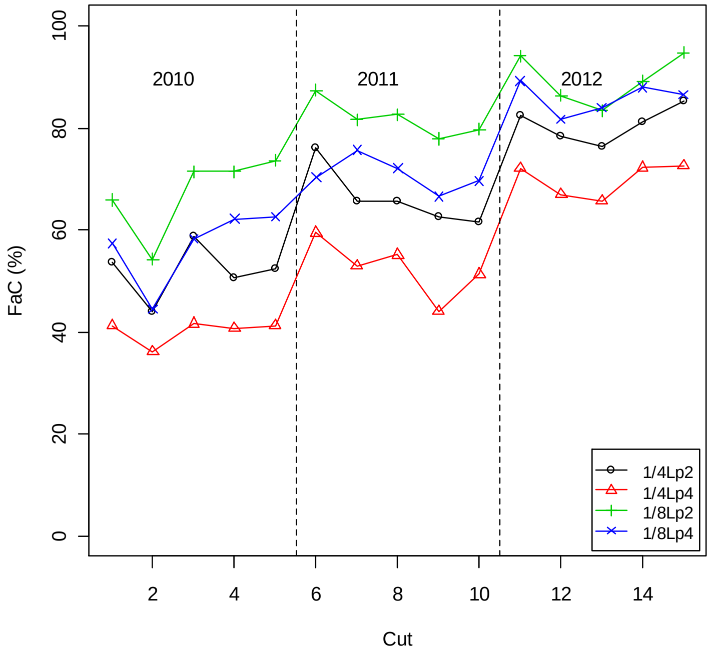


Figure 3.2 Evolution of the tall fescue content (FaC) in the dry matter yield in four mixtures of *Festuca arundinacea* (Fa) and *Lolium perenne* (Lp) (indicated in table 3.1). Evolution over 15 cuts in three successive years (2010-2012).

The tiller density, recorded after the final cut in 2012, indicated that the mixtures were dominated by Fa at the end of the trial (**Figure 3.3**). Lp2 had a significantly higher tiller density than the mixtures and all the other sward compositions ($p = 0.0011$): 4771, 3142 and 2754 tillers m^{-2} for Lp2, Lp4 and Fa respectively. Tiller density of the mixtures was between 2571 and 2712 tillers m^{-2} for 1/8Lp2 and 1/4Lp2 respectively. There was no difference in Fa tiller density between mixtures and pure Fa ($p = 0.16$).

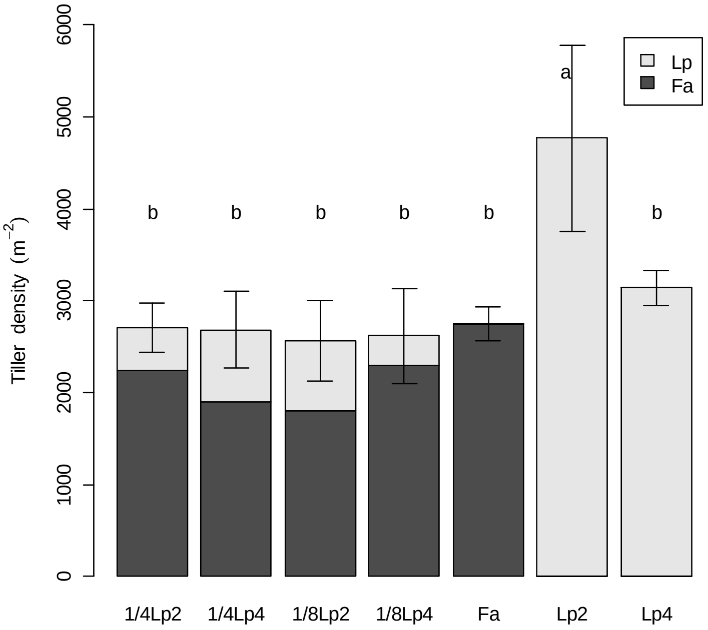


Figure 3.3 Tiller density (October 2012) in a sward of *Festuca arundinacea* (Fa), diploid and tetraploid *Lolium perenne* (Lp2, Lp4) and four mixtures of Fa and Lp (indicated in table 3.1). Error bars: ± standard deviation. Bars not sharing a common letter are significantly different from each other (Tukey; *p* = 0.05).

3.3.1.3 Quality

The DMC of Lp2 and Fa was on the same level in the three years; Lp4 on the other hand had a significantly lower DMC in all years (**Table 3.5**). The DMC of Lp4 was on average 2 % points lower than that of Lp2. The DMC peaked in the third cut of 2010 and in the second cut of 2011, probably due to the dry weather preceding these periods (**Figure 3.4**). The DMC of the mixtures had values between these of the pure swards.

In every year DOM was significantly lower for Fa compared to Lp2 and Lp4 (**Table 3.5**). Annual mean digestibility of Fa was at least 5 % points lower compared to Lp2. Particularly in the first two cuts of 2010, the third cut of 2011 and the first cut of 2012 differences between Fa and Lp2 were high: 8.9 % points, 8.6 % points, 8.7 % points and 14.0 % points respectively (**Figure 3.5**). Lp4 was averaged over the three years 1.4 % points more digestible than Lp2; this difference was significant in 2011.

The effect of FaC on the CPC of the mixtures was also linear. The minimal adequate model contained a different intercept for each year, and a common slope for the three years (**Table 3.4**). The model explained 73 % (R^2 value) of the difference in CPC in the mixtures (**Figure**

3.7b). An increase of 1 % point of FaC in the mixtures resulted in a decrease of the CPC with 2.48×10^{-3} % points

No quadratic terms were included in the minimal adequate model describing the effect of FaC and year on the DOM: the effect of FaC on the DOM was linear (**Table 3.4**). The model included a different intercept for every year, a common slope for 2010 and 2012 and a different slope for 2011. The model explained 95 % (R^2 value) of the total variability **(Figure 3.7a**). An increase of 1 % point of FaC in the mixtures resulted in a decrease of the DOM with -0.084 % points in 2010 and 2012 and with -0.065 % points in 2011.

In none of the years, significant differences were found in the CPC between the grass species or the mixtures (**Table 3.5**). Within the year 2010 CPC fluctuated more than in 2011 and 2012: CPC of Fa was 4.8 % point and 3.8 % points lower than that of Lp2 in the third and fourth cut respectively (**Figure 3.6**). Probably this was due to a dilution effect, as Fa had a higher DMY in both cuts compared to Lp2.

The higher DMY of Fa resulted in a significantly higher NY for Fa compared to Lp4 in 2010 and higher than both Lp2 and Lp4 in 2011 and 2012. Parallel with DMY, the NY difference between Fa and Lp2 grew with sward age: 26, 47 and 82 kg N ha^{-1} yr^{-1} in the 2010, 2011 and 2012 respectively. NY for the mixtures was generally between that of Fa and Lp (**Table 3.5**).

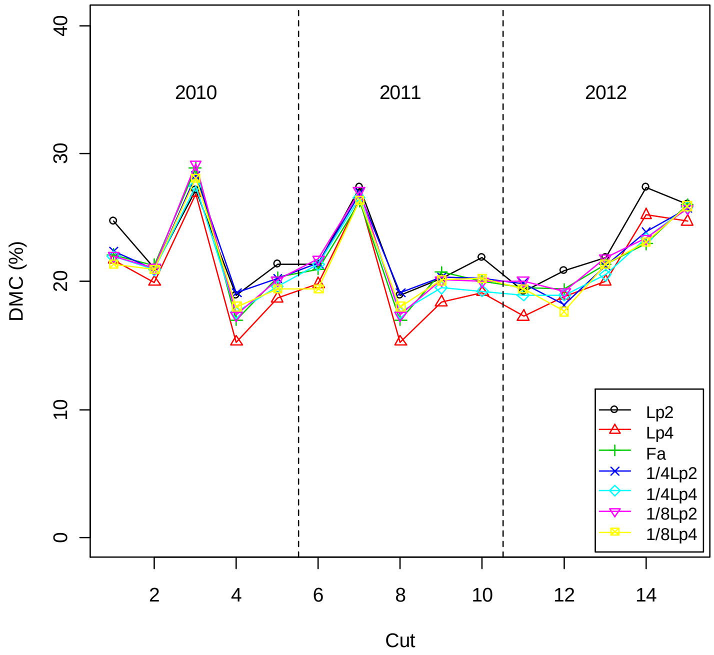


Figure 3.4 Evolution of the dry matter content (DMC) content over 15 cuts in three successive years (2010-2012) for diploid or tetraploid *Lolium perenne* (Lp2 or Lp4), *Festuca arundinacea* (Fa) and mixtures of both species (indicated in table 3.1).

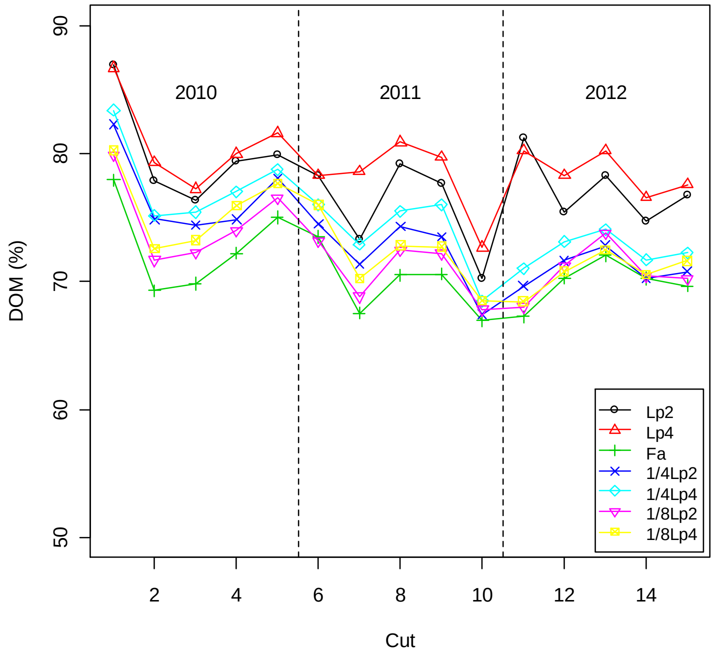


Figure 3.5 Evolution of the digestibility of the organic matter (DOM) content over 15 cuts in three successive years (2010-2012) for diploid or tetraploid *Lolium perenne* (Lp2 or Lp4), *Festuca arundinacea* (Fa) and mixtures of both species (indicated in table 3.1).

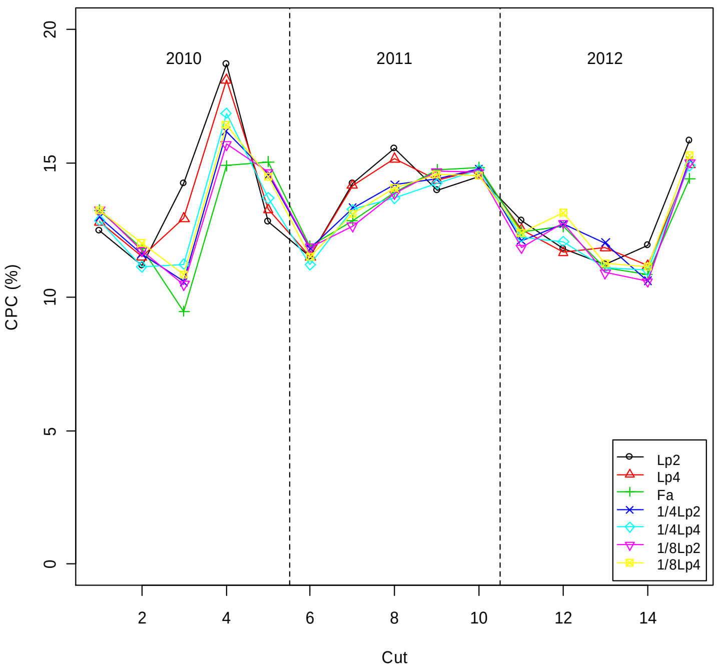


Figure 3.6 Evolution of the crude protein (CPC) content over 15 cuts in three successive years (2010-2012) for diploid or tetraploid *Lolium perenne* (Lp2 or Lp4), *Festuca arundinacea* (Fa) and mixtures of both species (indicated in table 3.1).

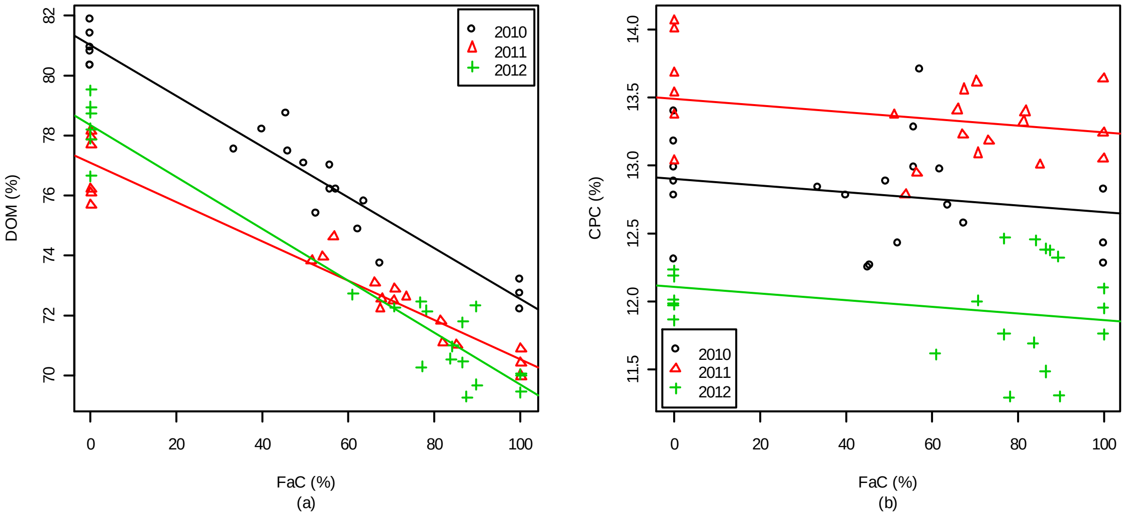


Figure 3.7 Regression of the digestibility of the organic matter (DOM) (a) and crude protein content (CPC) (b) on the *Festuca arundinacea* (Fa) content (FaC) in mixtures of Fa and *Lolium perenne.*

3.3.2 Grass-clover trial

3.3.2.1 DM Yield

Despite the significantly higher yield of Fa+Tr in the second and the third cut of the year 2010, there were no significant yield differences between the means of the sward compositions over the whole year (**Table 3.7**). In the second year, Fa+Tr was significantly overyielding Lp+Tr in the first two cuts, resulting in a 29 % significantly higher annual yield for Fa+Tr compared to Lp2+Tr. In 2012, Fa+Tr had a significant higher yield in the last three cuts, but over the whole year there was no significant difference between the means of the sward compositions. Over the 15 cuts in the three years Fa+Tr and 1/8Lp2+Tr were yielding significantly more than Lp2+Tr and Lp4+Tr; Fa+Tr was overyielding Lp2+Tr with 20 % and Lp4+Tr with 19 %.

3.3.2.2 Botanical composition

In none of the years, there was a significant effect of the grass species or grass species mixture on the weighted average clover content in the harvested dry matter (**Table 3.8**). For both grass species and mixtures, the clover content in the harvested material was higher in the second year (19.9 % - 26.3 %) compared to the first year (18.8 % - 22.5 %), but it decreased again in the third year (11.7 % - 15.3 %). Within each year, there was a clear seasonal pattern: low clover content in spring, steeply increasing in the summer and decreasing again in the autumn and winter. In the third cut of 2010, which was preceded by a drought period, the TrC increased stronger in combination with Lp than in combination with Fa (**Figure 3.8**): the TrC increased from 18, 14 and 18 % for Fa, Lp2 and Lp4 respectively in the second cut to 23 % for Fa and 30 % for Lp2 and Lp4 in the third cut.

Table 3.7 Dry matter yield (kg DM ha^{-1}) of *Festuca arundinacea* (Fa), diploid and tetraploid *Lolium perenne* (Lp2, Lp4) and mixtures of both grass species in combination with *Trifolium repens* (Tr) (indicated in table 3.1) in 15 successive cuts (C1-C15) in three successive years (2010-2012)

	1/4Lp2 +Tr	1/4Lp4	1/8Lp2	1/8Lp4	Fa	Lp2	Lp4	Sign.^{A}
2010								
C1	4189	4085	4561	4375	4831	3808	3745	NS
C2	4085	3677	4207	3746	3950	4531	3852	NS
C3	1581^{bcd}	1784^{abc}	1962^{ab}	1675^{bc}	2365^{a}	1013^{d}	1316^{cd}	***
C4	1310^{ab}	1316^{ab}	1479^{ab}	1353^{ab}	1660^{a}	1187^{b}	1079^{b}	***
C5	1902	1793	1915	1639	1928	1673	1571	NS
Subtotal	13067	12655	14123	12789	14733	12212	11562	NS
Relative (Fa = 100)	88.7	85.9	95.9	86.8	100.0	82.9	78.5	
2011								
C6	5144^{b}	5388^{ab}	5654^{ab}	5469^{ab}	6079^{a}	4388^{c}	5118^{b}	***
C7	2023^{abc}	1972^{bc}	2418^{ab}	1988^{bc}	2855^{a}	1293^{c}	1381^{c}	***
C8	1979^{a}	1891^{a}	2020^{a}	1761^{a}	2069^{a}	1835^{a}	1783^{a}	*
C9	2963	2781	2969	2854	2776	3002	2904	NS
C10	2268	2100	2266	2300	2215	1928	1795	NS
Subtotal	14378^{abc}	14132b^{cd}	15326^{ab}	14377^{abc}	15994^{a}	12446^{d}	12981^{cd}	**
Relative (Fa = 100)	89.9	88.4	95.8	89.9	100.0	77.8	81.2	
2012								
C11	5130	5243	5456	5173	5382	4325	4450	NS
C12	3621	3323	3719	3429	3605	3793	3520	NS
C13	3687^{ab}	3504^{ab}	3887^{a}	3843^{a}	3829^{a}	3509^{ab}	2971^{b}	**
C14	3033^{ab}	3024^{ab}	3214^{ab}	3209^{ab}	3330^{a}	2739^{bc}	2474^{c}	***
C15	794^{a}	726^{ab}	831^{a}	825^{a}	829^{a}	671^{ab}	536^{b}	**
Subtotal	16262	15820	17107	16479	16975	15037	13952	NS
Relative (Fa = 100)	95.8	93.2	100.8	97.1	100.0	88.6	82.2	
Total	43707^{ab}	42606^{ab}	46556^{a}	43640^{ab}	47701^{a}	39695^{b}	38496^{b}	**
Relative (Fa = 100)	91.6	89.3	97.6	91.5	100.0	83.2	80.7	

A: Significance of the anova model with the seven sward compositions as fixed factor and the three replicates as random factor. (Significance codes: ***: $p < 0.001$, **: $p < 0.01$, *: $p < 0.05$, NS: $p > 0.05$.). Values in the same row not sharing a same superscript are significantly different from each other ($p = 0.05$, Tukey test).

Table 3.8 Content of *Festuca arundinacea* (Fa) and *Trifolium repens* (Tr) in the dry matter yield (FaC and TrC), dry matter content (DMC), digestibility of the organic matter (DOM), crude protein content (CPC) and N yield (NY) for Fa, diploid and tetraploid *Lolium perenne* (Lp2, Lp4) and mixtures of both grass species in combination with Tr (indicated in table 3.1) in three successive years (2010-2012). Data are weighted averages over all cuts.

		1/4Lp2	1/4Lp4	1/8Lp2	1/8Lp4 +Tr	Fa	Lp2	Lp4	Sign.^{A}
2010									
FaC	(%)	32.4^{b}	28.7^{b}	47.0^{a}	45.6^{a}	$79.3^{\#}$	$0^{\#}$	$0^{\#}$	**
TrC	(%)	20.6	20.7	20.4	19.4	20.7	18.8	22.5	NS
DMC	(%)	19.4	18.8	20.1	19.6	20.2	21.7	18.9	NS
DOM	(%)	77.7^{b}	78.7^{b}	76.6^{bc}	77.5^{bc}	75.3^{c}	81.1^{a}	81.3^{a}	***
CPC	(%)	15.9	16.2	15.8	15.9	15.7	14.8	15.9	NS
NY	(kg N ha^{-1})	332.1^{a}	328.8^{a}	357.0^{a}	324.9^{a}	368.0^{a}	288.7^{a}	294.1^{a}	*
2011									
FaC	(%)	44.5^{ab}	38.3^{b}	56.2^{a}	48.7^{ab}	$77.5^{\#}$	$0^{\#}$	$0^{\#}$	*
TrC	(%)	26.3	19.9	24.2	22.2	22.5	24.2	23.5	NS
DMC	(%)	19.4	18.8	19.1	19.1	19.5	19.0	18.3	NS
DOM	(%)	72.3^{cd}	73.1^{c}	71.0^{e}	72.0^{def}	70.1^{f}	76.7^{b}	77.8^{a}	***
CPC	(%)	17.5	16.9	17.3	16.8	16.9	17.7	17.7	NS
NY	(kg N ha^{-1})	402.3^{ab}	381.2^{bc}	424.0^{a}	385.7^{bc}	431.1^{a}	352.9^{c}	366.8^{c}	***
2012									
FaC	(%)	67.6^{a}	60.8^{a}	77.1^{a}	77.0^{a}	$88.3^{\#}$	$0^{\#}$	$0^{\#}$	*
TrC	(%)	13.2	13.4	14.1	13.8	11.7	15.3	13.9	NS
DMC	(%)	19.1	18.5	19.3	18.8	18.8	19.1	17.5	NS
DOM	(%)	71.6^{b}	72.2^{b}	70.7^{b}	71.0^{b}	69.9^{b}	76.5^{a}	77.3^{a}	***
CPC	(%)	14.3	14.2	14.5	14.6	14.5	15.0	14.9	NS
NY	(kg N ha^{-1})	373.2	360.1	393.9	383.1	394.1	360.4	331.9	NS

A: Significance of the anova model with the seven sward compositions as fixed factor and the three replicates as random factor. (Significance codes: ***: $p < 0.001$, **: $p < 0.01$, *: $p < 0.05$, NS: $p > 0.05$.). Values in the same row not sharing a same superscript are significantly different from each other ($p = 0.05$, Tukey test).

The botanical composition of the monospecific grass swards was not taken into account in the statistical analyses.

The Fa content in the total dry matter yield differed significantly between the mixtures in the three years (**Table 3.8**). The highest Fa contents were found in the mixture 1/8Lp2+Tr: 47 %, 56 % and 77 % in 2010, 2011 and 2012 respectively. The lowest Fa contents were found in the mixture 1/4Lp4+Tr: 29 %, 38 % and 61 % in 2010, 2011 and 2012 respectively (**Table 3.8**). The effect of the ploidy of the Lp on FaC was not significant, but the effect of the initial proportion of Lp in the mixtures was significant in every year (**Table 3.6**). The contribution of Fa to the total dry matter yield (**Figure 3.9a**) was generally increasing through the years, but within a year there was a decreasing trend that was inverse to the importance of the Tr content in the dry matter yield. The contribution of Fa to the grass dry matter yield (**Figure 3.9b**) was very similar to that recorded in the pure grass trial (**Figure 3.2**).

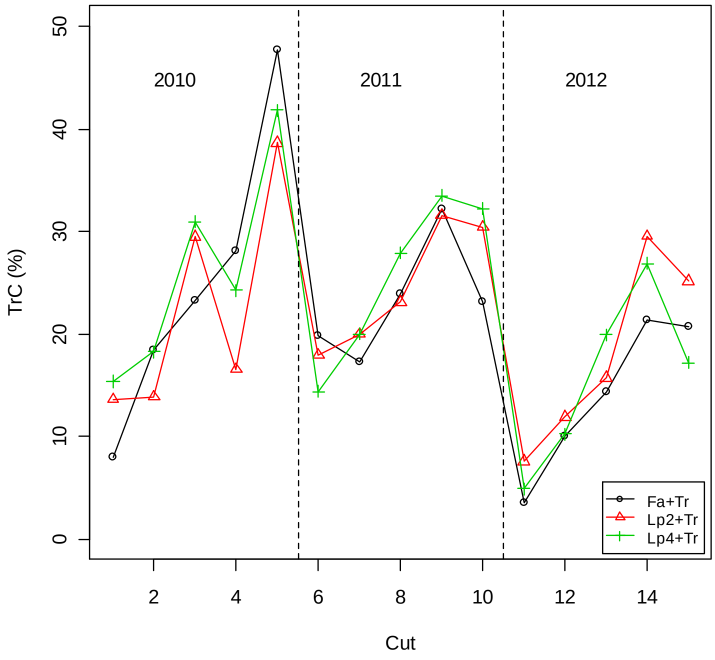


Figure 3.8 Evolution of the *Trifolium repens* content (TrC) in the dry matter yield of grass-clover mixtures combining white clover with diploid or tetraploid *Lolium perenne* (Lp2 or Lp4) and *Festuca arundinacea* (Fa) over 15 cuts in three successive years (2010-2012).

3.3.2.3 Quality

In none of the years, significant differences were found in CPC or DMC between the different grass-clover mixtures (**Table 3.8**). The trends were however the same: Fa+Tr and Lp2+Tr had a comparable DMC, Lp4 had a lower DMC (**Figure 3.10**). Due to the clover presence, the differences in DMC between the species, were smaller than in the pure grass trial. The CPC in the grass-clover trial was higher and less constant than in the pure grass trial. Parallel with the TrC, CPC increased near the end of each season (**Figure 3.11**).

The DOM of Lp2+Tr and Lp4+Tr was in all years significantly higher than that of Fa+Tr and the grass species mixtures with clover. The DOM of Fa+Tr was 5.8, 6.6 and 6.6 % points lower than that of Lp2+Tr in 2010, 2011 and 2012 respectively. DOM of the grass species mixtures with clover was between that of the two grass monospecific swards with clover (**Table 3.8, Figure 3.12**).

The NY in 2010 and 2011, differed significantly between the grass species and the mixtures of the two grasses + white clover. NY of Fa+Tr, the sward composition with the highest NY, was 79, 78 and 34 kg N ha^{-1} yr^{-1} higher than that of Lp2+Tr, the lowest N-yielding sward composition, in 2010, 2011 and 2012 respectively.

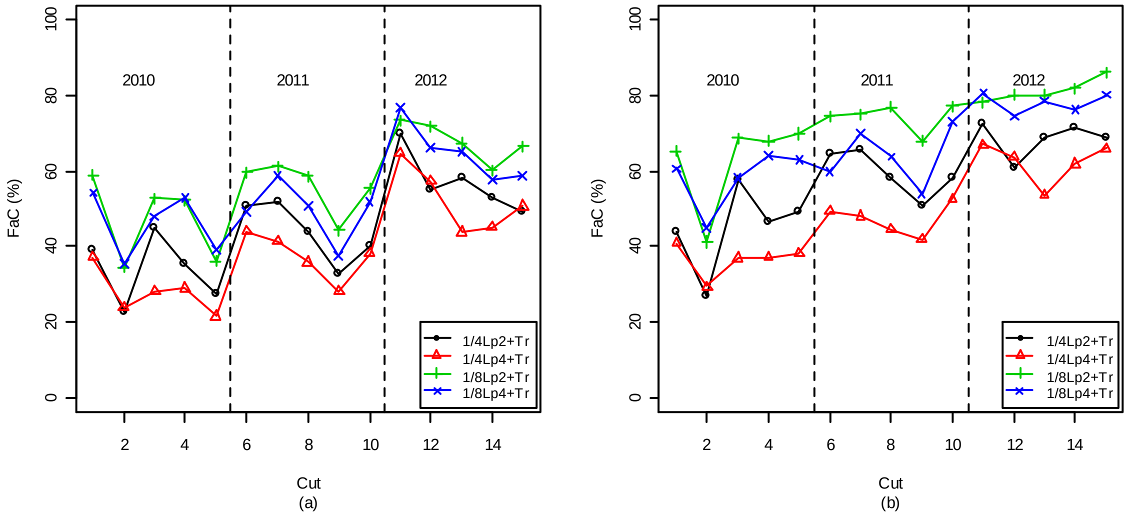


Figure 3.9 Evolution of the *Festuca arundinacea* (Fa) content in the TOTAL dry matter yield (DMY) (a) or in GRASS DMY (b) over 15 cuts in three successive years (2010-2012) in four mixtures of Fa and *Lolium perenne* (Lp) with *Trifolium repens* (Tr) (indicated in table 3.1).

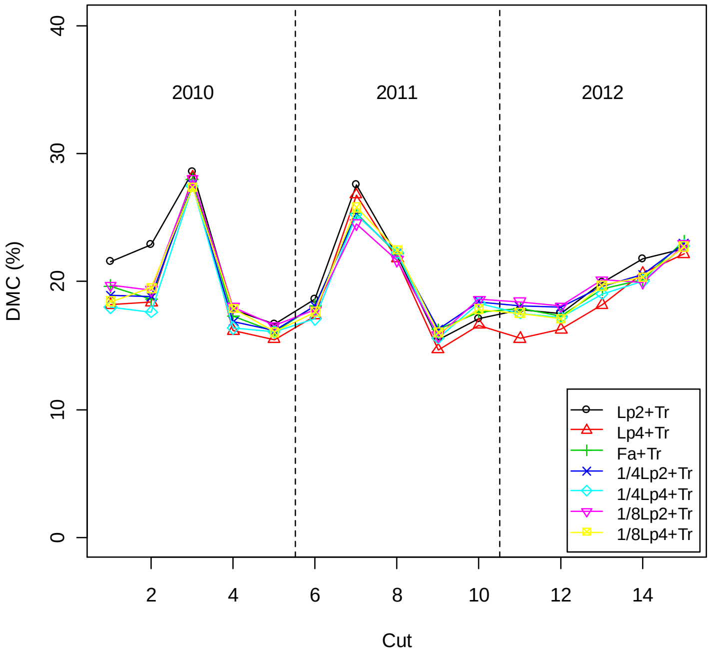


Figure 3.10 Evolution of the dry matter content (DMC) content over 15 cuts in three successive years (2010-2012) for diploid or tetraploid *Lolium perenne* (Lp2 or Lp4), *Festuca arundinacea* (Fa) and mixtures of both grass species with *Trifolium repens* (Tr) (indicated in table 3.1) .

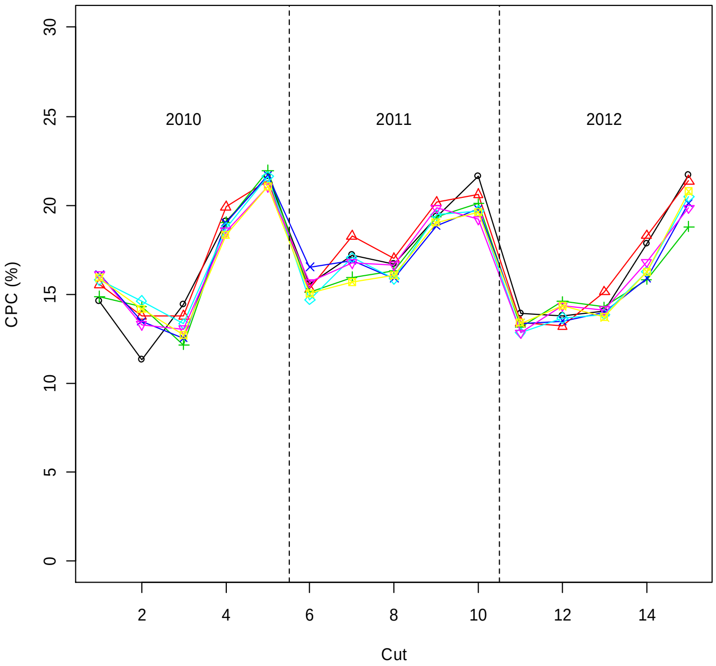


Figure 3.11 Evolution of the crude protein content (DMC) content over 15 cuts in three successive years (2010-2012) for diploid or tetraploid *Lolium perenne* (Lp2 or Lp4), *Festuca arundinacea* (Fa) and mixtures of both grass species with *Trifolium repens* (Tr) (indicated in table 3.1) .

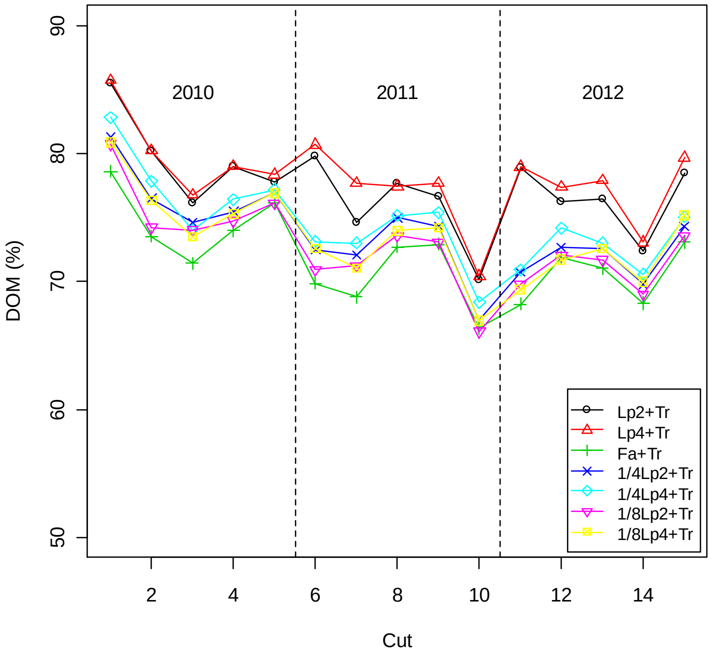


Figure 3.12 Evolution of the digestibility of the organic matter (DOM) content over 15 cuts in three successive years (2010-2012) for diploid or tetraploid *Lolium perenne* (Lp2 or Lp4), *Festuca arundinacea* (Fa) and mixtures of both grass species with *Trifolium repens* (Tr) (indicated in table 3.1).

3.3.2.4 N fixation

The estimated N fixation by the white clover ranged from 142 kg N ha^{-1}yr^{-1} for Lp2+Tr in 2010 to 258 kg N ha^{-1}yr^{-1} for Fa+Tr in 2012. Over the three years period the highest N fixation was found in Fa+Tr: 611 kg N ha^{-1}(**Table 3.9**).

Table 3.9 Calculated N-fixation (kg N ha^{-1}) by *Trifolium repens* (Tr) in association with *Festuca arundinacea* (Fa), diploid and tetraploid *Lolium perenne* (Lp2, Lp4) and mixtures of both species (indicated in table 3.1) as calculated according to the N difference method.

Year	1/4Lp2 + Tr	1/4Lp4	1/8Lp2	1/8Lp4	Fa	Lp2	Lp4
2010	175.7	171.0	180.6	150.2	195.1	142.1	161.0
2011	253.2	238.6	252.6	226.4	257.5	226.6	248.1
2012	173.8	160.6	165.9	178.2	158.8	207.0	200.0
Total	602.7	570.2	599.1	554.8	611.4	575.7	609.1
Relative (Fa = 100)	98.6	93.3	98.0	90.7	100.0	94.2	99.6

3.4 Discussion

3.4.1 Pure grass trial

Averaged over three years, Fa had a 23 % higher DMY than Lp2. This corresponded very well with the average value found for Fa DMY relative to Lp2 DMY under favourable conditions in **chapter 2**. In agreement with the trial of Wilman and Gao (1996), the yield difference between the two species increased with sward age. Organic matter digestibility was consistently lower for Fa compared to Lp, resulting in a weighted average DOM that was 5 % points lower for Fa compared to Lp2 per year. Particularly in the first cut of each year, which is generally the most important both in terms of quality and quantity, differences in digestibility were important: up to 14 % points lower for Fa compared to Lp2 in 2012 when the first cut was delayed due to the wet spring. The high difference in DOM in the first cut can be explained by the earlier growth of Fa compared to Lp (Gilliland *et al.*, 2011) which resulted in a more advanced growth stage for Fa compared to Lp when the first cut was harvested.

The higher DOM and lower DMC we found for Lp4 compared to Lp2 were in accordance with Burns (2012), who found, averaged over several years and varieties, 1.5 % points lower values for DMC and 1 % point higher values for DOM for Lp4 compared to Lp2. In contrast with Burns (2012) dry matter yield of Lp4 in our trial was on average lower compared to Lp2.

Every year, Fa exported more N than the N applied by fertilisation. Lp on the other hand exported up to 58 kg ha^{-1} yr^{-1} less than the applied quantity of N. The difference in NY between Fa and Lp2 was high: up to 27 % of the N dressing in 2012. Since the residual mineral N in the 0-90 cm soil layer, measured after the last cut in 2011 and 2012 was very low (< 5 kg N ha^{-1}) (results not shown) for both species, we hypothesize that more N has been incorporated in stubbles and/or in soil organic matter (SOM) in a Lp sward compared to a Fa sward. The higher tiller density found in Lp swards underpins this assumption. Differences in SOM accumulation between species have been reported earlier: Van Eekeren et al. (2010) found lower SOM and lower soil organic N under a sward of two years old *Dactilys glomerata* compared to Fa and Lp. As root biomass of Fa is expected to be higher than that of Lp, differences in SOM probably originate in leaf turnover, which is known to be faster in Lp than in Fa (Lemaire *et al.*, 2009).

Although we expected Fa and Lp to interact positively due to their complementary growth pattern and rooting depth, no transgressive overyielding (i.e. mixtures more productive than the most productive monospecific sward) was found in the mixtures. The relation between the DMY and the Fa content was linear (+ 28 kg DMY per % point FaC), except in the year 2012 where the yield of the mixtures was higher compared to what we expected from a linear model. This is in line with the results of Wilman and Gao (1996): in their experiment the mixture of Fa and Lp had a yield that was in between that of the pure Fa and Lp. The persistent and consistent transgressive overyielding found by Nyfeler et al. (2009) was mainly explained by the presence of legumes in the mixture rather than by the interaction of different grass species. We also found a strong yield effect of clover in the mixtures: both the DMY as the NY were higher in the grass-clover trial compared to the pure grass trial with half of the N input.

The relation between the DOM and CPC of the mixtures and FaC was also linear: DOM and CPC of the mixtures could be predicted from their botanical composition and the DOM and CPC of the pure swards. The higher DOM for Lp4 compared to Lp2 resulted in a slightly higher DOM in the mixtures with Lp4 compared to Lp2. Owing to the high imprecision of the regression, the relationship between CPC and FaC was inconclusive.

Under favourable conditions, yield and quality parameters of the mixtures were in between the results of the monospecific swards. Under drought stress, Fa suffered much less than Lp, which was reflected in the yield of the mixtures.

In the first year of the experiment, the grass species mixtures were well balanced, but in the last year Fa was dominating the mixtures with at least 70 % Fa in the total DMY. The management in the pure grass trial was more favourable for Fa than for Lp according to Surault *et al.*, (2006) who found that Fa increased in mixtures under high compared to low N fertilisation (250 kg N ha^{-1} *versus* 60 kg N ha^{-1}) and under infrequent compared to frequent cutting (every 45 days *versus* 25 days), which is close to the management in our trial. The effect of the initial proportion and the ploidy of the Lp variety on the Fa content in the mixtures persisted until the final year of the experiment. Lp4 was more resistant to the competition of Fa compared to Lp2. The faster ground cover and establishment after sowing of Lp4 compared to Lp2 can explain this difference. The seasonal pattern in the FaC in the mixtures corresponded with the growth pattern of both species found by Gilliland *et al.* (2011): The higher DM production per unit of photosynthetic radiation of Fa compared to Lp under winter-spring climatic conditions resulted in a sharp increase of FaC in the first cut of every year, followed by a decrease of the FaC in the second cut due to the higher DM production per unit of photosynthetic radiation growth of Fa compared to Lp under conditions met in the spring-summer.

Tiller densities of Fa swards or swards composed of a mixture of Fa and Lp, were approximately 40 % lower compared to Lp2 swards. Fa swards are thus more open than Lp2 swards.

3.4.2 Grass-clover trial

Averaged over three years Fa+Tr yielded 20 % more than Lp2+Tr, which is slightly lower than the yield gain found in the pure grass trial. There was no effect of the grass species or grass species mixture on the clover content in the harvested material. This is in contrast with the findings of Gilliland *et al.* (2010) who found lower clover content in combination with Fa or Lp4 compared to Lp2 averaged over three locations in Ireland over a period of three years. In a trial comparing the effect of different cutting frequencies and N rates on the yield of tall fescue mixed with white clover in Scotland, (Frame, 1973) compatibility between Fa and Tr was weak: with a cutting frequency of 6 cuts per year and an N fertilisation of 161 kg N $ha^{-1}yr^{-1}$ the clover content declined from 17.2 % in the first to only 2.5 % in the second year. Both in our trial as in the trials of Gilliland *et al.* (2010) the compatibility between Fa and Tr seemed to be good.

The effect of the Lp content in the seed mixture remained significant through the three years of the trial, but in contrast to the pure grass trial, the effect of the ploidy of the ryegrass on the Fa content was not significant. The lower N fertilisation in the grass-clover trial had no substantial influence on the evolution of Fa content in the dry matter yield as in both trials the evolution of the Fa content was similar. This is in agreement with Pontes *et al.* (2007), who found no difference in response to high N (360 kg N ha^{-1} yr^{-1}) or low N (120 kg N ha^{-1} yr^{-1}) between Fa and Lp.

Although the presence of the highly digestible clover mitigated the differences in digestibility between Fa and Lp, the differences in DOM between Fa+Tr and Lp2+Tr were comparable with the differences in the pure grass trial. No significant difference was found in CPC between the different sward compositions. Over the three years, the NY in Fa+Tr was 19 % higher than in Lp+Tr. As residual soil nitrogen was below 30 kg N ha^{-1} yr^{-1} in 2011 and below 11 kg N ha^{-1} yr^{-1} (results not shown), we stick to the same hypothesis as formulated in the pure grass trial. Clover content certainly did not influence the results: in none of the years Tr content differed significantly between the sward compositions and in 2011 and 2012, Tr was higher in Lp2+Tr compared to Fa+Tr.

Although the two trials were conducted as different trials, their small area and close vicinity on a very homogeneous soil and their similar management, stimulate to comment on some trends. The presence of the clover in the swards compensated largely for the lower N fertilisation in the grass-clover trial. DMY of the sward compositions in the grass-clover trial were equal or a little higher than the corresponding sward compositions in the pure grass trial. The NY in the grass-clover trial was up to 120 kg N ha^{-1} yr^{-1} higher compared to the same grass sward composition in the same year in the pure grass trial, with a fertilisation that was 135 kg N ha^{-1} yr^{-1} lower.

The N fixations we calculated were higher than the values found by other authors under a similar management. In a three years trial in the North of Germany Ingwersen (2002) found average N fixations between 32.6 and 95.4 kg N ha^{-1} yr^{-1} in perennial ryegrass - white clover swards with a mineral N fertilisation of 100 kg N ha^{-1} under a cutting management (4 cut yr^{-1}). Boller *et al.* (1987) found values for N fixation between 50 and 165 kg N ha^{-1} in perennial ryegrass white clover mixtures in Switzerland with a fertilisation of 120 kg N ha^{-1} and 5 cuts per year.

Particularly when expressed in terms of N fixation per unit of clover DMY, the values in our trial were high. We found 67-103 kg N (ton clover DMY)$^{-1}$ compared to 26-36 kg N (ton clover DMY)$^{-1}$ in Boller *et al.* (1987), 54-60 kg N (ton clover DMY)$^{-1}$ in Ingwersen (2002) or 31 kg N (ton clover DMY)$^{-1}$ in the review of Carlsson and Huss-Danell (2003). Climate, soil and previous crops may explain the differences. The soil in Boller *et al.* (1987) had a high soil organic matter (SOM) content compared to the soil of our experiment (3.4 % *versus* 1.4 % SOM). The small amount of mineralized N from SOM might have boosted the N fixation in our trial.

The DMY of Fa+Tr was 20 % higher than that of Lp2+Tr over the three years period. This difference was higher than that found by Gilliland *et al.* (2010). Over a three years period, averaged over three locations in Ireland they found 9.6 %, 7.8 % and 11.9 % higher dry matter yield for Fa compared to Lp2 in combination with white clover and with an annual N fertilisation of respectively 105 kg N ha^{-1}, 210 kg N ha^{-1} and 420 kg N ha^{-1} and a cutting management of 10 cuts yr^{-1}. The higher cutting frequency in the trials of Gilliland *et al.* (2010) may be responsible for lower yield differences than in our trial. Indeed, Pontes *et al.* (2007) found that the herbage yield of grass species like Fa and *Holcus lanatus* L., that are highly productive under a low cutting frequency, was more reduced under a high cutting frequency than that of Lp. Furthermore, the higher clover content in the sward with Lp compared to the sward with Fa in Gilliland *et al.* (2010), and the wetter climate in Ireland may also contribute to this difference.

3.5 Conclusion

Regarding our research hypotheses, we can conclude as follows:

1. We found no higher yield in the species mixtures compared to the monospecific swards. The yield of the mixtures was in between that of the single species. The effect of mixing the species was mainly additive without positive nor negative interactions.
2. Although the species composition in the mixtures was well balanced in the first cuts, the tall fescue content quickly rose, to dominate the mixtures from the second year on. The effect of the initial proportion of Lp in the seed mixture remained significant until the third year. The results of our trial suggest that under the management we applied, the proportion of Lp in the seed mixture should be higher than 1/4 to obtain an evenly mixed sward in the mid-long term. The exact proportion however can not be recommended based on the results of this study.
3. Contrary to what we expected, tetraploid perennial ryegrass competed better with Fa than diploid Lp.
4. The presence of white clover and a lower N fertilisation did not alter the interaction between Fa and Lp. The grass DMY continued to be dominated by Fa from the third year on and tetraploid Lp competed better with Fa than diploid Lp.

Chapter 4

Performance and quality of tall fescue and Italian ryegrass and mixtures of both species grown with or without red clover under cutting management

Redrafted after: Cougnon M., Van Waes C., Baert J., Poinsard L. and Reheul D., 2013. Rendement de mélanges de fétuque élevée et de ray-grass d’Italie avec ou sans trèfle violet en Belgique. In revision after submission to *Fourrages* April 2013.

4.1 Introduction

Interest in tall fescue (*Festuca arundinacea* Schreb.; **Fa**) in North-West European conditions is increasing for two main reasons (Reheul *et al.*, 2012). Firstly, the good drought resistance of Fa compared to other grass species can overcome dry periods that are believed to become more frequent due to climate change (**Chapter 1**).

Secondly, dairy production in western Europe is evolving towards less grazing for different reasons (Van den Pol-van Dasselaar *et al.*, 2008; Van den Pol-van Dasselaar, 2012). A result of this evolution is that more grass is cut and fed fresh or preserved as silage or hay. Italian ryegrass (*Lolium multiflorum* L.; **Lm**), tall fescue and *Festulolium* are the species that are traditionally recommended for cutting only management (Frame, 1992). Lm is praised for its very easy establishment and its high yield in the year of sowing. It produces highly nutritious and digestible forage. Disadvantages of Lm are its relative lack (depending on the varieties) of persistency, its susceptibility for crown rust (*Puccinia coronata* Corda) and its low drought resistance. Fa on the other hand establishes slowly, with a low yield in the year of sowing, but once established Fa is very high yielding, persistent and resists well to abiotic stress like drought, frost and flooding. Apart from the slow establishment, the main disadvantages of tall fescue are the lower palatability and digestibility compared to ryegrasses (**Chapter 1**).

A consequence of the the lower voluntary intake and the lower digestibility of Fa is that the higher herbage production of Fa is not necessarily translated into a higher animal production (Easton *et al.*, 1994). In a Dutch study (Luten and Remmelink, 1984), voluntary intake and milk production of cows fed with cut Fa, perennial ryegrass and Lm were compared for four years. When fed fresh, mostly a significantly lower intake and lower milk production was found for Fa compared to the ryegrasses (**Chapter 2**). On average, the dry matter intake was 13.7 kg day^{-1} for Fa and 14.7 kg day^{-1} for Lm resulting in a milk production of respectively 17.3 kg cow^{-1}day^{-1} and 18.2 kg cow^{-1}day^{-1}. When silage of the different species was fed, the differences in intake between the species disappeared.

Altough much breeding work was done to increase crown rust resistance, crown rust remains an important threat for the productivity of Lm swards in the late summer (Oertel *et al.* 1999; Schubinger *et al.*, 2012; Chaves *et al.*, 2009). Fa is also prone to crown rust infection and some genotypes are very susceptible, nevertheless it generally suffers less from rust infection than the ryegrasses (own observations).

In the same developmental stage, the dry matter content (DMC) of Fa is higher than that of Lm. At the end of the stem elongation phase, Fa has a DMC that is on average 2.8 % point higher than that of diploid Lm (INRA, 1978). Partly due to this higher DMC of Fa, the drying time to reach 33 % DM under controlled circumstances was found to be shorter for Fa compared to Lm (Jones and Prickett, 1981): the drying time needed to reach a DMC of 33 % for Fa was in all developmental stages around 20 h whereas the drying time needed to reach a DMC of 33 % for Lm was between 25 and 80 h depending on the developmental stage. This high drying rate makes Fa particularly suited for hay or silage making.

It is clear that *Festuca* sp. and *Lolium* sp. in general and especially the species Fa and Lm have complementary properties. Both can be combined genetically in the form of *Festulolium* (Thomas *et al.*, 2003; Humphreys *et al.*, 2012) or either physically by sowing mixtures of both species (**Chapter 1**).

Wilman and Gao (1996) compared the yield of monospecific swards of Fa and Lm and of a mixture of both species in which seed proportions were equal. Over a 5 years period, the yields of Fa and Lm were 77.0 t DM ha^{-1} and 75.1 t DM ha^{-1} respectively, whereas the mixture of both species yielded 78.1 t DM ha^{-1}, which is a yield gain of hardly 1.5 % compared to the most productive monospecific sward. In the sowing year, the yield of Fa was 27 % lower than that of Lm. In the fifth year of the experiment on the other hand, Fa yielded 31 % more than Lm in which the yield was partially maintained by the invasion of unsown species. The yield of the mixture was 93 % of the yield of the Lm monospecific sward in the year of sowing and 92 % of the yield of the Fa monospecific sward in the last year of the experiment. Tiller densities of the different species in the mixture were recorded in the first three years of the experiment. The proporition of the Fa tillers on the total amount of grass tillers in the mixture increased from 6.4 % in the year of sowing to 17.8 % in the third year of the experiment. The uneven distribution of both species in the mixture might have precluded the yield advantage of the mixtures compared to the monospecific swards. As both species were equally represented in the seed mixture, Lm was initially favoured as this species is developing much faster than Fa after sowing.

Adding a leguminous crop to a mixture of grass species generally has a net positive effect on the performance of the mixture (Nyfeler *et al.*, 2009; Lüscher *et al.*, 2012). The legimunous crops that are generally recommended for cutting management are lucerne (*Medicago sativa* L.) and red clover (*Trifolium pratense* L.; **Tp**) (Deprez *et al.*, 2004b; Frame, 1992). Persistence used to be problematic in both species, but persistence of the latest varieties of red clover has improved substantially. Marshall *et al.* (2012) compared the yield of twelve red clover varieties in combination with a companion grass in Wales, UK. In the three production years of the trial, the clover variety had a significant effect on the yield of the mixture. In the third production year the most persistent clover variety had a contribution to the yield of the mixture that was 2.6 times higher than that of the least persistent variety.

In our study, monospecific swards of Fa and Lm and different mixtures of both grass species were compared. The mixtures differed in the ploidy of the Lm variety, and the proportion of Lm seeds in the seed mixture. Moreover, two management types were applied: plots were either fertilised with a high mineral N dose or with a lower mineral N but sown in combination with Tp. Yield, botanical composition and quality parameters were followed for three successive years.

Our research hypotheses were that:

1. Mixtures of Lm and Fa are combining the advantages of both species, resulting in a higher and more stable yield compared to the monospecific swards.
2. A small fraction of Lm in the seed mixture offers good opportunities to obtain a sward with an even species composition after three years.
3. Mixing Fa with a tetraploid Lm variety offers Fa the best opportunities to develop owing to the open growth habit of tetraploid Lm.
4. Adding red clover to the mixture further increases the mixture effect and influences the interaction between Fa and Lm.

4.2 Materials and Methods

4.2.1 Trial design

The trial was sown on the 10th of September 2009 on a sandy loam soil in Merelbeke, Belgium. The land had been used for three years as arable land before the trial was established. In September 2007, the pH(KCl) was 5.9, the carbon content of the soil was 0.6 %. The trial consisted of two adjacent subtrials; a “**pure grass trial**” and a “**grass-clover**” trial. Both trials were randomized complete block designs with three replicates; plot size was 7.8m². In the pure grass trial, monospecific swards of Fa *cv.* ‘Castagne’ (Fa), diploid Lm (Lm2) *cv.* ‘Melclips’ and tetraploid Italian ryegrass (Lm4) *cv.* ‘Melquatro’ were compared with mixtures of Fa and Lm. Mixtures were created by substituting 1/4 or 1/8 of the Fa seeds by Lm2 or Lm4 seeds (**Table 4.1**). Sowing densities were 1500 germinating seeds m^{-2} in all cases. Annual fertilisation was 300 kg N ha^{-1}, 10kg P ha^{-1} and 270 kg K ha^{-1}. The concept of the grass-clover trial was identical to the concept of the pure grass trial, but all seeding densities were supplemented with 600 germinating seeds m^{-2} (approximately 12 kg ha^{-1}) of the red clover variety ‘Merviot’. Annual fertilisation was 120 kg N ha^{-1}, 35 kg P ha^{-1} and 270 kg K ha^{-1}. Throughout the text the abbreviations indicated in **table 4.1** will be used to name the different sward compositions in the trials.

Table 4.1 Sowing density (number of germinating seeds m^{-2}) for *Festuca arundinacea* (Fa) diploid and tetraploid *Lolium multiflorum* (Lm2, Lm4) and mixtures of both grass species with or without *Trifolium pratense* (Tp).

Sward compositions	Tall fescue	Diploid Italian ryegrass	Tetraploid Italian ryegrass	Red clover
Pure grass trial:				
Fa	1500	0	0	0
Lm2	0	1500	0	0
Lm4	0	0	1500	0
1/8Lm2	1312	188	0	0
1/8Lm4	1312	0	188	0
1/4Lm2	1125	375	0	0
1/4Lm4	1125	0	375	0
Grass-clover trial:				
Fa+Tp	1500	0	0	600
Lm2+Tp	0	1500	0	600
Lm4+Tp	0	0	1500	600
1/8Lm2+Tp	1312	188	0	600
1/8Lm4+Tp	1312	0	188	600
1/4Lm2+Tp	1125	375	0	600
1/4Lm4+Tp	1125	0	375	600

4.2.2 Measurements

Five cuts were harvested per year in 2010, 2011 and 2012 (**Table 4.2**). Harvest protocol, determination of nutritive quality, botanical composition and N fixation were as described in the ‘Material and Methods’ section of **chapter 3**.

Additionally, after the last cut in 2012, each plot in the pure grass trial was scored visually on a 1-5 scale (where 1=50 % soil cover and 5=80 % soil cover) for sward density.

Meteorological data were obtained from an official meteorological station at approximately 3 km from the experimental site (**Appendix 1**). Several dry spells and cold periods occurred during the experimental period. Details can be found in the 'Material and Methods' section of **chapter 3**.

Table 4.2 Harvesting dates in 2010-2012

Cut	2010	2011	2012
1	22 April	26 April	2 May
2	27 May	30 May	13 June
3	28 June	27 June	23 July
4	9 August	1 August	22 August
5	27 September	29 September	8 October

4.2.3 Data analysis

Same procedures were followed as described in 'Materials and Methods' section of **chapter 3**.

4.3 Results

4.3.1 Pure grass trial

4.3.1.1 DM yield

Both species established well during the autumn and survived well the winter of 2009-2010. In 2010, the first production year, Fa yielded significantly less than Lm, due to its low DMY in the first three cuts. Although Fa yielded significantly more than Lm in the last two cuts of 2010, this was insufficient to compensate for the lower DMY in the first cuts. For the whole year, Fa yielded 14000 kg DM ha^{-1} against 17000 kg DM ha^{-1} for Lm2. All of the mixtures had a higher DMY than the single grass species in 2010: they combined both the good DMY of Lm in the first cuts and that of Fa in the last two cuts. The highest yield was obtained with the mixture 1/8Lm4 with a yield of 17572 kg DM ha^{-1} (**Table 4.3**).

In 2011, Fa yielded slightly more than Lm, but the difference was not significant. Fa overyielded Lm2 in the second cut, and Lm2 and Lm4 in the fourth and the fifth cut. As in 2010, the highest DMY was again obtained by the mixture 1/8Lm4 with 17926 kg DM ha^{-1}.

The three other mixtures yielded less than the lowest yielding single grass species (Lm4) (**Table 4.3**).

Table 4.3 Dry matter yield (kg ha^{-1}) for *Festuca arundinacea* (Fa) diploid and tetraploid *Lolium multiflorum* (Lm2, Lm4) and mixtures of both species (indicated in table 4.1) in 15 successive cuts (C1-C15) in three successive years (2010-2012).

	1/4Lm2	1/4Lm4	1/8Lm2	1/8Lm4	Fa	Lm2	Lm4	Sign.^{A}
2010								
C1	3763 ^{a}	3562 ^{a}	3503 ^{a}	3501 ^{a}	2134 ^{b}	4370 ^{a}	4092 $^{.a}$	***
C2	4731 ^{a}	4889 ^{a}	4632 ^{a}	4720 ^{a}	3369 ^{b}	4486 ^{a}	4550 ^{a}	***
C3	3718 ^{a}	3985 ^{a}	3558 ^{a}	4162 ^{a}	2137 ^{b}	3661 ^{a}	3775 ^{a}	**
C4	1986 ^{ab}	1937 ^{ab}	2216 ^{ab}	2051 ^{ab}	2419 ^{a}	1621 ^{b}	1782 ^{ab}	*
C5	3120 ^{b}	3049 ^{b}	3173 ^{b}	3139 ^{b}	3940 ^{a}	2870 ^{b}	2743 ^{b}	**
Subtotal	17317 ^{a}	17422 ^{a}	17082 ^{ab}	17572 ^{a}	14000 ^{b}	17009 ^{ab}	16942 ^{ab}	**
Relative (Fa = 100)	123.7	124.4	122.0	125.5	100.0	121.5	121.0	
2011								
C6	4906	5566	5058	6031	4294	6880	6585	NS
C7	2175 ^{b}	2348 ^{ab}	2251 ^{ab}	2477 ^{ab}	2865 ^{a}	2183 ^{b}	2494 ^{ab}	*
C8	2040 ^{a}	1879	2024	2033	1954	2068	1951	NS
C9	1922 ^{bc}	1851 ^{bc}	2161 ^{ab}	1823 ^{bc}	2454 ^{a}	1612 ^{c}	1711 ^{c}	***
C10	5195 ^{abc}	5342 ^{abc}	5132 ^{abc}	5561 ^{ab}	5909 ^{a}	4611 ^{b}	4175 ^{c}	**
Subtotal	16238	16985	16626	17926	17476	17354	16915	NS
Relative (Fa = 100)	92.9	97.2	95.1	102.6	100.0	99.3	96.8	
2012								
C11	4438	4459	4187	4682	4291	4385	3985	NS
C12	6375	6315	6030	6360	5837	6194	5927	NS
C13	5130	4961	4853	4846	4675	5145	4842	NS
C14	2109	2096	2181	2100	2434	1973	1921	NS
C15	1664^{ab}	1738^{ab}	1768^{ab}	1716^{ab}	2056^{a}	1357^{b}	1472^{b}	**
Subtotal	19716	19569	19018	19703	19294	19054	18467	NS
Relative (Fa = 100)	102.2	101.4	98.6	102.1	100.0	98.8	95.7	
Total	53271	53977	52726	55201	50770	53417	52004	NS
Relative (Fa = 100)	104.9	106.3	103.9	108.7	100.0	105.2	102.4	

A Significance of the anova model with the seven sward compositions as fixed factor and the three replicates as random factor. (Significance codes: ***: $p < 0.001$, **: $p < 0.01$, *: $p < 0.05$, NS: $p > 0.05$.). Values in the same row not sharing a same superscript are significantly different from each other ($p = 0.05$, Tukey test).

In 2012 there were no significant DMY differences between the mixtures or the monospecific swards but Fa was yielding 1.2 % more than Lm2 and 4.2 % more than Lm4. Fa was yielding significantly more than Lm in the last cut. Mixture 1/8Lm4, the highest yielding mixture in 2010 and 2011, overyielded again the grass monospecific swards, but it was slightly overyielded by the mixture 1/4Lm2 (**Table 4.3**).

Over 15 cuts in the three growing seasons, DMY did not differ significantly between the species or the mixture. Fa had a DMY of 50770 kg DM ha^{-1}, Lm2 of 53417 kg DM ha^{-1} and Lm4 of 52004 kg DM ha^{-1}, but the highest yield was obtained with the mixture 1/8Lm4 with 55201 kg DM ha^{-1}. This mixture combined the high yield of Lm in the first cuts and the good yield of Fa in late summer: DMY of the first cut in 2010 was 85 % of the DMY of Lm4, while DMY of the final cut in each year was at least 80 % of the yield of pure Fa (**Table 4.3**). This resulted in a total DMY that was 8.7 % higher than Fa, and 6.1 % higher than Lm4. High DMYs of Fa in year 2 and 3 could not compensate for the low DMY in the first growing season, resulting in the lowest DMY for Fa. In all cuts, the DMY of Lm4 tended to be lower than that of Lm2 but in none of the cuts this difference was significant. Over all cuts, Lm2 was yielding 2.7 % more than Lm4 (**Table 4.3**).

The linear model that described the effect of the Fa content in the DMY (FaC) on total DMY contained a different intercept and a different quadratic term for each year (**Table 4.4**). The model explained 58 % (R^2 value) of the variation in DMY between the different sward compositions. The convex shape of the graphs of 2011 and 2012 in **figure 4.1**, suggest that there was generally a negative effect of mixing Fa and Lm in these years. In 2010 on the other hand, the graph was concave, suggesting a positive effect of the mixtures (**Figure 4.1**).

Three of the fifteen cuts were preceded by a drought period. In the third cut of 2010, the drought did not seem to hamper growth of Lm: Lm was overyielding Fa. In the second cut of 2011, Fa was the highest yielding sward composition, with a DMY that was significantly higher than that of Lm2, whereas in 2010 and 2012, when the second cut was not preceded by drought, Lm yielded more than Fa. In the fifth cut of 2012, the DMY of Fa was 52 % higher than that of Lm2. But it is not certain that this higher DMY of Fa can be ascribed to the higher drought resistance of Fa as in 2010 and 2012, Fa was also yielding more than Lm in the fifth cut.

Table 4.4 Regression coefficients for minimal adequate Ancova models for the dry matter yield (DMY), digestibility of the organic matter (DOM) and crude protein content (CPC) of mixtures of *Festuca arundinacea* (Fa) and *Lolium multiflorum* (Lm) as a function of the Fa content (FaC) in the DMY. ($Y= I+a.(FaC)+b.(FaC)^2$) (Significance codes: ***: $p < 0.001$, **: $p < 0.01$, *: $p < 0.05$, NS: $p > 0.05$).

	Year	I	a	b	R^2
DMY	2010	17300***	/[A]	-0.33***	
	2011	17001***	/[A]	$3.08*10^{-2}$***	0.58
	2012	19105***	/[A]	$3.72*10^{-2}$***	
DOM[C]	2010	75.95***	$5.64*10^{-2}$***	$3.43*10^{-4}$***	
	2011	75.26*	$5.64*10^{-2}$***	$-1.70*10^{-6}$***	0.83
	2012	73.72***	$5.64*10^{-2}$***	$3.43*10^{-4}$***	
CPC[C]	2010	11.87***	$1.40*10^{-2}$***	/[B]	
	2011	14.57***	$-1.10*10^{-2}$***	/[B]	0.90
	2012	10.5***	$1.40*10^{-2}$***	/[B]	

A: / : the linear term was not included in the minimum adequate model
B: / : the quadratic term was not included in the minimum adequate model
C: regression based on weighted average over all cuts.

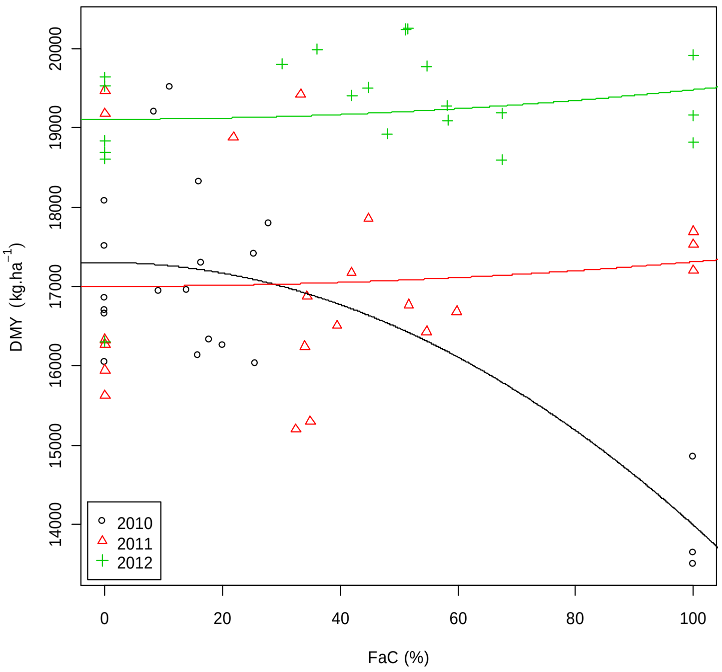


Figure 4.1 Regression of the dry matter yield (DMY) on the *Festuca arundinacea* content in the DMY (FaC) in mixtures of Fa and *Lolium multiflorum*.

4.3.1.2 Botanical composition

Although the FaC was increasing over the years, it was generally lower than the sown proportion. In 2010, FaC was between 11.1 % in the mixture 1/4Lm4 and 26.1 % in the mixture 1/8Lm2 whereas the proportion of Fa seeds in these mixtures was 75.0 % and 87.5 % respectively (**Table 4.5**). Within each year, the FaC was decreasing in the first three cuts, and increased sharply in the last two cuts (**Figure 4.2**). This pattern is in accordance with the observations of DMY: In the first cut, the mixtures behave more like Italian ryegrass whereas in the later cuts, Fa becomes more dominant. Over the three years, the highest Fa proportion was found in 1/8Lm2. In every year, the effect of the initial Fa proportion in the seed mixtures on FaC was significant: the higher the initial Fa proportion, the higher FaC. In 2010 and 2011, the effect of ploidy of the Lm variety in the mixtures was significant: a higher FaC in combination with Lm2 compared to Lm4. Over the three years, both the effects of the initial proportion of Lm in the seed mixture as the ploidy of the Lm were significant (**Table 4.6**).

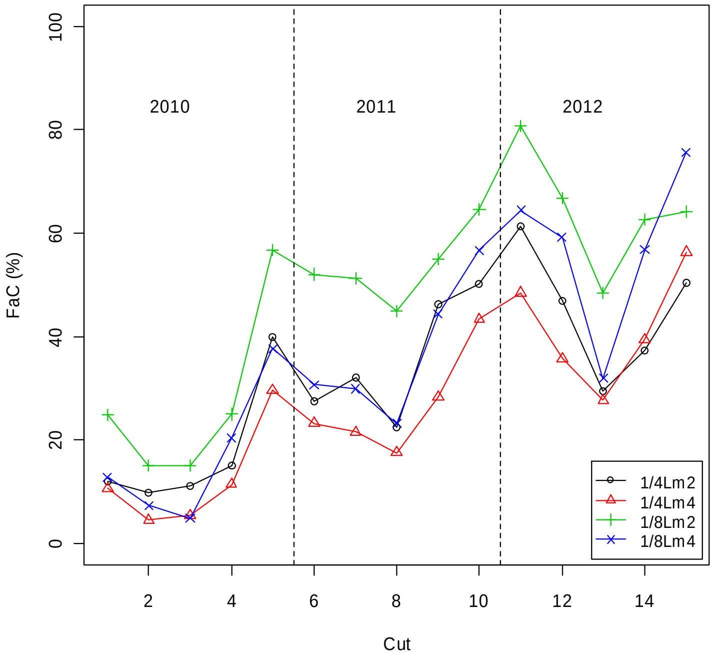


Figure 4.2 Evolution of the *Festuca arundinacea* content in the dry matter yield (FaC) over 15 cuts in three successive years (2010-2012) for four different mixtures of *Festuca arundinacea* (Fa) and *Lolium multiflorum* (Lm) (indicated in table 4.1).

Table 4.5 *Festuca arundinacea* content in the dry matter yield (FaC) (%), dry matter content (DMC) (%), digestibility of the organic matter (DOM) (%), crude protein content (CP) (%) and N yield (NY) (kg N ha^{-1}) for *Festuca arundinacea* (Fa) diploid and tetraploid *Lolium multiflorum* (Lm2, Lm4) and mixtures of both species (indicated in table 4.1) in three successive years (2010-2012). Data are weighted averages over all cuts.

		1/4Lm2	1/4Lm4	1/8Lm2	1/8Lm4	Fa	Lm2	Lm4	Sign.^{A}
2010									
FaC	(%)	16.7^{b}	11.1^{b}	26.1^{a}	14.9^{b}	$100^{\#}$	$0^{\#}$	$0^{\#}$	**
DMC	(%)	19.7^{ab}	18.7^{ab}	20.2^{a}	18.9^{ab}	20.7^{a}	18.5^{ab}	17.4^{b}	**
DOM	(%)	74.9^{abc}	75.4^{abc}	74.3^{bc}	74.9^{abc}	73.8^{c}	76.2^{ab}	76.5^{a}	**
CPC	(%)	12.1^{b}	11.9^{b}	12.0^{ab}	12.4^{b}	13.3^{a}	11.9^{b}	11.8^{b}	**
NY	(kg ha^{-1})	336.3	331.8	328.4	347.8	298.9	325.1	320.4	NS
2011									
FaC	(%)	37.0^{b}	29.6^{b}	55.4^{a}	39.2^{b}	$100^{\#}$	$0^{\#}$	$0^{\#}$	***
DMC	(%)	20.2^{abc}	19.5^{bc}	21.4^{ab}	20.9^{abc}	22.2^{a}	20.7^{bc}	18.6^{c}	**
DOM	(%)	73.0^{b}	73.6^{b}	72.1^{b}	72.9^{b}	69.5^{c}	75.3^{a}	75.6^{a}	***
CPC	(%)	14.7^{a}	14.1^{ab}	13.9^{ab}	13.9^{ab}	13.5^{b}	14.6^{a}	14.5^{a}	**
NY	(kg ha^{-1})	381.6	383.1	368.7	397.7	376.8	406.0	391.9	NS
2012									
FaC	(%)	44.6^{b}	37.2^{b}	64.4^{a}	52.8^{ab}	$100^{\#}$	$0^{\#}$	$0^{\#}$	*
DMC	(%)	21.1^{a}	20.3^{ab}	21.7^{a}	21.1^{a}	21.6^{a}	20.5^{a}	18.6^{b}	***
DOM	(%)	71.7	72.5	71.5	71.6	70.7	73.7	73.2	NS
CPC	(%)	11.4^{ab}	10.9^{ab}	11.3^{ab}	11.6^{ab}	12.0^{a}	10.6^{ab}	10.3^{b}	*
NY	(kg ha^{-1})	360.5^{a}	340.9^{ab}	342.9^{ab}	364.3^{a}	371.2^{a}	321.8^{ab}	298.6^{b}	*

A: Significance of the anova model with the seven sward compositions as fixed factor and the three replicates as random factor. (Significance codes: ***: $p < 0.001$, **: $p < 0.01$, *: $p < 0.05$, NS: $p > 0.05$.). Values in the same row not sharing a same superscript are significantly different from each other ($p = 0.05$, Tukey test).

The botanical composition of the monospecific swards was not taken into account in the statistical analyses.

Table 4.6 Effect of ploidy and proportion of *Lolium multiflorum* (Lm) in the initial seed mixture and the interaction of both factors on the *Festuca arundinacea* content (FaC) in the total DMY of swards sown with mixtures of both species (indicated in table 4.1). (Significance codes: ***: $p < 0.001$, **: $p < 0.01$, *: $p < 0.05$, NS: $p > 0.05$).

	Pure grass trial			Grass-clover trial		
Year	Ploidy	Proportion	Interaction	Ploidy	Proportion	Interaction
2010	**	**	NS	*	**	NS
2011	***	***	*	NS	***	*
2012	NS	**	NS	NS	*	NS
2010-2012	***	***	NS	NS	**	NS

4.3.1.3 Quality

Fa had a higher DMC than Lm in almost every single cut (**Figure 4.3**). Only in some summer cuts (e.g. in the 4th cut of 2010 and 2012), DMC of Lm was higher due to crown rust infection. Over a whole year, Fa had a DMC that was at least 1 % point higher than that of Lm2 and 3 % point than that of Lm4 (**Table 4.5**).

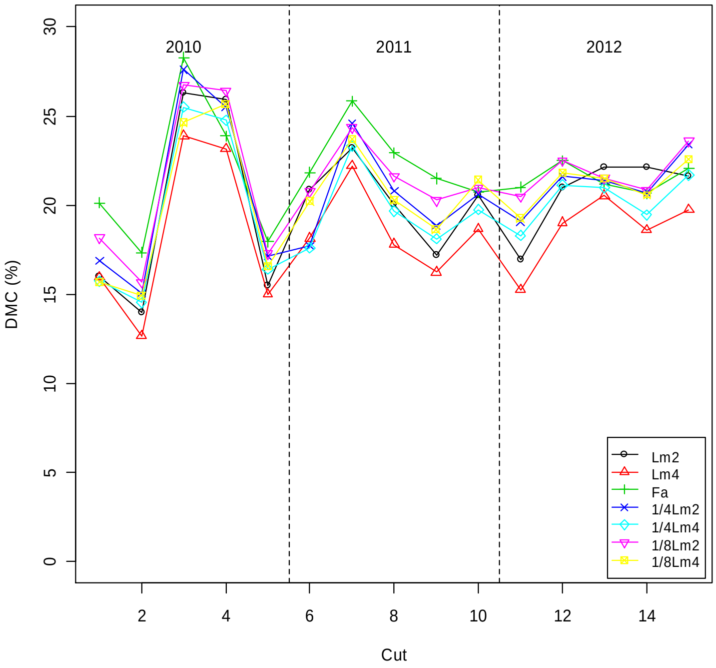


Figure 4.3 Evolution of the dry matter content (DMC) over 15 cuts in three successive years (2010-2012) for diploid or tetraploid *Lolium multiflorum* (Lm2 or Lm4), *Festuca arundinacea* (Fa) and mixtures of both species (indicated in table 4.1).

Each year, the DOM was between 3 and 6 % points lower for Fa compared to Lm (**Table 4.5**); this difference was significant in 2010 and 2011. The greatest differences in DOM between Fa and Lm were found in the first cut of each year: in the first cut of 2011 the DOM of Fa was 8 % points lower than that of Lm4, in 2012 the difference was even 11 % points. As the first cut is very important in terms of DMY, this had a great impact on the DOM of the total DMY (**Figure 4.4**). In later cuts, the differences were much smaller, and in some summer cuts (for example the fourth cut in 2010 and third cut in 2012), the digestibility of Fa was higher than that of Lm because of flowering stems in Lm which did not occur in Fa (**Figure 4.4**).

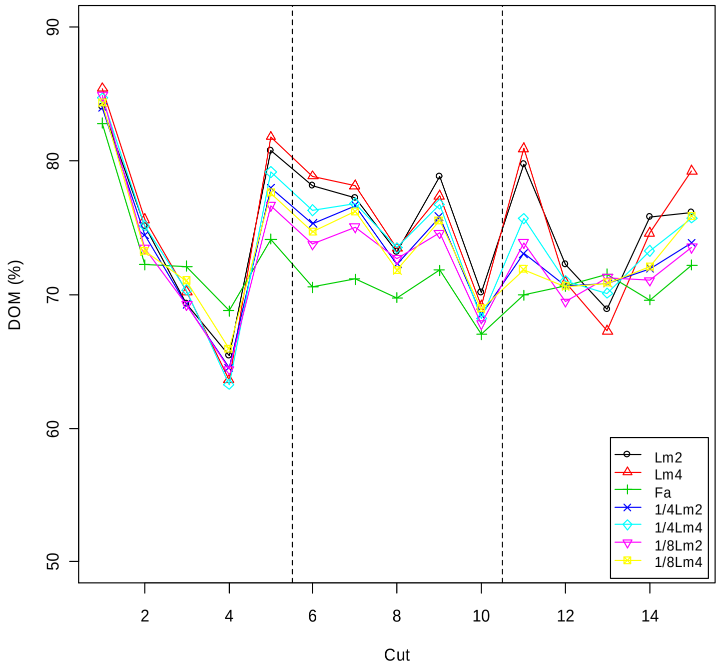


Figure 4.4 Evolution of the digestibility of the organic matter (DOM) over 15 cuts in three successive years (2010-2012) for diploid or tetraploid *Lolium multiflorum* (Lm2 or Lm4), *Festuca arundinacea* (Fa) and mixtures of both species (indicated in table 4.1).

In 2010 and 2012, CPC of Fa was significantly higher than that of Lm, whereas the inverse was true in 2011 (**Table 4.5**). The CPC of Fa fluctuated less than that of Lm (**Figure 4.5**). Although Fa had a significantly higher CPC in 2010, its NY was lower than that of Lm due to its lower DMY. In 2012, the highest DMY in combination with the highest CPC of Fa resulted in a NY that was more than 50 kg N ha^{-1} higher than that of Lm. NY was always higher than N fertilisation: a NY up to 400 kg N ha^{-1} was measured in 2011, whereas N fertilisation was 300 kg N ha^{-1} (**Table 4.5**).

DMC, DOM and CPC of the mixtures were intermediate to that of the monospecific swards. In the linear model describing the effect of FaC in the mixtures on total DOM, both linear as quadratic effects were significant for each year (**Table 4.4**). The model explained 83 % (R^2 value) of the variation in DOM between the different sward compositions. The convex shape of the graphs of 2010 and 2012 in **figure 4.6a**, suggest that there was generally a negative effect of mixing the Fa and Lm on DOM in these years.

The effect of FaC on CPC contained no significant quadratic effect and was purely linear, with a different intercept for every year (**Table 4.4**, **Figure 4.6b**). The same positive slope was found for 2010 and 2012: an increase of FaC with 1 % point resulted in an CPC increase of 0.014 % points. A negative slope was found for 2011: an increase of FaC with 1 % point resulted in an CPC decrease of 0.011 % points. The model explained 90 % (R^2 value) of the variation in CPC in the mixtures.

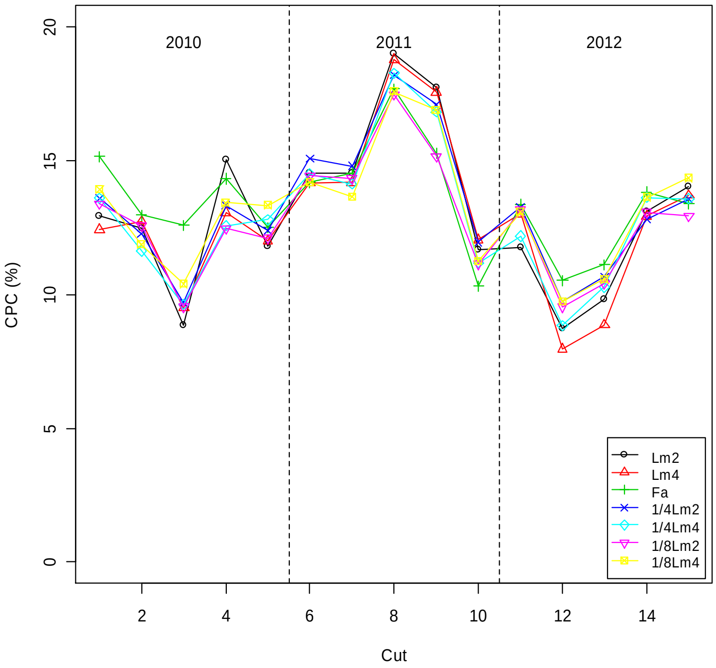


Figure 4.5 Evolution of the crude protein (CPC) content over 15 cuts in three successive years (2010-2012) for diploid or tetraploid *Lolium multiflorum* (Lm2 or Lm4), *Festuca arundinacea* (Fa) and Fa, Lm2 and mixtures of both species (indicated in table 4.1).

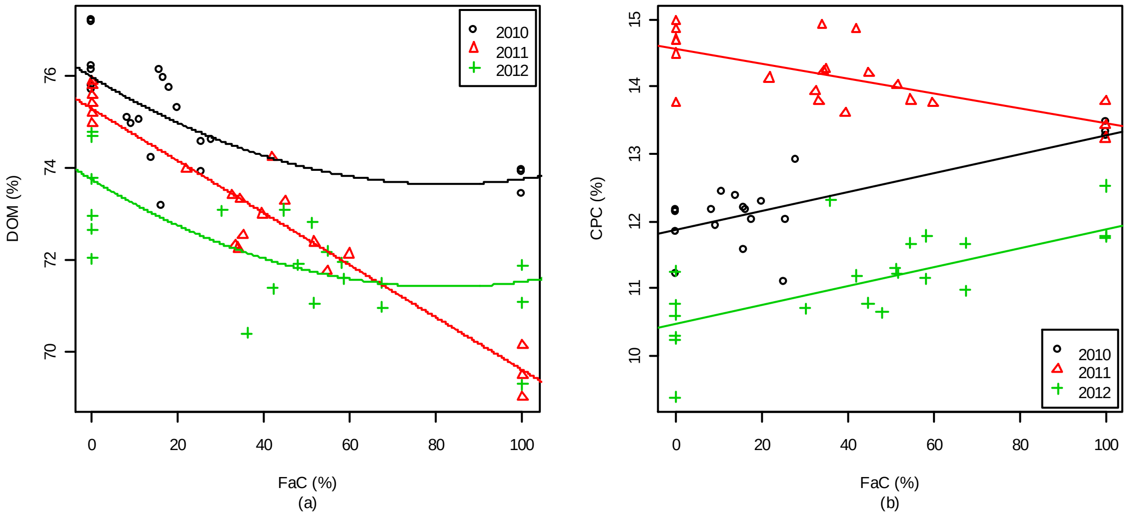


Figure 4.6 Regression of the digestibility of the organic matter (DOM) (a) and crude protein content (CPC) (b) on the *Festuca arundinacea* content (FaC) in mixtures of Fa and *Lolium multiflorum*

4.3.1.4 Tiller density and ground cover

At the end of the trial in October 2012, the tiller densities of Fa, Lm2 and the four mixtures were on the same level: 3067 tillers m^{-2} for Fa and 3134 tillers m^{-2} for Lm2. The lowest tiller density was found for Lm4 (1805 tillers m^{-2}): significantly lower tiller density compared to 1/4Lm2 (**Figure 4.7**). The number of Fa tillers in the mixtures was not significantly lower compared to the pure Fa sward (p = 0.201).

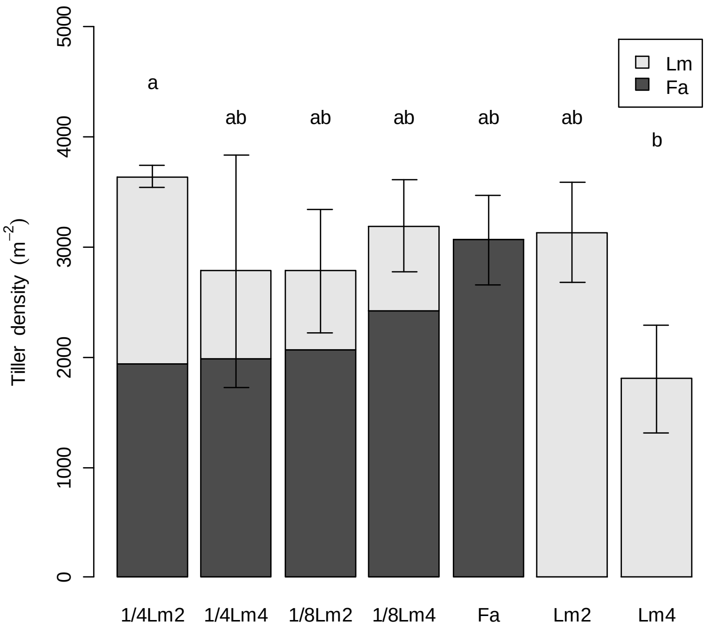


Figure 4.7 Tiller density (October 2012) in swards of *Festuca arundinacea* (Fa) diploid and tetraploid *Lolium multiflorum* (Lm2, Lm4) and mixtures of both species (indicated in table 4.1). Error bars: ± standard deviation. Bars not sharing a common letter are significantly different from each other (Tukey; *p* = 0.05)

Although the tiller density of Fa and Lm was on the same level, the ground cover in Fa and Lm swards was different. The average scores for ground cover were 5.0 for Fa, 2.0 for Lm2 and 1.0 for Lm4. The scores for ground cover for the mixtures were between 4.3 for 1/8Lm2 and 3.0 for 1/4Lm2 (**Photograph 4.1**).

Photograph 4.1 General aspect and ground cover of diploid *Lolium multiflorum* (Left) and *Festuca arundinacea* (Right) after 15 cuts in 3 successive years (2010-2012). Photographs taken on 8/10/2012.

4.3.2 Grass-clover trial

4.3.2.1 DM Yield

Like in the pure grass trial, Fa+Tp yielded less than Lm+Tp in the year of sowing, due to its significantly lower DMY in the first two cuts (**Table 4.7**). Fa + Tp had a DMY of 15889 kg DM ha^{-1}, whereas Lm2+Tp, the highest yielding mixture, had a yield of 18749 kg DM ha^{-1}. The DMY of the mixtures was intermediate in 2010.

Table 4.7 Dry matter yield (kg ha^{-1}) of *Festuca arundinacea* (Fa) diploid and tetraploid *Lolium multiflorum* (Lm2, Lm4) and mixtures of both species (indicated in table 4.1) in combination with *Trifolium pratense* (Tp) in 15 successive cuts (C1-C15) in three successive years (2010-2012).

	1/4Lm2	1/4Lm4	1/8Lm2	1/8Lm4 +Tp	Fa	Lm2	Lm4	Sign.^{A}
2010								
C1	3627^{ab}	3685^{ab}	3471^{ab}	3435^{ab}	2695^{b}	4108^{a}	3893^{a}	*
C2	4018^{ab}	4710^{a}	4251^{ab}	4375^{ab}	3649^{b}	4502^{ab}	4166^{ab}	*
C3	3597	4151	3926	4147	2837	4189	4158	NS
C4	2513	2608	2795	2707	2879	2514	2233	NS
C5	3462^{a}	3462^{a}	3744^{a}	3563^{a}	3829^{a}	3436^{a}	3364^{a}	*
Subtotal	17216	18615	18187	18226	15889	18749	17815	NS
Relative (Fa = 100)	108.4	117.2	114.5	114.7	100.0	118.0	112.1	
2011								
C6	7399	7092	6239	6558	6151	6300	5899	NS
C7	2873^{ab}	2972^{ab}	2825^{ab}	2657^{ab}	2409^{b}	2961^{ab}	3090^{a}	*
C8	2442^{ab}	2493^{ab}	2528^{ab}	2381^{b}	2268^{b}	2728^{a}	2522^{ab}	*
C9	2584^{b}	2913^{ab}	2697^{ab}	2765^{ab}	3104^{a}	2766^{ab}	2769^{ab}	*
C10	4920	5050	5005	5184	5614	4819	4928	NS
Subtotal	20218	20521	19295	19545	19546	19573	19207	NS
Relative (Fa = 100)	103.4	105.0	98.7	100.0	100.0	100.1	98.3	
2012								
C11	5051	5484	5527	5616	4904	5517	4981	NS
C12	6555	6642	6727	6894	6519	6344	6704	NS
C13	5098	5497	5369	5375	5174	5532	5443	NS
C14	3358	3481	3431	3407	3717	3567	3252	NS
C15	1744^{b}	1863^{b}	1824^{b}	1782^{b}	2358^{a}	1504^{b}	1594^{b}	***
Subtotal	21805	22967	22879	23074	22671	22465	21975	NS
Relative (Fa = 100)	96.2	101.3	100.9	101.8	100.0	99.1	96.9	
Total	59239	62103	60360	60844	58106	60787	58997	NS
Relative (Fa = 100)	101.9	106.9	103.9	104.7	100.0	104.6	101.5	

A: Significance of the anova model with the seven sward compositions as fixed factor and the three replicates as random factor. (Significance codes: ***: $p < 0.001$, **: $p < 0.01$, *: $p < 0.05$, NS: $p > 0.05$). Values in the same row not sharing a same superscript are significantly different from each other ($p = 0.05$, Tukey test).

In 2011, the mixtures 1/4Lm2+Tp and 1/4Lm4+Tp were the highest yielding sward compositions with yields over 20000 kg DM ha^{-1} owing to their very high yields in the first cut (**Table 4.7**). In 2012, Fa was overyielding Lm; but the highest DMY was obtained by the mixture 1/8Lm4+Tp (23074 kg DM ha^{-1}).

Over all cuts in three years, Fa+Tp and 1/4Lm4+Tp were the lowest respectively highest yielding swards with yields of 58106 kg DM ha^{-1} and 60844 kg DM ha^{-1} respectively. The low DMY of Fa+Tp in the first two cuts in the year after sowing was not compensated by the higher DMY later on. The yields in the grass-clover trial were between 10 % and 15 % higher compared to the same grass swards without red clover.

4.3.2.2 Botanical composition

In 2010 and 2012 significant differences were found in the Tp content in the DMY (TpC) of swards with a single grass species: in 2010 TpC in Fa+Tp was more than twice as high as in Lm2+Tp: 53 % *versus* 25 % respectively (**Table 4.8**); in 2012 TpC were 31 % *versus* 18 % respectively. TpC in the swards with two grass species was mostly intermediate. Given the relatively high N fertilisation, the contribution of Tp to the DMY was considerable: it decreased from values between 53 and 24 % in the first year of the trial to values between 31 and 17 % in the third year of the trial. There was a clear seasonal pattern in the TpC: low in the first three cuts, sharply increasing after the third cut. In Fa+Tp, the TpC was fluctuating less compared to Lm2+Tp and Lm4+Tp (**Figure 4.8**): in 2010 for example, TpC fluctuated from 7 % in the first cut to 55 % in the fifth cut in Lm2+Tp whereas in Fa+Tp, TpC remained between 49 % in the first cut and 64 % in the fourth cut in Fa+Tp.

The contribution of Fa to the total DMY in the mixtures of two grass species and clover was very modest compared to the sown quantity of seed: between 12 % and 6 % in the first year, and between 10 and 24 % in the third year (**Table 4.8**). A significant effect of the proportion of Fa seeds in the seed mixture on the Fa proportion in the DMY was found (**Table 4.6**): the lower the Lm proportion in the seed mixture, the higher the FaC. The effect of the ploidy of the Lm in the mixture on the FaC was significant in 2010: on average a 6.5 % points higher FaC in the mixtures with Lm2 compared to Lm4 (FaC).

Each year, the Fa proportion in the total DMY decreased in the first two cuts and increased in the last two cuts (**Figure 4.9a**). The increase in the proportion of Fa in the later cuts was less sharp in the grass-clover trial because the proportion of Tp generally increased in the last two cuts. The proportion of Fa in the grass DMY followed a seasonal pattern similar to that in the pure grass trial (**Figure 4.9b**).

Table 4.8 Content of *Festuca arundinacea* (Fa) and *Trifolium pratense* (Tp) in the dry matter yield (FaC and TpC), dry matter content (DMC), digestibility of the organic matter (DOM), crude protein content (CPC) and N yield (NY) for Fa, diploid and tetraploid *Lolium multiflorum* (Lm2, Lm4) and mixtures of both grass species (indicated in table 1) in combination with Tp in three successive years (2010-2012). Data are weighted averages over all cuts.

		1/4Lm2	1/4Lm4	1/8Lm2	1/8Lm4	Fa	Lm2	Lm4	Sign.
					+Tp				
2010									
FaC	(%)	6.1^{b}	5.1^{b}	12.4^{a}	8.3^{b}	$41.3^{\#}$	$0^{\#}$	$0^{\#}$	**
TpC	(%)	29.9^{bc}	30.9^{bc}	35.6^{b}	32.4^{bc}	52.9^{a}	25.9^{bc}	24.2^{c}	***
DMC	(%)	17.3^{ab}	17.5^{ab}	17.9^{ab}	17.4^{ab}	16.8^{b}	18.3^{a}	16.8^{b}	*
DOM	(%)	75.4^{bc}	75.6^{abc}	74.6^{cd}	74.5^{cd}	73.7^{d}	76.3^{ab}	76.8^{a}	***
CPC	(%)	16.6^{b}	15.9^{bc}	16.7^{b}	16.8^{b}	19.5^{a}	14.6^{c}	15.5^{bc}	***
NY	(kg ha^{-1})	458.4	472.6	485.7	489.1	496.3	438.2	439.9	NS
2011									
FaC	(%)	7.0^{bc}	4.6^{c}	9.9^{ab}	11.0^{a}	$50.6^{\#}$	$0^{\#}$	$0^{\#}$	***
TpC	(%)	38.3^{b}	42.0^{ab}	40.8^{ab}	42.7^{ab}	49.4^{a}	40.5^{ab}	39.5^{ab}	***
DMC	(%)	18.4	17.6	17.6	17.2	17.5	17.3	16.3	NS
DOM	(%)	75.0^{ab}	75.0^{ab}	75.0^{ab}	75.2^{ab}	72.7^{b}	76.4^{a}	76.4^{a}	***
CPC	(%)	17.2^{b}	17.5^{ab}	18.2^{ab}	18.1^{ab}	19.2^{a}	17.6^{ab}	18.2^{ab}	**
NY	(kg ha^{-1})	555.8	575.6	560.7	567.6	600.8	551.3	558.7	NS
2012									
FaC	(%)	9.8	10.4	17.1	23.7	$69.5^{\#}$	$0^{\#}$	$0^{\#}$	NS
TpC	(%)	17.9^{b}	22.9^{ab}	22.9^{ab}	23.7^{ab}	30.5^{a}	18.0^{b}	17.2^{b}	***
DMC	(%)	17.0	16.9	17.4	16.7	16.5	18.2	16.9	NS
DOM	(%)	70.6^{abc}	71.1^{ab}	70.4^{abc}	70.2^{bc}	69.4^{c}	71.7^{a}	71.2^{ab}	**
CPC	(%)	15.4^{ab}	14.8^{ab}	15.2^{ab}	16.5^{a}	16.5^{a}	13.7^{b}	14.2^{b}	*
NY	(kg ha^{-1})	537.6^{ab}	544.2^{ab}	555.5^{ab}	610.2^{a}	600.2^{a}	460.6^{b}	499.8^{b}	**

A: Significance of the anova model with the seven sward compositions as fixed factor and the three replicates as random factor. (Significance codes: ***: $p < 0.001$, **: $p < 0.01$, *: $p < 0.05$, NS: $p > 0.05$). Values in the same row not sharing a same superscript are significantly different from each other ($p = 0.05$, Tukey test).

The botanical composition of the monospecific swards was not taken into account in the statistical analyses.

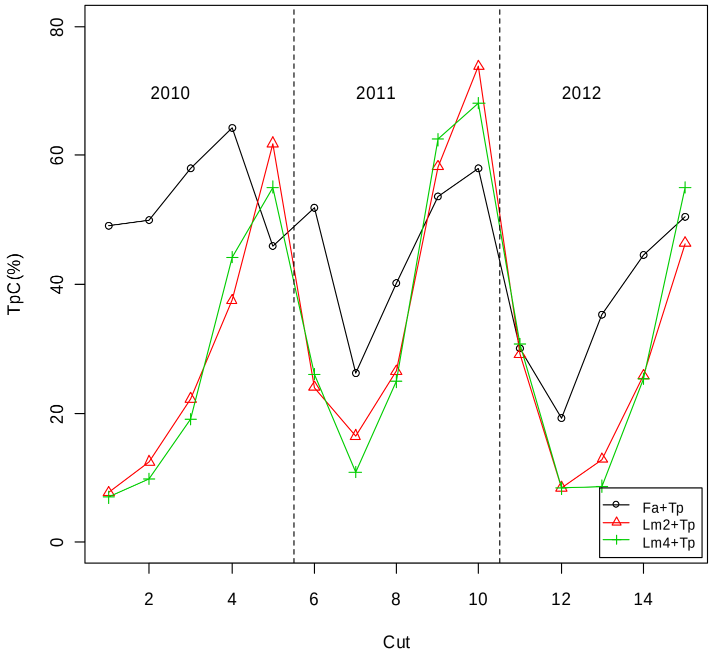


Figure 4.8 Evolution of the *Trifolium pratense* (TpC) content in the dry matter yield of grass-clover mixtures combining red clover with diploid or tetraploid *Lolium multiflorum* (Lm2 or Lm4) and *Festuca arundinacea* (Fa) over 15 cuts in three successive years (2010-2012).

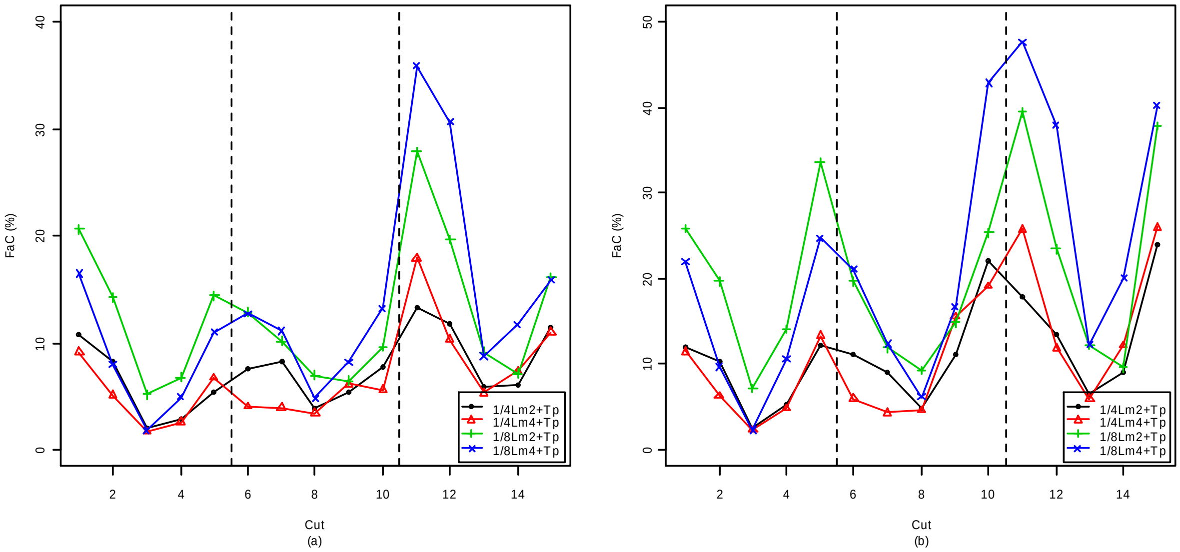


Figure 4.9 Evolution of the *Festuca arundinacea* content (FaC) in (a) the TOTAL dry matter yield or (b) the GRASS dry matter yield, over 15 cuts in three successive years (2010-2012) for four different grass mixtures of *Festuca arundinacea* and *Lolium multiflorum* (Lm) with *Trifolium pratense* (Tp) (indicated in table 4.1).

4.3.2.3 Quality

Apart from the year 2010, no significant differences were found for DMC. The significantly lower DMC found for Fa+Tp in 2010 can be explained by the higher TpC content in Fa+Tp (**Table 4.8, Figure 4.10**).

Each year, the DOM was significantly lower for Fa+Tp compared to Lm+Tp. The DOM of Fa+Tp was 2.6, 3.7 and 2.3 % points lower than the DOM of Lm2+Tp in 2010, 2011 and 2012 respectively. The DOM of the mixtures was intermediate (**Table 4.8, Figure 4.11**).

The CPC was clearly influenced by the TpC in the DMY. In the years 2010 and 2012, when Fa+Tp had a significantly higher TpC than Lm+Tp, CPC followed the same pattern (**Table 4.8**). The seasonal pattern found in CPC (**Figure 4.12**) was parallel to that found in TpC: a decrease in the first two cuts, a sharp rise in the late seasons and less fluctuation in Fa+Tp compared to Lm+Tp.

In 2010 and 2011, the highest NYs were obtained with Fa+Tp (496 kg ha^{-1} and 601 kg ha^{-1} respectively) but differences between the sward compositions were not significant (**Table 4.8**). In 2012, Fa+Tp, the sward composition with the highest CP, and 1/8Lm4+Tp, the sward composition with the highest DMY, had NYs that were significantly higher than these of Lm2+Tp and Lm4+Tp. NY was intermediate in the other mixtures.

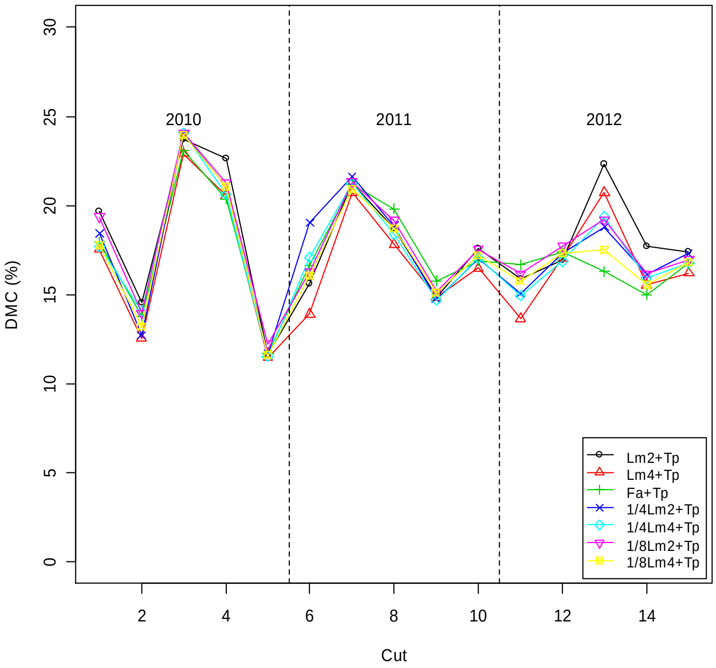


Figure 4.10 Evolution of the dry matter content (DMC) over 15 cuts in three successive years (2010-2012) for diploid or tetraploid *Lolium multiflorum* (Lm2 or Lm4), *Festuca arundinacea* (Fa) and mixtures of both grass species with *Trifolium pratense* (Tp) (indicated in table 4.1).

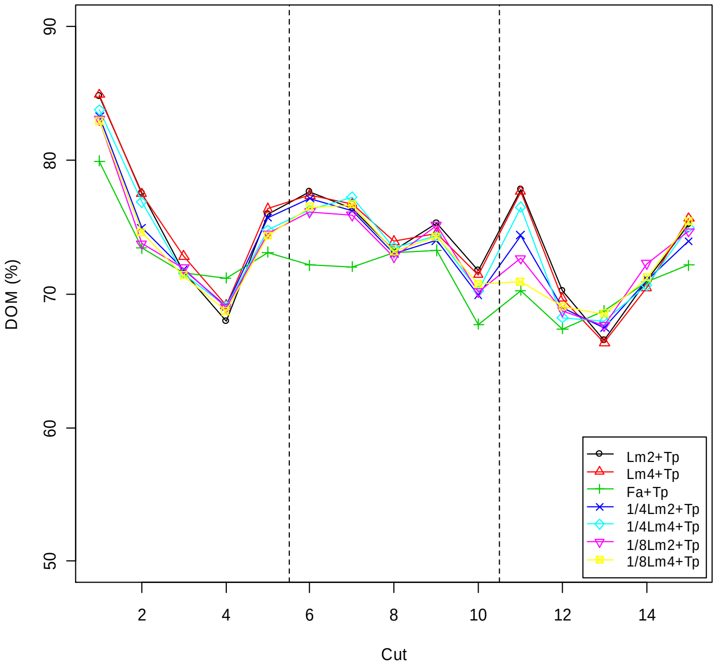


Figure 4.11 Evolution of the digestibility of the organic matter (DOM) over 15 cuts in three successive years (2010-2012) for diploid or tetraploid *Lolium multiflorum* (Lm2 or Lm4), *Festuca arundinacea* (Fa) and mixtures of both grass species with *Trifolium pratense* (Tp) (indicated in table 4.1).

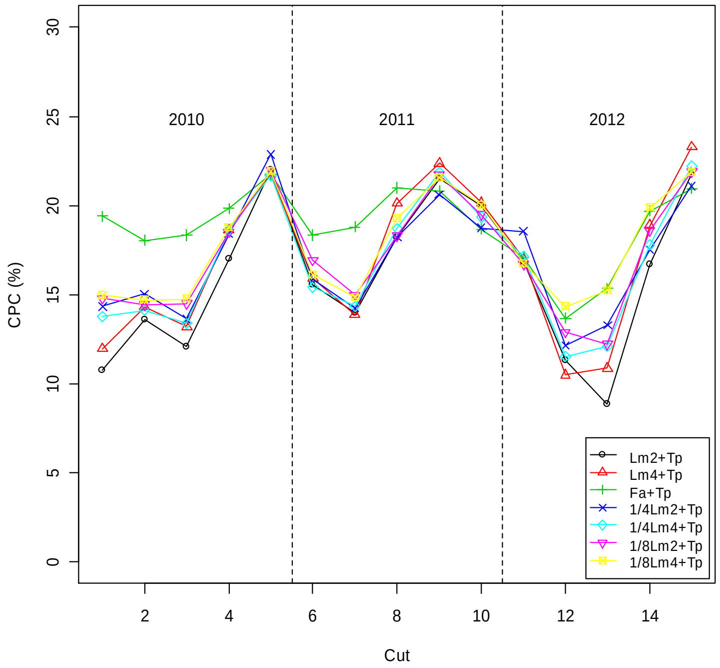


Figure 4.12 Evolution of the crude protein content (CPC) over 15 cuts in three successive years (2010-2012) for diploid or tetraploid *Lolium multiflorum* (Lm2 or Lm4), *Festuca arundinacea* (Fa) and mixtures of both grass species with *Trifolium pratense* (Tp) (indicated in table 4.1).

4.3.2.4 N fixation

The red clover N fixation as calculated with the N difference method ranged from 293 kg N $ha^{-1}yr^{-1}$ for Lm4+Tp in 2010 to 425 kg N $ha^{-1}yr^{-1}$ for 1/8Lm4+Tp in 2012. Over the three years the highest N fixation was found in Fa+Tp (**Table 4.9**): and the lowest in Lm2+Tp, with N fixations of 1190 kg ha^{-1} and 937 kg ha^{-1} respectively.

Table 4.9 Calculated N-fixation (kg N ha^{-1}) by *Trifolium pratense* (Tp) in association with *Festuca arundinacea* (Fa), diploid and tetraploid *Lolium multiflorum* (Lm2, Lm4) and mixtures of both grass species (indicated in table 4.1) as calculated according to the N difference method.

Year	1/4Lm2	1/4Lm4	1/8Lm2	1/8Lm4 +Tp	Fa	Lm2	Lm4
2010	302.1	320.8	337.3	321.3	377.4	293.1	299.5
2011	354.2	372.5	372.0	349.9	404.0	325.3	346.8
2012	357.1	383.3	392.6	425.9	409.0	318.8	381.2
Total	1013.4	1076.6	1101.9	1097.1	1190.4	937.2	1027.5
Relative (Fa = 100)	85.1	90.4	92.6	92.2	100.0	78.7	86.3

4.4 Discussion

Over the 15 cuts, no DMY advantage of Fa compared to Lm was found in the pure grass trial. Several factors have contributed to this result.

First, the establishment of Fa was slower than that of Lm. Fa yielded 18 % less than Lm in the first year. In the study of Willman and Gao (1996), Fa yielded 27 % less than Lm in the year of sowing. The smaller yield difference found in our study is probably due to the autumn sowing of our trial, while Wilmann and Gao (1996) applied spring sowing and considered the sowing year as the first production year.

Second, the persistence of Lm in the third year was above the expectation: even though the ground cover by the Lm tillers was fairly low at the end of the experiment, yield remained on a high level in the third year. Under practical circumstances (wheel tracks, less favourable growing conditions) Lm is expected to be less persistent.

Third, no severe summer drought occurred during the experimental period; so Fa could not take advantage of its better drought resistance compared to Lm. In our trial, there was no clear evidence that Fa was more productive in dry periods compared to Lm. In the second cut of 2011, which was preceded by a very dry spring, Fa was yielding more than Lm, whereas in 2010 and 2012 Lm was the more productive species in the second cut. The lower yield of Lm in 2011 might also result from the very high Lm yield in the first cut. In the fifth cut of 2012, preceded by a dry September month, Fa overyielded Lm more than in the previous years, but this could also be explained by the decreasing persistence of the Lm.

Although the mixtures seemed to combine the high yield of Lm in the first cuts after sowing and the good yield potential of Fa in the late summer cuts, no significant transgressive overyielding was detected. Again, the very good conditions for grass growth during the experimental period may explain this non-effect of mixing Fa and Lm.

Even if the mixing of Fa and Lm did not lead to significant transgressive overyielding, the experiment proved that the low yield in the first cuts after installing a pure Fa sward could be tackled by sowing a mixture of Fa with a small proportion of Lm seeds: mixtures in which 1/8 of Fa seeds were substituted by Lm seeds yielded nearly as much as pure Lm swards. The initial dominance of Lm in the mixtures studied by Wilman and Gao (1996) did not prevent the increase of the abundance of the most persistent species in the mixture leading eventually to a pure Fa sward. At the end of the trial the number of Fa tillers and the ground cover in our plots with mixtures were not significantly different from the pure Fa plots. Lm4 produced significantly less tillers than Lm2, leaving more space for the Fa to develop. Particularly the plots sown with 1/8Lm4 looked like the pure Fa plots at the end of the trial. So Lm can be seen as a cover crop for Fa. Apart from tetraploid Italian ryegrass, the short lived, Westerwold ryegrass could be an interesting partner to obtain a pure Fa sward without having the drawback of the low Fa yield in the first cuts.

The effect of the ploidy of the Lm was the inverse to what we expected: we found significantly lower Fa proportions in combination with Lm4 compared to Lm2. In combination with Lm4, Fa was particularly suppressed in the first three cuts, but in the later cuts, Fa was increasing steeper in combination with Lm4 than with Lm2. This means that once the Lm4 grew less vigorously, the Fa had enough space to develop, which is in accordance with the more open sward formed by Lm4 compared to Lm2. As indicated above, the highest yield was obtained in mixtures with Lm4 which indicates that differences in functional traits like rooting pattern, growth pattern, plant height and crop density are probably larger between Fa and Lm4 compared to Lm2, explaining the higher benefit of mixtures with Lm4. Fa and Lm proved to be complementary: early vigour of Lm, good summer and autumn yield and persistence of Fa. As proposed by Huyghe *et al.* (2012) further research on the right genotypes and sowing densities for both species might optimize the advantage created by this functional diversity.

The values found for DMC were in accordance with values found in literature: DMC of Fa between 1 and 2 % points higher than Lm2 (INRA, 1978; Jones and Prickett, 1981) and Lm2 between 1 and 2% points higher than Lm4 (Tabacco *et al.*, 2004). Even tough we used a recent Fa variety, the digestibility of Fa was significantly lower compared to Lm. Particularly in the first cut of each year the difference between Fa and Lm was important. A higher cutting regime in spring (e.g. an earlier first cut and three instead of two spring cuts) could mitigate this difference in DOM. There was no clear difference in CPC between Fa and Lm: the observed differences were mostly explained by differences in DMY, neither was there a clear indication that the NY for one of both species was higher. For all quality parameters, the values found in the mixtures were generally intermediate to values found in the monospecific swards. The convex shape of the regressions of the DOM in function of the FaC in the mixtures suggested that the DOM of the mixtures was generally lower than expected from a weighted average of the DOM of the monospecific swards. This could be explained by the seasonal pattern both in the DOM and in the FaC: in the first cut, when the FaC in the mixtures was generally high, the DOM of Fa was relatively low. Whatever the underlying reason or the magnitude of this negative effect may be, the quality of the forage produced by the mixtures, evolved in line with the botanical composition of the mixture. The effect of the FaC in the mixtures on the CPC content was purely linear: an increase of FaC with 1 % point resulted in an CPC increase of 0.014 % points in 2010 and 2012 and resulted in an CPC decrease of 0.011 % points in 2011. The CPC of the mixture corresponded to the weighted average values of the monospecific swards

In combination with clover, the yield disadvantage of Fa in the 1st year after establishment was on the same level as in the pure grass trial. The growth of the red clover in the first three cuts was insufficient to compensate for the slow establishment of the Fa, resulting in a lower yield for Fa+Tp over all the cuts. The use of a mixture of Fa and Lm supplemented with red clover was an interesting option to tackle the low yield of Fa in the first cuts.

Unlike in the pure grass trial, the contribution of Fa to the total DMY or to the grass DMY was not steadily increasing over the years, and remained on a low level. The Fa proportion in the grass DMY was below that in the pure grass trial. Probably the competition from both Lm and Tp was too strong for Fa to establish well and to evolve to a pure Fa sward in the long term. A lower proportion of Lm seeds in the initial seed mixture and/or a less persistent Lm variety may create more opportunities for Fa to become dominant over time.

Overall, the TpC of the swards was relatively high. It is well known that clover grows better when established in former arable land (Bommelé, 2007). The clover content was higher and more regular in combination with Fa than with Lm, resulting in higher CPC and higher N yields. Mc Bratney (1984) also found that Fa was a good companion grass for red clover, based on a six years trial in Northern Ireland, in which combinations of different red clover varieties with each of three companion grasses were compared. Tall fescue was the companion grass species with the most regular clover content in the DMY. In our trial TpC in the Fa+Tp sward was around 40 % when averaged over the three years, which corresponds with the proportion of legumes needed in a grass + legumes sward to obtain the largest benefit of the legumes in the sward (Lüscher *et al.*, 2010).

Our trial was not designed to determine the N fixation, but a good estimation was possible as the pure grass trial, the reference in the N difference method, was adjacent to the grass-clover trial. The N fixations we calculated were very high (> 300 kg ha^{-1} yr^{-1}). The values were in the same range as those found by Deprez *et al.* (2004) or Boller and Nösberger (1987) for red clover with perennial ryegrass, but they were at least twice as high as the values obtained with the equation of Carlsson and Huss-Danell (2003). We found N fixations between 40 kg N (ton clover DMY)$^{-1}$ for Fa+Tp in 2011 and 100 kg N (ton clover DMY)$^{-1}$ for Fa+Tp in 2012, whereas Carlsson and Huss-Danell (2003) calculated an averaged N fixation of 31 kg N (ton clover DMY)$^{-1}$. A factor that might have contributed to this success was that the clover contents found in our trial were close to the 40 % level that is generally regarded as the optimal clover content to maximize the nitrogen fixation (Nyfeler *et al.*, 2011). The very low organic matter content in the soil, resulting in a very low soil N availability from mineralisation is another factor that might have stimulated the N fixation.

The presence of red clover in the mixtures lowered the DMC of the harvested material compared to the pure grass trial. This is obvious, as the DMC of bolting Tp is around 4 % point lower compared to Italian ryegrass that starts heading (INRA, 1978). The DOM on the other hand was positively influenced by the presence of the clover compared to the pure grass trial. The difference in digestibility between Fa and Lm in combination with red clover was decreased to less than 4 % points.

Over the 15 cuts the grass-clover mixtures yielded at least 10 % more DM than the pure grass swards. This higher yield was obtained with 60 % less N fertilisation and resulted in a 40 % higher N yield. In the study of Nyfeler *et al.* (2009), grass-clover mixtures, fertilised with 50 kg N ha^{-1} yr^{-1}, produced yields comparable to grass monospecific swards fertilised with 450 kg N ha^{-1} yr^{-1} if the legume proportion was 50 to 70 %.

4.5 Conclusion

Over a period of three years, Fa did not overyield Lm. Although Fa was more productive than Lm in the second and third year after sowing, this could not compensate for the low yield of Fa in the first production year. No evidence was found that mixtures of Fa and Lm were more productive than the highest yielding monospecific sward. The substitution of a small part of Fa seeds by Lm seeds is a good strategy to compensate for the low yields of a pure Fa sward in the first production year. The Lm can be regarded as a cover crop: its abundance declines leaving finally a nearly pure Fa sward. Adding red clover to the single grass species or to the mixtures of Fa and Lm brought a substantial yield benefit with 180 kg N ha^{-1} yr^{-1}. In the three species (Fa+Lm+Tp) mixture the competition of both Lm and Tp prevented Fa to become dominant.

Regarding our research hypotheses, we can conclude that

1. Although every year mixtures of Fa and Lm had the highest yields in the pure grass trial, we found no significantly higher yield with the mixtures compared to the monospecific swards. Mixtures of Fa and Lm combined the high yield of Lm in the first cuts after sowing and the good yield potential of Fa in the late summer and autumn.
2. In all years, the FaC was significantly higher in the mixtures with 1/8 compared to 1/4 of Lm seeds in the initial mixture. A proportion of 1/8 of Lm seeds in the initial mixture was sufficient to have a yield on the same level as that of pure Lm in the first cuts after sowing and resulted in a sward that was dominated by Fa after three years.
3. Contrary to what we expected, FaC was significantly higher in combination with Lm2 compared to Lm4 in 2010 and 2011. Especially in the first cuts of each year, Lm4 suppressed Fa more than Lm 2.
4. Adding Tp to the grass compositions led to a higher DMY and NY with less N fertilisation compared to the pure grass swards. The interaction between Fa and Lm was not altered by the presence of Lm, but the competition of both Fa and Tp was too strong for Fa to establish well after three years.

Chapter 5

Performance of tall fescue and perennial ryegrass and mixtures of both species grown with white clover under a management of mowing the first cut followed by grazing

5.1 Introduction

The yield of tall fescue (*Festuca arundinacea* Schreb.; **Fa**) was found to be higher than that of perennial ryegrass (*Lolium perenne* L.; **Lp**) under cutting management (**Chapters 2, 3**). In **chapter 3**, we hypothesized that mixtures of Fa and Lp might lead to transgressive overyielding, but no transgresssive overyielding was found under cutting only management. As management may influence the interaction between species, the results found under cutting management may not be generalized. Hence the hypothesis tested in this Chapter was that transgressive overyielding can occur under a management of cutting combined with grazing.

Significant management x genotype interactions exist in perennial ryegrass varieties (McEvoy *et al.*, 2010) and it is very likely that such interactions also occur between species, meaning for example that Fa is more productive than Lp under a cutting management, but that this situation is inconsistent under a grazing management.

Generally, the yield of grazed grassland is lower compared to that of cut grassland. Wims *et al.* (2010) found a 25 % higher yield for cut compared to grazed perennial ryegrass when both were defoliated at the same frequency in Ireland. Binnie and Chessnut (1991) found, both under a 3 weeks as well as under a 4 weeks defoliation interval, a 10 % higher yield for cut compared to grazed perennial ryegrass in Northern Ireland. Trampling damage (Binnie and Chestnutt, 1991; Phelan *et al.*, 2011) and deposition of faeces and urine (Van den Pol-van Dasselaar *et al.*, 2008) are the two main reasons for the lower yield of grazed compared to cut grassland.

Gaps in the sward created by hoof trampling, cow pads deposition or freezing damage can easily get colonised by species like dandelion (*Taraxacum officinale* F.H.Wigg.), annual meadow grass (*Poa annua* L.), greater plantain (*Plantago major* L.) and docks (*Rumex* sp.) (Bullock *et al.*, 1995), decreasing the yield potential of the sward and the quality of the harvested herbage. Hence, colonisation by weed species can be seen as an indicator of non-persistency of a sward.

Lp is the cool season grass species that is best adapted to intensive grazing, whereas Fa is the most robust of all cool season grass species (Frame, 1992). Mixing both grass species might result in a positive interaction eventually leading to a higher and more stable yield (Huyghe *et al.*, 2012). Research by Wilmann and Gao (1996) suggested that seed mixtures of Fa and Lp should be dominated by Fa owing to the higher early vigour of Lp compared to Fa.

Our research hypotheses were that under a combined management of mowing early spring growth and grazing:

1. Mixtures of Fa and Lp lead to transgressive overyielding.
2. A small Lp fraction in the seed mixture offers good opportunities to obtain a sward with an even species composition in the mid-long term.
3. A tetraploid Lp variety is less competitive compared to a diploid Lp variety and allows a quicker progression of Fa in the sward.
4. Weed invasion is lower in Fa compared to Lp2 or Lp4, owing to the better persistence of Fa.

5.2 Materials and Methods

5.2.1 Trial design

The experimental field was sown on the 24th of April 2009 on a sandy loam soil in Melle, Belgium. The land had been used as grazed grassland for over 10 years before the establishment of the trial. In March 2009, the existing grass sward was killed with a herbicide (1440 g glyphosate ha^{-1}). The dead sward was cultivated, ploughed and rotary harrowed to obtain a fine seedbed. One variety of diploid perennial ryegrass (Lp2) *cv.* ‘Plenty’ one variety of tetraploid perennial ryegrass (Lp4) *cv.* ‘Roy’, two varieties of tall Fescue (Fa_1 and Fa_2) *cv.* ‘Castagne’ and *cv.* ‘Callina’ respectively and six mixtures of Fa_1 and Lp2 or Lp4 (**Table 5.1**) were sown. Mixtures were created by substituting 1/4, 1/8 or 1/16 of the Fa_1 seeds by Lp2 or Lp4 seeds. Plots were sown in a randomized complete block design with four blocks; individual plot size was 49 m^2 (7 x 7 m). Pure grass species and grass species mixtures were all sown at a density of 1500 viable seeds m^{-2}. This corresponded to a seed density of *circa* 25 kg ha^{-1} for Lp2, 40 kg ha^{-1} for Fa and 50 kg ha^{-1} for Lp4. Grass seeds were broadcast sown by hand and incorporated by harrowing. Afterwards, all plots were oversown with white clover *cv.* ‘Merwi’ at 700 viable seeds m^{-2} (*circa* 5 kg ha^{-1}). Clover seeds were mixed with sand for broadcast sowing. Finally, the seedbed was consolidated with a Cambridge roller.

5.2.2 Management

In the year of sowing, the trial was cut a first time in mid June and grazed by dairy heifers for the rest of the season. Stocking rate was regulated to maintain a sward height above 6 cm. Fertilisation was 100 kg N ha^{-1} in the year of sowing. In the years 2010 till 2012, dairy cattle slurry was injected as soon as the bearing capacity of the land was sufficient (end February – mid March). The average injected dose was 42.5 ton $ha^{-1}yr^{-1}$ with an average composition of 4.0 kg N, 0.6 kg P and 3.5 kg K per 1000 l slurry respectively, resulting in a slurry fertilisation of 170 kg N $ha^{-1}yr^{-1}$, 38.3 kg P $ha^{-1}yr^{-1}$ and 149 kg K $ha^{-1}yr^{-1}$ on average. An additional 150 kg N $ha^{-1}yr^{-1}$ as mineral fertiliser was applied in two or three fractions from May till August.

In 2010 and 2011 a first cut was harvested for silage making in May, and the trial was grazed with dairy heifers for the rest of the season. The rejected herbage was topped at the end of the growing season.

The experimental field, lying within a larger pasture that was continuously stocked, was fenced after the slurry application in 2012, in order to restrict the grazing to well defined periods. This was needed allow the determination of dry matter yield (DMY) by cutting.

Table 5.1 Sowing density (number of germinating seeds m^{-2}) of *Festuca arundinacea* (Fa_1, Fa_2) diploid and tetraploid *Lolium perenne* (Lp2, Lp4) and mixtures of Fa_1 with Lp2 or Lp4, differing in the proportion and ploidy of the Lp component in the mixture, grown in association with *Trifolium repens* (Tr)

	Fa		Lp		Tr
	‘Callina’	‘Castagne’	‘Plenty’ (2x)	‘Roy’ (4x)	‘Merwi’
Fa_1	0	1500	0	0	700
Fa_2	1500	0	0	0	700
Lp2	0	0	1500	0	700
Lp4	0	0	0	1500	700
1/4Lp2	0	1125	375	0	700
1/4Lp4	0	1125	0	375	700
1/8Lp2	0	1312	188	0	700
1/8Lp4	0	1312	0	188	700
1/16Lp2	0	1406	94	0	700
1/16Lp4	0	1406	0	94	700

5.2.3 Measurements

At the end of May 2009, the botanical composition of the establishing swards was determined by counting the numbers of Fa, Lp and Tr seedlings in four randomly selected squares of 0.25 m x 0.25 m per plot. No measurements were conducted in 2010 and 2011.

In 2012 DMY was recorded at approximately 6 weeks intervals during the growing season (21 May, 3 July, 21 August and 2 October). DMY was determined by cutting a strip, of *circa* 3 m long in the centre of each plot with a reciprocating blade mower with a blade of 1.25 m wide;

cutting height was 5 cm. Attention was paid to cut on a different location in each plot in two successive cuts. The cut herbage was weighed and the exact length of each cut strip measured. A subsample was dried for 16 h at 75°C for the determination of the dry matter content. Another subsample was separated into the different species: Fa, Lp, Tr and unsown species (including weeds) and the fresh and dry weights of these fractions were determined.

After each DMY determination, the sward was intensively grazed by dairy heifers till a sward height of approximately 5 cm (a period between 5 and 7 days). Grass growth during the grazing windows was not recorded as we were not allowed by the owner of the animals to install cages, protecting strips from grazing.

In addition to the weed content in the DMY, that was determined on four occasions in 2012, the abundance of the individual plants of dandelion and greater plantain was determined at the end of June 2012. These two species were monitored, as both weed species are typical for grazed pastures and because they were present in all plots of the trial. Their abundance was determined in the plots Fa_1, Fa_2, Lp2, Lp4, 1/4Lp2 and 1/4Lp4. A metal frame measuring 1 x 1 m was dropped at three random spots in each plot and the numbers of individual dandelion and greater plantain plants within the frame were counted.

Meteorological data were obtained from an official meteorological station on the experimental farm (**Appendix 1**).

5.2.4 Data analysis

The abundance of Fa and Lp in the establishing swards were compared with the proportion in the seed mixtures using a chi-square test ($p = 0.05$) (Snedecor and Cochran, 1967). Plots for which the null hypothesis (i.e. seedling distribution in swards corresponds to distribution expected from seed mixture) was rejected, were excluded from the trial.

Analyses of variance (anova) to test the effect of the different grass species and mixtures on herbage yield, botanical composition and presence of dicot weeds were performed using the *aov()* function in R (R core development team, 2011). Sward compositions were considered as a fixed factor, replications as a random factor. Multiple comparison of means was done using the *TukeyHSD()* function. Dicot weeds counts were square root transformed before analysis.

5.3 Results

5.3.1 Seedling density

The abundance of the seedlings corresponded to the proportions of Fa and Lp in the seed mixtures in all but four plots. In these four plots, the chi square test indicated a significant deviation from the expected proportion. Hence, these plots were excluded for analysis (**Table 5.2**). As two of the four 1/8Lp4 plots did not have the expected ratio of Fa and Lp seedlings, this sward composition was completely excluded from the analysis.

Two months after sowing, the average number of grass and clover seedlings was 592 m^{-2} and 112 m^{-2} respectively (**Table 5.3**). Although the grass seedling densities tended to be higher in the Lp plots compared to the Fa plots, no significant differences were found nor in grass, nor in clover seedling density.

5.3.2 Dry matter yield

Annual DMY in 2012 varied between 12016 kg DM ha^{-1} for Fa_1 and 10004 kg DM ha^{-1} for Lp4. The DMY of the mixtures was between that of the constituent species. Differences in annual DMY were not significant (**Table 5.4**).

The first cut accounted for more than 50 % of the total DMY. Only in the third cut, significant yield differences appeared: the DMY of the swards 1/8Lp2 and 1/16Lp2 was significantly higher than that of Lp4 (**Table 5.4**).

Table 5.2 Grass seedling numbers of *Festuca arundinacea* (Fa) and *Lolium perenne* (Lp) in swards sown with mixtures of both species in association with *Trifolium repens* (indicated in Table 5.1) measured 2 months after sowing in four squares of 25 cm x 25 cm.

		Fa	Lp	Total	Chi square[A]
Block 1	1/4Lp2	125	35	160	0.83 ^{NS}
	1/4Lp4	83	32	115	0.49 ^{NS}
	1/8Lp2	114	25	139	6.96***
	1/8Lp4	120	22	142	1.16 ^{NS}
	1/16Lp2	173	15	188	0.96 ^{NS}
	1/16Lp4	186	15	201	0.50 ^{NS}
Block 2	1/4Lp2	109	24	133	3.43 ^{NS}
	1/4Lp4	101	39	140	0.61 ^{NS}
	1/8Lp2	121	20	141	0.37 ^{NS}
	1/8Lp4	96	24	120	11.22***
	1/16Lp2	143	11	154	0.21 ^{NS}
	1/16Lp4	158	11	169	0.02 ^{NS}
Block 3	1/4Lp2	121	36	157	0.36 ^{NS}
	1/4Lp4	94	32	126	0.01 ^{NS}
	1/8Lp2	157	26	183	0.49 ^{NS}
	1/8Lp4	104	23	127	7.31***
	1/16Lp2	144	12	156	0.55 ^{NS}
	1/16Lp4	132	10	142	0.15 ^{NS}
Block 4	1/4Lp2	99	45	144	3.00 ^{NS}
	1/4Lp4	108	49	157	3.23 ^{NS}
	1/8Lp2	117	14	131	0.39 ^{NS}
	1/8Lp4	162	25	187	0.13 ^{NS}
	1/16Lp2	114	10	124	0.70 ^{NS}
	1/16Lp4	102	15	117	15.27***

A: Chi-square test to test whether seedling abundance in swards corresponded to abundance expected from seed mixtures (Significance codes: ***: $p < 0.001$, **: $p < 0.01$, *: $p < 0.05$, NS: $p > 0.05$).

Table 5.3 Average seedling number (m^{-2}) of *Festuca arundinacea* (Fa), *Lolium perenne* (Lp) and *Trifolium repens* (Tr) in swards sown with two Fa varieties (Fa$_1$, Fa$_2$), diploid or tetraploid Lp (Lp2, Lp4) and mixtures of both species in association with *Trifolium repens* (indicated in Table 5.1) measured 2 months after sowing.

	Fa	Lp	Total	Tr
1/4Lp2	454	140	594	124
1/4Lp4	386	152	538	131
1/8Lp2	507	89	596	122
1/16Lp2	574	48	622	126
1/16Lp4	599	49	648	112
Fa$_1$	587	0	587	112
Fa$_2$	509	0	509	90
Lp2	0	606	606	86
Lp4	0	624	624	102
Significance^A			NS	NS

A: Significance of the anova model with the sward compositions as fixed factor and the four replicates as random factor. (Significance codes: ***: $p < 0.001$, **: $p < 0.01$, *: $p < 0.05$, NS: $p > 0.05$)

Table 5.4 Dry matter yield (kg DM ha^{-1}) in grazed swards of *Festuca arundinacea* (Fa$_1$, Fa$_2$), diploid or tetraploid *Lolium perenne* (Lp2 and Lp4) and mixtures of both species in association with *Trifolium repens* (indicated in Table 5.1) in four successive cuts in 2012.

	Cut				
	1	2	3	4	Total
1/16 Lp2	5450	2899	1607^a	1478	11433
1/16 Lp4	5919	2390	1422^{ab}	1604	11334
1/8 Lp2	5962	2500	1715^a	1584	11761
1/4 Lp2	5855	2581	1345^{ab}	1418	11199
1/4 Lp4	6112	2220	1336^{ab}	1334	11002
Fa$_1$	5761	2938	1404^{ab}	1913	12016
Fa$_2$	5557	2927	1480^{ab}	1339	11302
Lp2	5508	2292	978^{ab}	1639	10416
Lp4	6268	1822	713^b	1202	10004
Significance^A	NS	NS	**	NS	NS
Mean	5821	2508	1333	1501	11163

A: Significance of the anova model with the sward compositions as fixed factor and the four replicates as random factor. (Significance codes: ***: $p < 0.001$, **: $p < 0.01$, *: $p < 0.05$, NS: $p > 0.05$) Values in the same column not sharing a same superscript are significantly different from each other ($p = 0.05$, Tukey test).

5.3.3 Botanical composition

In all cuts, the Fa content (FaC) in the DMY differed significantly between the sward compositions. Based on the annual DMY the FaC was between 77 % in the mixture 1/16Lp2 and 48 % in the mixture 1/4Lp4 (**Table 5.5**). In all sward compositions, FaC generally increased through the season.

Table 5.5 *Festuca arundinacea* (Fa) content (%) in the dry matter yield (DMY) in grazed swards sown with mixtures of Fa and diploid or tetraploid *Lolium perenne* (Lp2 and Lp4) in association with *Trifolium repens* (indicated in Table 5.1) in four successive cuts in 2012.

	Cut				Cut
	1	2	3	4	1-4
1/16 Lp2	74.1^{a}	75.2^{a}	84.6^{a}	87.7^{a}	77.7^{a}
1/16 Lp4	$66.6a^{b}$	76.3^{a}	81.1^{ab}	74.7^{a}	71.4^{a}
1/8 Lp2	62.6^{ab}	61.8^{a}	73.9^{bc}	70.4^{ab}	65.0^{ab}
1/4 Lp2	48.3^{b}	52.9^{b}	55.5^{bc}	57.5^{b}	51.4^{b}
1/4 Lp4	43.5^{b}	49.4^{b}	47.4^{c}	62.4^{ab}	47.7^{b}
Significance^{A}	***	**	***	**	***

A: Significance of the anova model with the sward compositions as fixed factor and the four replicates as random factor. (Significance codes: ***: $p < 0.001$, **: $p < 0.01$, *: $p < 0.05$, NS: $p > 0.05$) Values in the same column not sharing a same superscript are significantly different from each other (p = 0.05, Tukey test).

In all swards, the clover contents (TrC) in the DMY were very low (**Table 5.6**). Clover was completely absent in the first cut, resulting in a TrC below 5 % in the total DMY. The highest TrC were found in the fourth cut: 8 % in combination with Lp4 and only 1 % in combination with 1/16Lp2. Differences in TrC were never significant (**Table 5.6**).

Table 5.6 *Trifolium repens* content (%) in the dry matter yield (DMY) in grazed swards of *Festuca arundinacea* (Fa$_1$, Fa$_2$), diploid or tetraploid *Lolium perenne* (Lp2 and Lp4) and mixtures of both species in association with *Trifolium repens* (indicated in Table 5.1) in four successive cuts in 2012.

	Cut				Cut
	1	2	3	4	1-4
1/16 Lp2	0	0.7	0.8	1.0	0.6
1/16 Lp4	0	0.8	1.8	2.6	1.2
1/8 Lp2	0	0.9	1.8	2.5	1.3
1/4 Lp2	0	0.8	3.0	2.5	1.8
1/4 Lp4	0	2.0	4.0	3.2	2.2
Fa$_1$	0	2.3	1.8	2.7	1.3
Fa$_2$	0	1.2	3.3	3.8	1.7
Lp2	0	2.4	5.1	6.3	3.5
Lp4	0	3	7.2	8.4	4.3
Significance^A	NS	NS	NS	NS	NS

A: significance of the anova model with the sward compositions as fixed factor and the four replicates as random factor. (Significance codes: ***: $p < 0.001$, **: $p < 0.01$, *: $p < 0.05$, NS: $p > 0.05$)

The main weed species in the harvested biomass were, in order of importance: dandelion, greater plantain, creeping buttercup (*Ranunculus repens* L.) and broad leaved dock (*Rumex obtusifolius* L.). Particularly in the first cut, the contribution of weeds to the biomass was high: between 6 % in Lp4 and 19 % in 1/4Lp4. In none of the cuts, significant differences in the weed content were found between the sward compositions. Over all cuts, the lowest weed content was found in Fa$_1$ (**Table 5.7**).

In June 2012, when the abundance of dandelion and greater plantain was determined, the highest abundance of dandelions (101 m^{-2}) was found in Lp4 and the lowest (15 m^{-2}) in Fa$_1$. In the swards 1/4Lp2 and 1/4Lp4, the abundance of dandelions was intermediate: 48 and 81 dandelions m^{-2} respectively. The effect of sward composition on dandelion abundance was never significant (p = 0.174). Neither was the greater plantain abundance (p = 0.302) (**Table 5.8**).

Table 5.7 Weed content (%) in dry matter yield (DMY) in grazed swards of *Festuca arundinacea* (Fa_1, Fa_2), diploid or tetraploid *Lolium perenne* (Lp2 and Lp4) and mixtures of both species in association with *Trifolium repens* (indicated in Table 5.1) in four successive cuts in 2012 .

	Cut				Cut
	1	2	3	4	1-4
1/16 Lp2	17.7	5.1	2.4	3.1	8.0
1/16 Lp4	8.6	6	4.8	4.2	6.0
1/8 Lp2	14.8	2.8	5.5	5.1	6.8
1/4 Lp2	17.6	6.2	6.9	5.5	8.1
1/4 Lp4	18.5	6.7	9	9	11.1
Fa_1	12.2	6.4	1	2.3	5.8
Fa_2	15.2	6.4	2.6	6.2	7.9
Lp2	10.8	2.7	10.1	8.8	8.3
Lp4	6.2	4.3	11.7	11	8.5
Significance^A	NS	NS	NS	NS	NS

A: Significance of the anova model with the sward compositions as fixed factor and the four replicates as random factor. (Significance codes: ***: $p < 0.001$, **: $p < 0.01$, *: $p < 0.05$, NS: $p > 0.05$)

Table 5.8 Abundance (m^{-2}) of dandelion (*Taraxacum officinale*) and greater plantain (*Plantago major*) plants in grazed swards of *Festuca arundinacea* (Fa_1, Fa_2), diploid or tetraploid *Lolium perenne* (Lp2 and Lp4) and mixtures of these species in association with *Trifolium repens* (indicated in Table 5.1) in June 2012.

	Dandelion	Plantain
1/4Lp2	47.6	0.5
1/4Lp4	81.2	0.8
Fa_1	14.5	1.8
Fa_2	40.0	1.2
Lp2	66.6	3.2
Lp4	100.6	1.5
Significance^A	NS	NS

A: Significance of the anova model with the sward compositions as fixed factor and the four replicates as random factor. (Significance codes: ***: $p < 0.001$, **: $p < 0.01$, *: $p < 0.05$, NS: $p > 0.05$)

5.4 Discussion

Seedling density in Fa_1, Fa_2, Lp2 and Lp4 was very similar, which indicated that the germination energy of the different seed lots was similar. It is not clear why the Fa and Lp seedling abundance in some plots did not correspond to the expected proportion.

Fa_1 was consistently overyielding Lp2, high coefficients of variation however prevented the difference from being significant.

Only in the third cut, significant yield differences were found in the different sward compositions. Higher yields were found for the swards sown with the mixtures 1/8Lp2 and 1/16Lp2 compared the swards sown with Lp4. Trampling damage may explain this lower yield of Lp4 and its mixtures: June and July 2012 were very wet months with 248 mm of rain compared to 151 mm normally (**Appendix 1**), resulting in an intensive trampling damage in the grazing period that followed the 2nd cut. Lower tolerance to trampling of Lp4 compared to Lp2 was reported earlier (Frame, 1992). We can only speculate on the effect of the non-recording of the grass growth during the grazing windows on the DMY results, but we think that a full record would not have changed ranks.

Three years after the sowing of the trial, FaC was still significantly influenced by the initial Lp proportion in the seed mixture. The Lp proportion in the DMY was always higher than the Lp proportion in the seed mixture. Both species were contributing approximately equally to the DMY in the swards with 1/4 Lp.

The clover seedling abundance two months after sowing was between 86 and 131 seedlings m^{-2} for the swards sown with Lp2 and 1/4Lp4 respectively. Although this was below the target of 150 white clover plants m^{-2} three months after sowing set by Frame (1995) for the successful establishment of perennial ryegrass white clover swards, the clover was very well established by the autumn of 2009 (no quantitative data available), but it declined sharply in 2012. Previous research confirms that it is difficult to maintain white clover content on a high level in the conditions encountered in this trial. In a trial established in 1996 in Schleswig-Holstein, Germany with a management similar to the management in our trial: grazed grass clover fertilised with $20 m^3$ cattle slurry $ha^{-1}yr^{-1}$ and 100 kg mineral N $ha^{-1}yr^{-1}$, Ingwersen (2002) measured a TrC of 14.9, 7.0 and 3.9 in the years 1997-1999 respectively.

A factor that might have contributed to the low TrC is the establishment of the trial in former grassland. Bommelé (2007) showed that TrC was higher in perennial ryegrass-white clover swards established on former arable land compared to former grassland. In combination with a mineral fertilisation of 100 kg N ha^{-1}, the TrC averaged over 2002-2004 was 47 % in the swards on former arable land and 18 % in swards on former permanent grassland. When the mineral fertilisation was 300 kg N ha^{-1}, TrC was 17 % in swards on former permanent arable land and 6 % in swards on former permanent grassland. Williams *et al.* (2003) however proved that there were important varietal differences in the adaptability of white clover to grazing and high N fertilisation. They found that some white clover varieties in sheep grazed swards, consisting of perennial ryegrass and white clover, continued to deliver a contribution of 15 % to the DMY after ten years of grazing and with a fertilisation of 200 kg N ha^{-1} yr^{-1}.

Although Fa swards are reported to have a significantly lower tiller density than Lp2 swards (**Chapter 3**), these more open swards did not lead to higher invasion by weed species in our trial. Both the weed biomass as the abundance of dandelion plants were lowest in Fa$_1$, and highest in 1/4 Lp4 and Lp4 respectively. It is well known, that once Fa has established, it is very competitive towards other species. The differences in weed biomass between the species were not significant due to the high variation found in weed presence between the plots. The non-uniform distribution of the dandelion plants could partially be explained by the experimental design that did not allow uniform seed rain over the whole experimental area. Honek *et al.* (2005) found that 40-60 % of dandelion seed rain falls in the direct neighbourhood of parent plant. As the original pasture surrounding the experimental plot contained a lot of dandelions, the outer plots of the trial were expected to be subjected to a higher dandelion seed rain. Indeed: seven out of the ten plots with the highest dandelion presence were plots adjacent to the original pasture.

5.5 Conclusions

Regarding our research hypotheses, we can conclude as follows:

1. No transgressive overyielding was found in the mixtures Fa + Lp.
2. A proportion of 1/4 Lp in the seed mixture led to a sward in which Fa and Lp contributed approximately equally to the dry matter yield, three years after sowing.
3. The Fa content in the swards with tetraploid Lp was not significantly higher than in the swards with diploid Lp.
4. No significant difference in weed abundance nor weed contribution to the DMY was found between the sward compositions.

Chapter 6

Comparison of NIRS calibration strategies for the botanical composition of grass-clover mixtures

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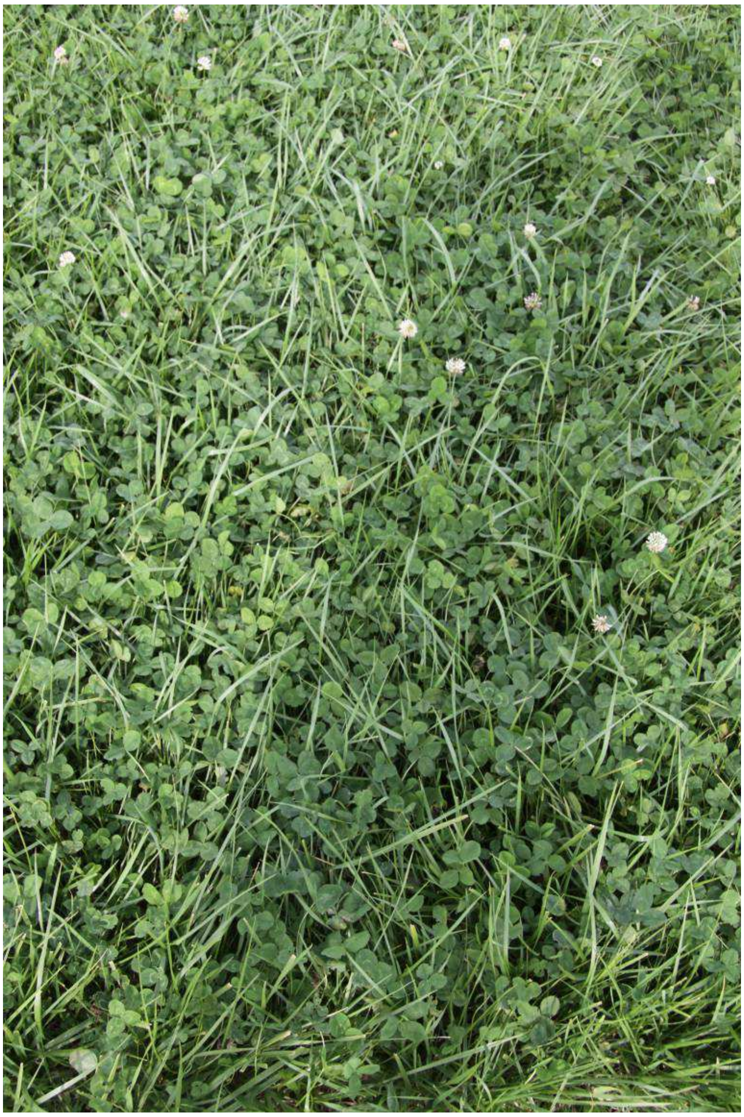


6.1 Introduction

In grassland research dealing with mixtures of different forage species, the contribution of the different species in the yield is often of interest, for example in research on sward dynamics of mixed swards (**Chapters 3, 4** and **5**). Forage crop breeders, grassland researchers and farmers have an interest in the competitive ability of species, since in many countries swards are sown with mixtures of grass and legume species. Important differences in the compatibility of white clover (*Trifolium repens* L.; **Tr**) and perennial ryegrass (*Lolium perenne* L.; **Lp**) genotypes to grow in association with each other were found (Elgersma and Schelpers, 1997; Annicchiarico and Proietti, 2010; Wulfes and Taube, 2010; Williams *et al.*, 2003). Also in grass species mixtures, compatibility differences were found between varieties. Sugiyama and Nakashima (1991) found significant differences in relative contribution of difference tall fescue (*Festuca arundinacea* Schreb.; **Fa**) varieties growing in association with orchardgrass (*Dactylis glomerata* L.; **Dg**). The availability of a handsome and efficient method to assess the compositon of mixed swards may stimulate breeders to breed for a “coexistence between species”. The incentive is important since mixed swards may take advantage of niche differentiation and functional biodiversity (Huyghe *et al.*, 2012).

Visual estimation of the botanical composition is a fast method, but is very subjective. Separation of a cut sample into the different constituents is very precise but is often too labour intensive for large field experiments or breeding programmes. NIRS (near infrared reflectance spectroscopy) can predict the botanical composition of a sample both fast and precise, provided an equation is available.

NIRS equations are widely used in grass variety evaluation trials to assess nutritive quality of new candidate varieties (Burns, 2012). NIRS equations are based on calibration samples. These are samples for which both the NIRS spectra and the reference values are available. Reference values are obtained by analysing the samples with a reference (laboratory) method for the parameter for which the equation is built. Chemometrics are used to link the multivariate information in the NIRS spectra to reference values found for the different samples (Naes, 2002).

Several equations to predict the botanical composition of multi species swards by NIRS have been developed in the past. These calibrations can be classified according to (1) the species and the number of different species for which the calibration was built, (2) the magnitude of the calibration population and its specific use and (3) the calibration strategy used.

(1) Mostly, equations were built to discriminate between grass and one or more leguminous species. Discriminations have been made between a specific grass and a specific legume, for example tall fescue *versus* white clover in Petersen *et al.* (1987); between non specified grasses *versus* white or red clover in Wachendorf *et al.* (1999) or between functional groups for example grasses *versus* leguminous species in Locher *et al.* (2005a).

Equations which aim to discriminate between different grass species were built by Coleman *et al.*, (1985) and Chataigner *et al.*, (2010).

(2) The objective of the calibration can be to predict the composition of a "closed population" for example a well defined trial with species mixtures (Surrault *et al.*, 2006) or a "open population" for example clover content in swards varying in phenological growth stages, in grass species and in nitrogen fertilization and climatic conditions (Wachendorf *et al.*, 1999).

(3) Different strategies can be used to build a calibration according to the way in which the composition of the samples is determined. The most straightforward way to build a calibration is by separating samples (harvested from multispecies swards) into their different components, weighing the different species, recomposing the original samples, drying and grinding the samples and finally recording the spectra. Calibration samples obtained in this way are called "real samples" (Shaffer *et al.*, 1990; Pitman *et al.*, 1991, Wachendorf *et al.*, 1999; Surrault *et al.*, 2006). An alternative is mixing dried and ground components to compose a whole new set of samples differing in the proportions of their components. Samples obtained in this way are called "artificial samples" (Pitman *et al.*, 1991; Coleman *et al.*, 1985; Locher *et al.*, 2005a; Surrault *et al.*, 2006). The plant material used in this strategy is harvested in swards sown with a single species, which eliminates the labour intensive hand separation.

An even simpler strategy is to collect the NIR **spectra** of dried and ground material of "botanically pure samples" (i.e. samples taken from swards with a single species) and to use these spectra to estimate the composition of mixtures. This strategy is based on the assumption that spectra of mixtures can be obtained by linearly combining the spectra of the botanically pure samples. This strategy is called "end point calibration" (Coleman *et al.*, 1990; Locher *et al.*, 2005a, 2005b).

Pitman *et al.* (1991) compared the three strategies mentioned above: equations based on real, artificial and pure samples were built and validated with a same, independent set of actual pasture samples (real samples). Despite the excellent calibration statistics of the equations based on the artificial samples, the validation statistics were very poor. Using a calibration based on pure samples improved the results but validation statistics remained poor. Calibration based on real samples gave the best results. The comparison between the methods was not entirely justified, as the numbers of calibration samples and the origins of the material were not equal in the tested strategies. Up to now, any explanation is missing why calibrations based on artificial samples and/or pure samples are mostly failing in predicting the composition of real pasture samples.

In order to disentangle this problem, we repeated the comparison made by Pitman *et al.* (1991) using fully comparable plant material and using equal numbers of samples with a fully comparable botanical composition. The equations were validated with the same set of real samples. On top of this we developed a calibration strategy, based on adding variation to the spectra of artificial samples. The rationale for this fourth strategy was based on our own observation that spectra of artificial samples contain less spectral variation than spectra of real samples (Cougnon *et al.*, 2012).

Our research hypothesis was that NIRS calibration can be used to develop an equation to predict the botanical composition of mixtures of tall fescue, perennial ryegrass and white clover. The questions we wanted to answer were:

1. Which calibration strategy leads to the most robust equations resulting in the smallest prediction errors?
2. What is the prediction error when the best calibration strategy is applied to the samples of the trial described in **chapter 3**?

6.2 Materials and Methods

6.2.1 Plant material

Plant material was collected in the year 2011, from two trials comparing the yield and botanical composition of Lp or Fa with Tr or mixtures of both grass species with Tr. These trials were described in **chapter 3** (cutting only management; referred to in this chapter as trial 1) and **chapter 5** (mixed cutting and grazing management; referred to in this chapter as trial 2).

At each cut (5 cuts/year for the trial described in **chapter 3**; first cut of 2011 of the trial described in **chapter 5**) a representative sample (150-300 g of fresh material) of the harvested material of each plot was separated into the different species and the fresh weights of the species were recorded. After the separation, mixtures containing two grass species (both Fa and Lp) were treated differently from mixtures containing only one grass species. The separated material from the plots with Fa + Lp +Tr was recomposed. The separated material from the plots with Fa+Tr or Lp+Tr was not recomposed, but pooled per species over the replications resulting in one pure sample for each of the four species (Fa, Lp2, Lp4 and Tr) per cut and per trial. Finally all samples were dried (16 h, 75 °C) and ground (Brabender shear mill) to pass a 1mm sieve.

The origin and the construction of the samples is presented in **table 6.1**. Each cut in both trials delivered one pure sample for each of the four single species. On top of this we collected 83 separated and recomposed samples with three constituent species: 60 samples came from trial 1 (4 mixtures Fa+Lp+Tr x 3 replicates x 5 cuts); the remainder of the samples came from the spring cut in trial 2. As the botanical composition within the plots in trial 2 was expected to be less homogenous compared to trial 1 due to the grazing management, an extra sample was taken per plot on two of the four blocks in trial 2. As one sample got lost we finally ended with 23 samples: 4 mixtures x 4 reps + (4 mixtures x 2 reps) - 1.

The plant material described above was used to compare different calibration strategies. Finally, we used the best of these four strategies to build an equation for the determination of the botanical composition of the trial described in **chapter 3**. Over the 15 cuts in the three successive years, 180 samples containing Fa, Lp and Tr were hand sorted for determination of the botanical composition (3 years x 5 cuts/year x 4 sward compositions x 3 replicates). We collected the NIR spectra from all these samples.

Table 6.1 Number of samples used and their origin. C1-C5: first to fifth cut.

		Trial 1					Trial 2	
	Samples were…	C1	C2	C3	C4	C5	C1	Total
1 grass species and clover								
Fa	Separated, pooled per species over replicates, dried, ground	1	1	1	1	1	1	6
Lp2		1	1	1	1	1	1	6
Lp4		1	1	1	1	1	1	6
Tr		1	1	1	1	1	1	6
Total		4	4	4	4	4	4	24
2 grass species and clover								
1/4Lp2+3/4Fa	Separated, recomposed, dried, ground	3	3	3	3	3	6	21
1/4Lp4+3/4Fa		3	3	3	3	3	6	21
1/8Lp2+3/8Fa		3	3	3	3	3	5[a]	20
1/8Lp4+3/8Fa		3	3	3	3	3	6	21
Total		12	12	12	12	12	23	83

[a] one lost sample

6.2.2 Four calibration sets

Four different sets of calibration samples were built with the available plant material (**Figure 6.1**). The first set consisted of the 83 hand separated and recomposed samples; these samples are “real samples” (**Figure 6.1a**). As hand separating is the reference method to determine the botanical composition, equations should always be validated with real samples. Therefore, the set of 83 real samples was split in a calibration set and a validation set. Half of the samples of each cut were assigned to the calibration set (42 samples) and the remaining 41 samples were assigned to the validation set.

A second calibration set consisted of artificial samples (**Figure 6.1b**): for each of the 42 real samples in the calibration set, an artificial sample with the same botanical composition was made, by physically mixing the powders of pure samples in the proportions found in the real sample. As a result the number of samples and the botanical composition was identical in both the real and artificial calibration set.

A third calibration set (again with the same number of samples and identical botanical composition as in the real calibration set) was built by making linear combinations of the spectra of the pure samples. So this third calibration set had no physical samples but was a set of spectra. The spectra were calculated as ($a_iX_{Fa,j}+b_iX_{Lp2,j}+c_iX_{Lp4,j}+d_iX_{Tr,j}$); a_i, b_i, c_i, d_i, being the proportion of Fa, Lp2, Lp4 and Tr respectively in the *i*-th sample of the 42 real samples and $X_{Fa,j}$ $X_{Lp2,j}$ $X_{Lp4,j}$ $X_{Tr,j}$ being the spectra of the four pure species in the *j*-th cut (**Figure 6.1c**). We called these linear combinations of spectra “artificial spectra”.

A fourth calibration set was obtained by adding variation to the spectra of the artificial samples (**Figure 6.1d**). The added variation was the difference found between spectra of real and artificial samples with the same botanical composition. Among the spectra of the real calibration samples, twelve spectra (two from each cut of the first trial and two from the single cut in the second trial) were chosen randomly and corrected by subtracting the spectra of the 12 corresponding artificial samples resulting in 12 spectra differences. These 12 spectra differences were added to each of the spectra of the 42 different artificial calibration samples, resulting in 504 (12 x 42) new spectra. This set of spectra was called “artificial spectra with added variation”.

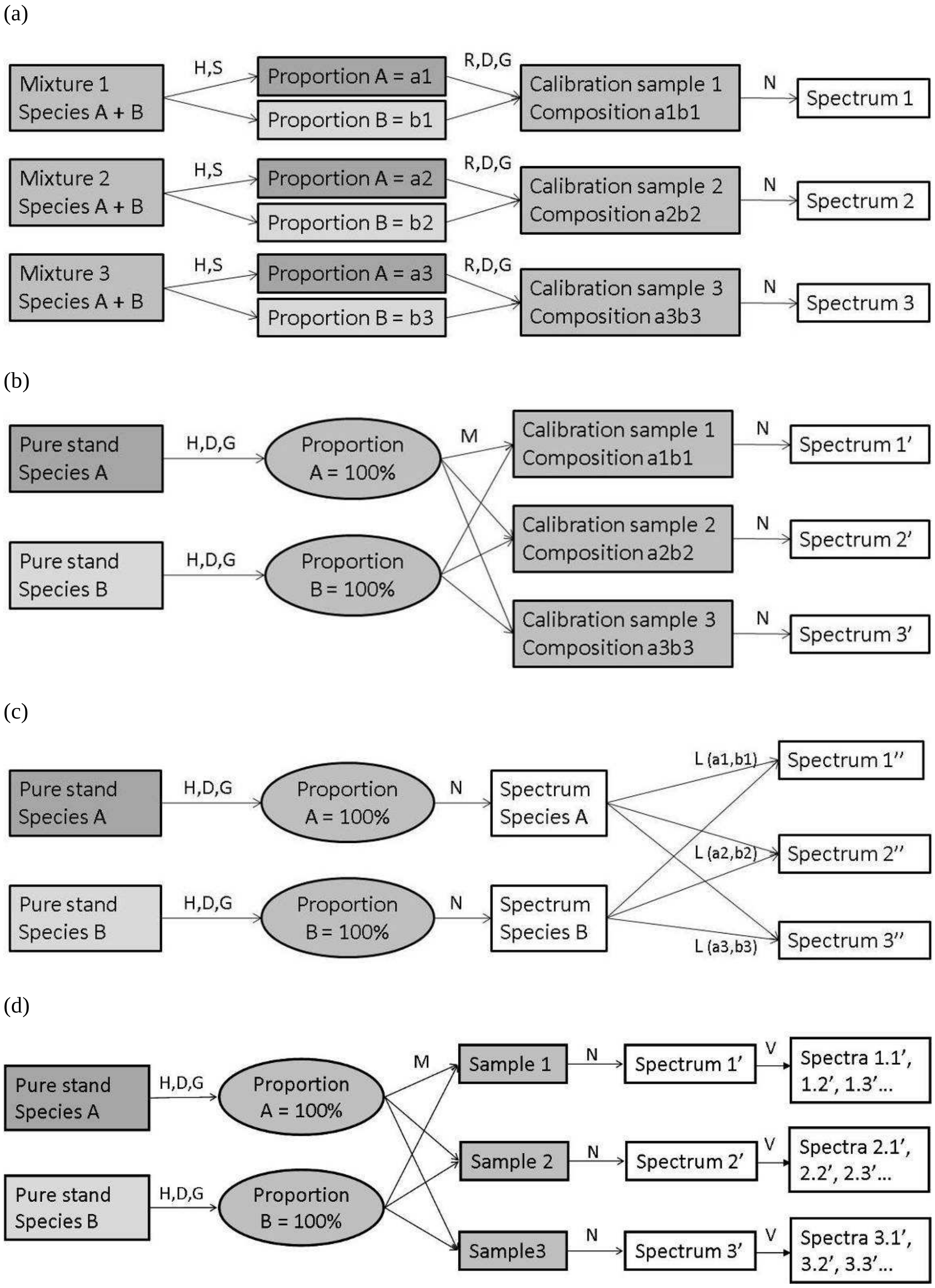


Figure 6.1 Calibration strategies for the distinction of 2 species A and B based on: (a) real samples, (b) artificial samples, (c) artificial spectra, (d) artificial spectra with added variation. H=Harvest; S=Separate; R=Recompose; D=Dry; G=Grind; N=collection of NIR spectrum; M=Mixing powders in known proportions; L= Making linear combinations of spectra with scalars (a, b); V=Adding variation

6.2.3 Collection of spectra and calibration

The spectra of the pure species and of the real and artificial samples were collected on the same day with a Foss NIRSystems 5000 and ISIscan 2.85.1 software. One quartercup was scanned per sample. The inverse reflectance (log(1/R)) was measured from 1100-2500 nm in steps of 2 nm, resulting in 700 datapoints per spectrum. All equations were calculated with WinISI II 1.5 using the partial least square regression method. A data pre-treatment was performed before regression: the first derivative of the spectra was taken (with gap 4 and smoothing 4) and scatter correction using SNV (standard normal variate) and detrend was applied (Shenk and Westerhaus, 1993). Principal component analysis and calculation of Mahalanobis distance were performed in WinISI (Shenk and Westerhaus, 1991).

6.2.4 Linear combinations and adding variation

The linear combinations of the spectra of the botanically pure samples, needed to create the artificial samples, were calculated in R (R Development core team, 2011). The 24 (6 cuts x 4 species) spectra of botanically pure species were exported from WiniISI as a 24 x 700 matrix, which was imported in R using the *read.table()* function. This matrix was split in six 700 x 4 matrices, each containing the spectra of Fa, Lp2, Lp4 and Tr for one cut). The 42 x 4 matrix with the proportions of Fa, Lp2, Lp4 and Tr for each of the 42 real calibration samples was also imported. Using a loop, each row of the matrix with the proportions was multiplied with the matrix containing the pure spectra of the appropriate cut, resulting in a 42 x 700 matrix. This matrix was exported using the *write.table()* function and imported in WinISI.

The addition of spectral variation to each of the artificial samples, creating the artificial spectra with added variation, was also performed in R. A 24 x 700 matrix including the spectra of the 12 selected real samples and the 12 corresponding artificial samples and the 42 x 700 matrix containing the spectra of the 42 artificial samples were imported in R using the *read.table()* function. The 12 spectra of the artificial samples were subtracted from the 12 corresponding real samples, resulting in a 12 x 700 matrix containing the spectral differences between real and artificial samples. Using a loop, these 12 spectral differences were added to each of the 42 spectra of the artificial samples, resulting in a 504 (12 x 42) new spectra of artificial samples with added variation. This 504 x 700 matrix was exported using the *write.table()* function.

6.2.5 Comparison of the models

For each of the calibration sets, an equation for the prediction of the proportion Fa, Lp and Tr in the dry matter was built, called eq. 1 till eq. 4 (**Table 6.2**). Standard errors of calibration (SEC), Standard errors of cross-validation (SECV) and the determination coefficient of the linear regression between the predicted and the reference values (RSQ) were calculated for the four equations. The four equations were validated with the validation set of 41 real samples. Root mean square error of prediction (RMSEP), bias and the ratio of prediction to deviation (RPD), which is the ratio of the standard deviation of the reference values to the RMSEP, were calculated (Shenk and Westerhaus, 1993). For each of the spectra of the real calibration samples, artificial calibration samples and artificial calibration spectra, the Neighbourhood H (NH) value, which is the Mahalanobis distance of a spectrum to its closest neighbour, was calculated (Shenk and Westerhaus, 1993).

Table 6.2 Calibration sets on which different equations were based

Equation	Calibration set
1	42 real samples
2	42 artificial samples
3	42 artificial spectra
4	504 artificial spectra with added variation

The different calibration strategies were compared statistically by calculating confidence intervals for the ratios of the standard errors of the prediction errors (SEP), taking into account the paired nature of the prediction errors (Fearn, 1996; Naes *et al.*, 2002). Confidence intervals for the ratios of the SEPs obtained with the equation based on real samples (eq. 1) and each of the three other equations were calculated. If a confidence interval excluded 1, the SEPs were different at the 5% significance level, and the results obtained with the two methods were considered as different.

6.3 Results and Discussion

As the calibration strategies used to build eq. 1, eq. 2 and eq. 3 were based on an equal number of calibration points with the same composition, a direct comparison of these strategies was appropriate. Both the calibration sets and the validation set contained samples with a very diverse composition (**Table 6.3**). The validation set used to compare the different calibration strategies was a subset of our real samples. Hence, the validation set was not completely independent. The results shown here are only valuable in the closed population of samples of the concerned trials. This is not problematic as it was not the aim of this experiment to present the performance of the calibrations, but merely to compare different strategies to develop calibrations. The same principles would apply to a larger dataset, validated with a real, independent dataset (for example plant material harvested on a different site).

Table 6.3 Distribution of the botanical composition (% by dry weight) of the samples in the calibration sets and in the common validation set (SD=standard deviation).

	Calibration set				Validation set			
	Mean	SD	Min	Max	Mean	SD	Min	Max
Fa	49.3	16.9	18.4	89.0	53.5	15.2	26.5	93.9
Lp	32.4	11.8	11.0	60.1	28.2	13.8	5.7	66.9
Tr	18.2	11.7	0.0	41.3	18.3	10.9	0.4	41.1

6.3.1 Real samples vs. artificial samples and artificial spectra

The standard errors of calibration (SEC) were higher for the equation based on the real samples (eq. 1) compared to the calibrations based on artificial samples (eq. 2) or artificial spectra (eq. 3) (**Table 6.4**). The RMSEP on the other hand, was lower for eq. 1 compared to eq. 2 and 3 for the three species involved. For the three equations Tr was the species which was best predicted (the lowest RMSEP, highest RPD) (**Table 6.4**), which was no surprise as the difference in chemical composition between clover and grass is higher than between two grass species.

Eq. 1 had an RPD higher than 2 for Fa, Lp and Tr; for eq. 2 and eq. 3 the RPD was below 2 for the three species. As an RPD of 2 is generally regarded as the minimum for a suitable calibration, only eq. 1 was considered as suitable to predict the composition of the validation set (**Table 6.4**).

Table 6.4 Calibration and validation statistics of four equations for the prediction of the botanical composition of forage samples containing *Festuca arundinacea* (Fa), *Lolium perene* (Lp) and *Trifolium repens* (Tr). SEC: standard error of calibration (%); SECV: standard error of cross-validation (%); RSQ: R squared value; RMSEP root mean square error of prediction (%); Bias: average difference between the predicted values and the reference value of the validation samples; RPD: ratio of standard deviation of validation samples and RMSEP.

		Calibration			Validation		
		SEC	RSQ	SECV	RMSEP	Bias	RPD
Equation 1	Fa	3.7	0.92	6.1	5.4	-1.2	2.8
	Lp	5.0	0.87	7.8	6.1	1.4	2.3
	Tr	2.5	0.95	3.9	2.9	0.4	3.8
Equation 2	Fa	3.6	0.94	4.9	15.6	-9.1	1.0
	Lp	3.3	0.94	5.0	15.9	7.4	0.9
	Tr	1.4	0.98	1.9	6.8	0.7	1.6
Equation 3	Fa	1.8	0.98	2.3	14.3	0.6	1.1
	Lp	1.8	0.98	2.2	21.7	-6.8	0.6
	Tr	0.7	0.99	0.9	13.9	8.0	0.8
Equation 4	Fa	1.9	0.98	2.4	6.0	0.3	2.5
	Lp	1.9	0.98	2.4	7.7	0.5	1.8
	Tr	0.6	0.99	0.8	3.5	-0.7	3.1

Confidence intervals for the ratio of the SEPs obtained with eq. 1 and eq. 2 were [0.35, 0.60], [0.41, 0.68] and [0.26, 0.48] for Fa, Lp and Tr respectively. For eq. 1 and eq. 3, the confidence intervals of the ratio of the SEPs were [0.33, 0.61], [0.26, 0.49] and [0.20, 0.36] for Fa, Lp and Tr respectively. This means that the standard deviations of the prediction errors obtained with the calibrations based on artificial samples or on artificial spectra were significantly higher for the three species; hence the equation based on real samples predicted the botanical compositions of the samples in the validation set significantly better.

The different performances of the equations based on artificial samples and artificial spectra on one hand and the real samples on the other hand can be understood from a principal component analysis (**Figure 6.2**). Two clear trends can be observed. Firstly, the scores of the artificial samples and the artificial spectra almost overlapped, indicating that the spectral information contained in both types of spectra was very similar. This was confirmed by the study of the NH distances, the Mahalanobis distance between a sample and its closest neighbour. Averaged over all cuts, the NH values between the artificial samples and the artificial spectra were almost equal (**Table 6.5**).

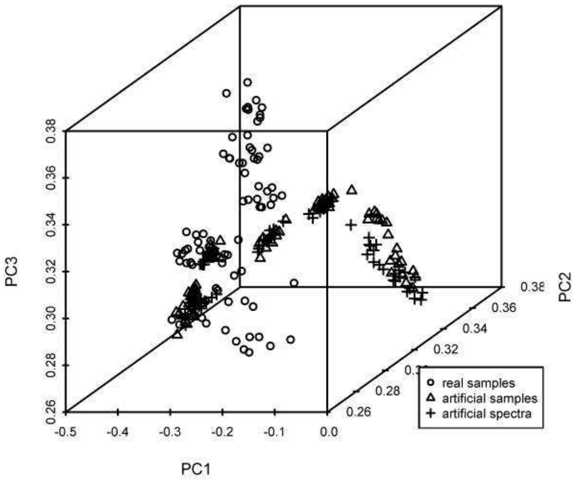


Figure 6.2 Scores of spectra of real and artificial samples and of artificial spectra plotted in their first three principal components

Table 6.5 Neighbourhood H values (means and standard deviation) of real samples, artificial samples and artificial spectra calculated in their principal components. C1-C5: first to fifth cut.

		Trial 1					Trial 2	Mean
		C1	C2	C3	C4	C5	C1	
Real samples	Mean	0.096	0.110	0.091	0.086	0.122	0.185	0.126
	Stdev	0.061	0.040	0.048	0.041	0.050	0.120	0.086
Artificial samples	Mean	0.013	0.011	0.024	0.028	0.074	0.025	0.028
	Stdev	0.065	0.007	0.009	0.017	0.060	0.019	0.031
Artificial spectra	Mean	0.014	0.010	0.025	0.023	0.072	0.027	0.028
	Stdev	0.021	0.008	0.008	0.023	0.052	0.021	0.031

Secondly, the real samples occur in larger, wider clouds than the artificial samples or artificial spectra. The mean NH distance was at least twice as high for the real samples compared to the artificial samples. The standard deviation on the NH distances between the points was also larger for the real samples, except for the fifth cut of the first trial. Averaged over all cuts, the mean NH distance between the real samples was four times higher than the distance between artificial samples; standard variation was twice as high.

We can conclude that less variation is present in the spectra of artificial samples and artificial spectra compared to real samples even though the botanical composition of both types of samples was the same. The variation present in the validation samples is not spanned by the cloud of artificial samples which may be the reason for the weak performance of eq. 2. To prove this hypothesis, we tried to add relevant variation to the artificial samples, and compared the performance of the resulting equation with the equation based on real samples.

6.3.2 Artificial spectra with added variation

Adding relevant spectral variation to the artificial samples was expected to enhance the prediction ability of the calibrations based on these samples. Indeed, the equation based on the artificial spectra with added variation (eq. 4) performed well: RMSEPs were on the same levels as these obtained with the real samples (**Table 6.4**) and the RPD's were higher than 2 for Fa and Tr. Confidence intervals for the ratio of the standard deviations of the prediction errors of eq. 1 and eq. 4 were [0.82, 1.39], [0.76, 1.24] and [0.69, 1.16] for Fa, Lp and Tr respectively, which means that there was no significant difference in the performance of the equation based on real samples and the equation based on artificial spectra. The values of the artificial spectra with added variation spanned the whole space filled by the real (validation) samples in the first three principal components (**Figure 6.3**).

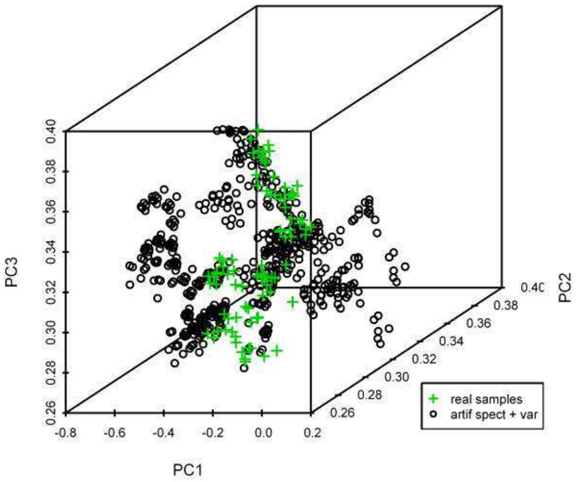


Figure 6.3 Scores of the artificial spectra with added variation (artif. spect. + var.) plotted in the first three principal components of the spectra of the real and the artificial samples.

From the observations above, we concluded that the weak performance of eq. 2 and eq. 3 were based on a lack of variation in the spectra of the artificial samples and artificial spectra. As the variation in botanical composition in both sets of samples was exactly the same, the difference has to be explained by other spectral variation originating from factors excluding the botanical composition. However, the main sources of spectral variation (different cuts, different trials, ploidy of the perennial ryegrass, drying, grinding) were identical for both sets. The only source of spectral variation present in the real samples and absent in the artificial samples, was the variation caused by different plots. As indicated above, the pure material from the plots sown with only one grass species and clover was pooled over the replicates of the same cut, resulting in one pure sample per cut for each species. By acting like this we diminished the labour which was one of the prerequisites of our research. We did not expect this to have any effect on the calibration, as the trials were very uniform, and the variation coefficients for dry matter yield, crude protein content and digestibility of the organic material were very low (data not shown). The difference between the real and the artificial samples may find its origin in the post harvest handling and behaviour of the plant material. Different authors (Locher *et al.*, 2005a; Coleman et al., 1990) suggest that there is a difference in particle size distribution whether a specific species was ground solely or in a mixture, but these effects are believed to be largely eliminated by the mathematical treatment applied on the spectra. Another source of variation between the real and artificial samples might be due to the interaction of species growing together in a mixture compared to species growing in a pure stand. A similar suggestion was made by Surrault *et al.* (2006), who explained the difference between real and artificial samples by stating that the spectral signatures of grasses growing in pure stands and mixtures are different. This interaction effect is especially true when one of the species is clearly dominant. Indeed, there is a clear morphological difference between a species growing in a mixture where it is dominated by another species, and the same species growing in a pure stand. Whether the spectra of species growing in pure stands or in mixtures are different, remains to be proven.

Whatever the reason or origin may be, it is remarkable that apparently small differences in spectral variation in the calibration samples have a huge influence on the resulting equations.

One way to increase the spectral variation in the artificial samples is to harvest pure material from more plots since apparently a pure sample for each species in the real samples is needed to obtain the same amount of variation in the artificial samples as in the real samples.

Surrault *et al.* (2006) noticed that broadening a calibration based on artificial samples with some real samples greatly improved the performance of their calibration. The strategy we used to improve the performance of the artificial samples was to increase the variation in the spectra of the artificial samples by adding the variation present in the spectra between real and artificial samples with an identical botanical composition. No new reference values were added, just differences between spectra. The number of spectra in the fourth calibration strategy was inflated a lot, but the number of reference values did not change.

6.4 Implications for practise

Although good calibrations for botanical composition based on botanical pure samples were described (Locher *et al.*, 2005), our findings suggest there is more advantage in taking time and effort to use real samples, that represent all the variables which would affect the NIRS spectra, rather than creating artificial samples. The labour required to separate samples can be reduced by following a strategy in which relevant spectral variation is added to the available reference values (Fernández Pierna *et al.*, 2010). By scanning a restricted set of samples for example on different days (with different temperature and humidity), the variation due to different scanning circumstances can be added to all the reference samples. The variation present between the spectra of a particular species grown as a single species in different locations or managed under different circumstances is another source of variation that may be added to make a calibration more robust for botanical composition.

In some cases the use of a calibration strategy based on real samples is not possible (e.g. because the species for which the calibration is built are very difficult to separate). In that case a calibration strategy based on botanical pure samples can be used keeping some important points in mind.

There is not much gain in mixing physically only a few botanically pure samples to obtain series of artificial samples with a whole range of compositions. Our results and results of Locher *et al.*(2005a) and Coleman *et al.(*1990) and Pitman et al.(1991) indicate that acting like this merely creates linear combinations of spectra of pure samples. It is the spectral variation in the pure samples that is of the greatest importance and spectral variation can be added to the spectra using a strategy as presented in this article.

6.5 Application to mixtures of chapter 3

We learned above that best calibration equations for the determination of the botanical composition are based on real samples that include all the spectral variation that is present in the validation set. With this knowledge, we built an equation for the prediction of the botanical composition of the samples collected in the trial described in **chapter 3**. The 15 harvested cuts in the three successive years generated 180 hand sorted samples containing Fa, Lp and Tr and the corresponding spectra. In each cut, the samples harvested in block 1 were attributed to the calibration set, resulting in a calibration set of 60 samples: 4 samples taken from every cut. Like this we were sure that all mixtures and all cuts were evenly represented in the calibration. The equation based on this calibration set was used to predict the botanical composition of the 120 samples taken in block 2 and 3 (**Table 6.6**). SEC, RSQ and SECV were from the same order of magnitude for the three species. For the three species, a significant relationship between the predicted and the reference values was found, with R^2 values of 75 %, 67 % and 84 % respectively (**Figure 6.4**).

Table 6.6 Calibration and prediction statistics for the equation determining the botanical composition of forage samples harvested in the trial described in chapter 3 containing *Festuca arundinacea* (Fa), *Lolium perenne* (Lp) and *Trifolium repens* (Tr). SEC: standard error of calibration (%); SECV: standard error of cross-validation (%); RSQ: R squared value; RMSEP root mean square error of prediction (%); Bias: average difference between the predicted values and the reference value of the validation samples; RPD: ratio of standard deviation of validation samples and RMSEP; Fa: tall fescue; Lp: perennial ryegrass; Tr: white clover.

	Calibration (n=60)			Prediction (n=120)		
	SEC	RSQ	SECV	RMSEP	Bias	RPD
Fa	3.6	0.95	7.4	8.0	-0.1	1.9
Lp	3.7	0.93	8.7	9.8	1.1	1.7
Tr	3.3	0.90	5.4	4.2	0.7	2.4

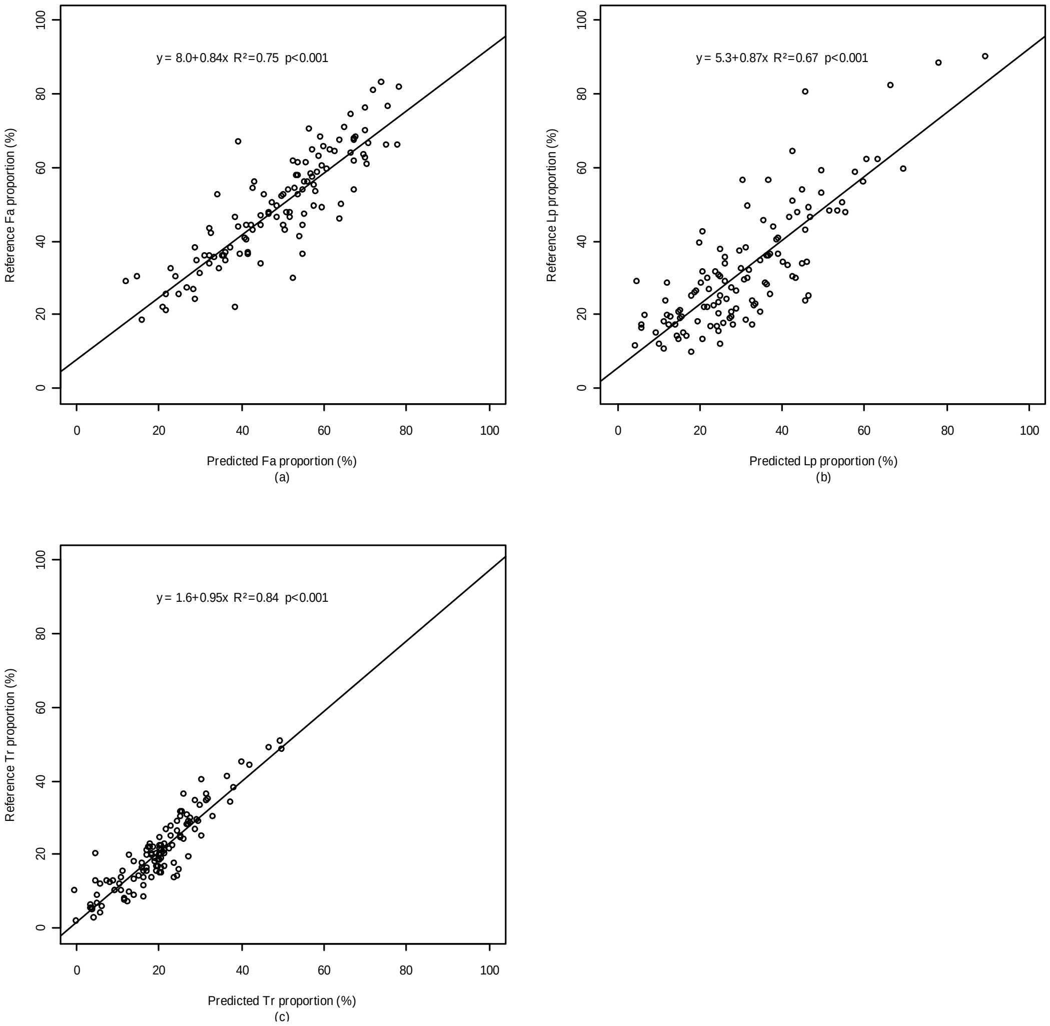


Figure 6.4 Relationship between predicted proportion of (a) tall fescue (Fa), (b) perennial ryegrass (Lp) and (c) white clover (Tr) in 120 forage samples with equation based on 60 calibration samples and botanical composition determined by hand sorting.

The prediction was better for Tr compared to the grass species. RMSEP was more than twice as large for Fa and Lp compared to Tr. Only for Tr, the RPD was above 2, indicating that the equation was not suited to predict the Fa and Lp proportion in the samples.

This better prediction of Tr compared to the grass species is obvious. Firstly, the difference in chemical composition between clover and grass is greater than between Fa and Lp resulting in larger spectral differences between Tr and the grass species compared to Fa and Lp. Secondly, sorting the grass species was hard in some cuts, and it is very probable that in some samples, the Fa and Lp proportion found by hand sorting deviates a little bit from the reality. Particularly in the cuts taken later in the season, when the grass growth was lower, sorting was difficult. This was not the case in sorting Tr from grass: in all cuts it was very easy to separate clover material from grass material and every sorted sample was thoroughly checked for clover presence. The sorting errors can partially explain the higher SEC and RMSEP found for Fa and Lp compared to Tr. The magnitude of this source of error is not known for this trial. The calibration was based solely on samples from block 1, so possible variation present between blocks was not included in the calibration. Given the small size of this trial, and the very uniform land on which it took place, it is very improbable that the absence of this source of variation had a negative influence on the prediction results.

These results are in line with the results found by Chataigner *et al.* (2010) who built equations to predict the clover and grass species composition of multispecies forage mixtures containing Tr, Fa, Lp and Dg. An equation based on 3607 artificial and real samples from two different harvest years was validated with 80 independent real samples. They found RMSEPs of 3.3 %, 5.4 %, 9.0 % and 7.3 % for Tr, Fa, Lp and Dg respectively. Wachendorf *et al.* (1999) found an SEC and SECV of 5.9 % and 6.5 % for the determination of white clover content in 183 forage samples originating from different trials.

We can conclude that in trials where determination of clover content is of importance, labour can be saved by sorting only a part of the samples from every cut, to build an equation and to determine botanical composition of the remainder of the samples using the calibration. For determining the content of individual grass species, prediction errors were larger, and the statistics were suggesting that the calibration was not adequate to predict the Fa and Lp contents. The results of the equation for Fa and Lp might be improved by adding more calibration samples to the equation, but this implies more labour and decreases the interest of building an equation.

6.6 Conclusion

The performance of equations for the prediction of botanical composition based on artificial samples or artificial spectra was not satisfying. This weak performance could be explained by a lack of spectral variation originating from factors excluding the botanical composition in the artificial samples relative to the real samples. Adding the variation among artificial and real samples to the artificial calibration samples allowed to obtain a calibration which performed nearly as good as the calibration based on real samples. Based on this experience, we recommend a calibration strategy based on diverse hand sorted samples, rather than making a lot of artificial samples that contain relatively few spectral information. Adding environmental variation to the spectra of the calibration samples allows obtaining a robust calibration with a minimum of hand separated samples.

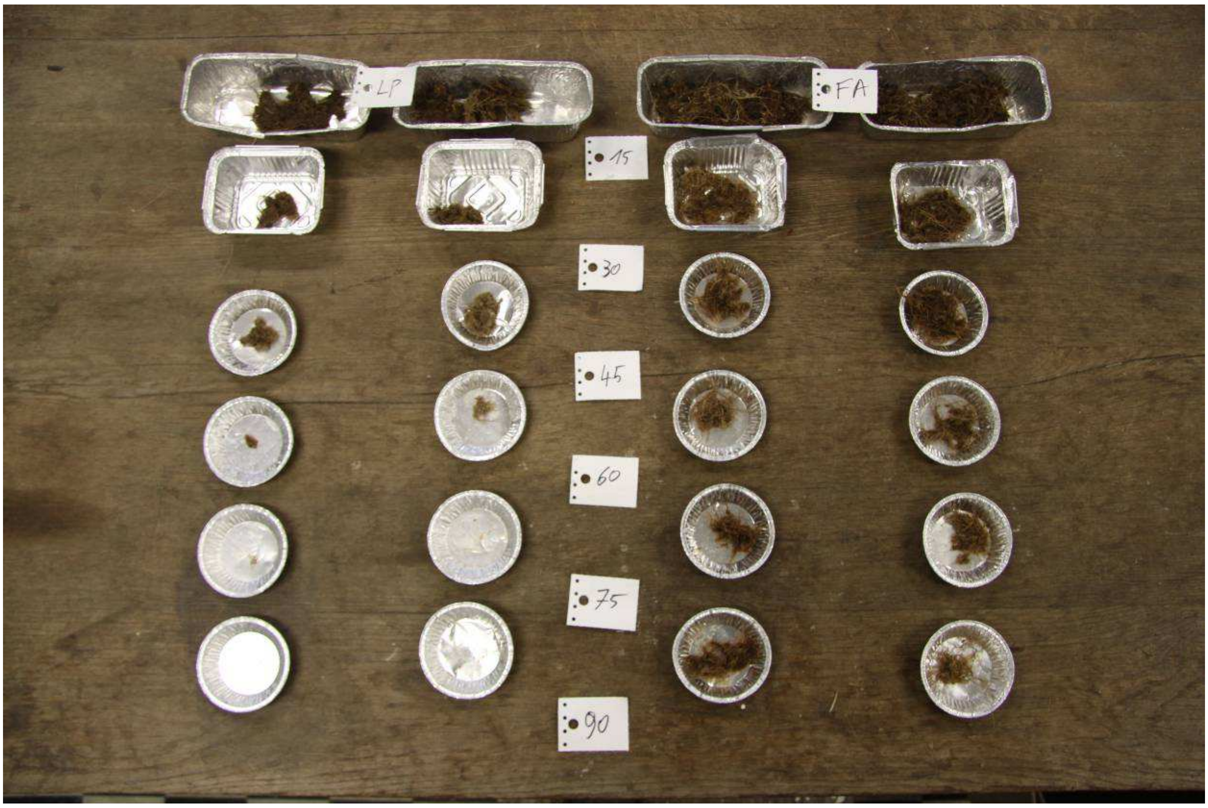
Regarding our research questions, we can conclude that:

1. A calibration strategy based on hand sorted samples that contain as much environmental variation as possible gave the best results.
2. When 1/3 of the hand sorted samples from **chapter 3** were use to build an equation, Tr was predicted with an RMSEP below 5 %. For Fa and Lp, the prediction was unsatisfactory: RMSEP of 8 % and 10 % respectively were found.

Chapter 7

Root depth and biomass of tall fescue compared to perennial ryegrass

Redrafted after: Cougnon M, Deru J, van Eekeren N, Baert J and Reheul D, 2013. Root depth and biomass of tall fescue vs. perennial ryegrass. In: Helgadóttir A. and Hopkins A. (eds) *The role of grasslands in a green future – Threats and perspectives in less favoured areas. Proceedings of the 17*th *symposium of the European Grassland Federation, Aykureyi, Iceland, 2013:* 285-287.



7.1 Introduction

Tall fescue (*Festuca arundinacea* Schreb.; **Fa**) has good drought resistance compared to other grass species (**Chapter 2**). Although the drought resistance of Fa compared to ryegrasses is explained by the deeper rooting of Fa particularly in deeper soil layers (**Chapter 1**), quantitative data on rooting depth and root biomass of grass species are scarce.

Durand and Ghesquière (2002) measured with a neutron probe that Fa extracted water until 180 cm deep whereas water extraction of Italian ryegrass (*Lolium multiflorum* L.; **Lm**) was limited to 80 cm in a deep soil in Lusignan, France. They found similar root biomass for Fa and Lm in the 0-50 cm layer of the soil and in both species the top 0-25 cm horizon contained approximately 80 % of the root biomass. In another research in Lusignan, France, Gonzalez-Dugo *et al.* (2005) measured root biomass, specific root length and root length density of Fa and Lm. Soil cylinders with a length of 25 cm were extracted until 1 m deep. Lm and Fa had respectively 78 % and 62 % of their root biomass in the first 25 cm layer. The maximum difference between Lm and Fa was found in the 25-75 cm layer where Fa root biomass doubled that of Lm. The specific root length on the other hand was markedly different between the species, with a mean value of 95 g m^{-2} for Fa and 181 g m^{-2} for Lm. This resulted in a larger estimated root length density for Lm (9.1 cm cm^{-3}) compared to Fa (5.8 cm cm^{-3}) in the first 25 cm of the soil, but in very similar values deeper in the soil.

Van Eekeren *et al.* (2010) measured root biomass of Fa and Lp growing on a sandy soil in the south of the Netherlands. Soil cylinders with a length of 10 cm were extracted until 30 cm deep. Fa had the highest root biomass in the 10-20 cm soil layer and in the 20-30 cm soil layer.

From these scarce results it is not clear whether the deeper rooting of Fa compared to Lp occurs in all soil types and under different management conditions. We compared the rooting depth of Fa and Lp in different field trials including both species. We hypothesised that:

1. Below 30 cm root biomass is consistently higher for Fa compared to Lp
2. Soil type, grassland management and sampling season are influencing root biomass of both species

7.2 Material and Methods

Four yield trials comprising both Fa and diploid Lp were sampled for root biomass. Trials were located on different soil types and conducted under different management regimes (**Table 7.1**). Throughout this chapter, the different trials will be named by the trial numbers reported in **table 7.1**. All trials were complete randomized block designs with three replicates. In the Belgian trials located at Melle, Merelbeke and Poperinge the sampled Fa and Lp varieties were 'Castagne' and 'Plenty' respectively. More details on the trials in Merelbeke and Melle can be found in **chapter 3** and **chapter 5** respectively. In the Dutch trial located in Helvoirt the Fa and Lp varieties were 'Barolex' and 'Bargala' respectively. Using a root auger (Eijkelkamp, Giesbeek, the Netherlands), soil cylinders with a radius of 4 cm and a height of 15 cm were extracted on six depths (0-15, 15-30, 30-45, 45-60, 60-75, 75-90 cm) in the Belgian trials (1A, 1B, 1C, 2 and 4). In the Dutch trials (2A and 2B) soil cylinders with a radius of 4 cm and a height of 10 cm were extracted on seven depths (0-10, 10-20, 20-30, 30-40,40-50, 50-60, 60-70 cm). Two samples of each depth were pooled per plot. Soil samples were stocked at -18°C prior to washing. Soil was washed from unfrozen samples with tap water on a sieve with mesh width (0.4 mm); no distinction was made between dead and living roots. Finally the roots were dried for 24 h at 75°C.

Table 7.1 Trial identification, soil type and management regime.

Trial	Location	Sowing date	Soil type	Sampling date	Management regime
1 A B C	Melle (B)	April 2009	Sandy loam	27/4/2011 16/10/2011 1/10/2012	Mowing (1st cut) followed by grazing 350 kg N yr^{-1}
2 A B	Helvoirt (NL)	September 2007	Sand	13/5/2011 5/8/2011	Cutting (4 cuts year^{-1}) 300 kg N yr^{-1}
3	Merelbeke (B)	April 2009	Sandy loam	15/10/2012	Cutting (5 cuts year^{-1}) 300 kg N yr^{-1}
4	Poperinge (B)	April 2009	Loam	7/11/2012	Cutting (5 cuts year^{-1}) 300 kg N yr^{-1}

For each individual location and sampling date, the effect of the grass species on the root biomass at the different depths was tested using one way analysis of variance (anova). The effect of the season (spring vs. autumn or summer) on the root biomass below 30 cm was tested in Melle (trial 1A versus 1B) and Helvoirt (2A versus 2B) using two way anova with season and species as fixed factors. Statistics were performed in R using the *aov()* function (R Development Core Team, 2011).

7.3 Results

7.3.1 Effect of location

The total root biomass (0-90 cm layer in trials 1, 3, 4; 0-75 cm layer in trial 2) was higher for Fa compared to Lp in the trials 1A, 3 and 4, whereas in the trials 1B, 1C, 2A and 2B root biomass of Lp was higher (**Table 7.2, Table 7.3**). Only in trial 3, a significantly higher (p = 0.03) root biomass was found for Fa. Averaged over all trials, 85 % and 92 % of the root biomass of Fa and Lp respectively was found in the 0-30 cm soil layer.

The root biomass below 30 cm depth, was significantly higher for Fa compared to Lp in the trials 1C, 3 and 4. In these trials, the root biomass below 30 cm represented 16 %, 19 % and 20 % for Fa and 8 %, 10 % and 6 % respectively of the total root biomass. When considering only the samples measured in autumn (trials 1B, 1C, 2B, 3 and 4), the largest differences were found in trial 3 and 4, where Fa root biomass was respectively 3.6 and 4.2 times higher than that of Lp. The smallest differences were found in the trials 1B and 1C, where Fa root biomass was respectively 1.5 and 1.6 times higher than that of Lp. Averaged over all trials sampled in autumn, the root biomass below 30 cm represented 8 % and 17 % of the total measured root biomass for Fa and Lp respectively.

7.3.2 Effect of season

Both in trial 1 as in trial 2, measured root biomass below 30 cm was lower in spring (1A and 2A) than in summer/autumn (1B and 2B) (**Figure 7.1**). In trial 1, the season effect was significant ($p < 0.001$): the root biomass below 30 cm was1.6 and 1.7 times higher in autumn compared to spring for Fa and Lp respectively. The effect of grass species (p = 0.06) and the interaction between species and season (p = 0.08) were marginally significant: it seemsed that Fa had a higher root biomass than Lp and that the effect of species was smaller in spring compared to autumn. The interaction between species and season was not significant. In trial 2, the season effect was again significant (p = 0.04): root biomass below 30 cm was 3.0 and 1.3 times higher in late summer compared to spring for Fa and Lp respectively. The effects of grass species (p = 0.18) and the interaction between species and season were not significant (p = 0.12).

Table 7.2 Root biomass (g dry matter m^{-2}) of *Festuca arundinacea* (Fa) and *Lolium perenne* (Lp) measured at 6 depths in the 0-90 cm soil layer measured in sandy loam and loamy soils. (Trial codes indicated in table 7.1) (Significance codes: ***: $p < 0.001$, **: $p < 0.01$, *: $p < 0.05$, NS: $p > 0.05$).

Trial		0-15 cm	15-30 cm	30-45 cm	45-60 cm	60-75 cm	75-90 cm	0-90 cm
1 A	Fa	1082.3	167.0	47.0	26.9	17.6	10.1	1350.9
	Lp	847.5	183.3	32.2	14.6	11.0	6.2	1094.8
		NS	NS	NS	NS	NS	NS	NS
1 B	Fa	763.3	108.8	48.6	36.5	37.0	37.4	1031.6
	Lp	811.7	162.6	39.9	31.6	23.7	13.1	1082.6
		NS	NS	NS	NS	NS	*	NS
1 C	Fa	730.9	142.8	59.5	39.3	37.4	26.1	1036.1
	Lp	894.1	118.2	35.1	28.3	13.8	13.8	1103.3
		NS	NS	NS	NS	*	NS	NS
3	Fa	841.0	181.0	113.1	55.0	42.4	38.3	1270.8
	Lp	509.3	106.5	53.6	13.1	1.5	1.0	685.0
		NS	*	*	*	*	NS	*
4	Fa	742.3	71.7	53.1	58.8	45.8	28.5	1000.1
	Lp	692.0	62.4	24.4	12.5	5.2	2.1	798.7
		NS	NS	NS	*	**	***	NS

Table 7.3 Root biomass (g dry matter m^{-2}) of *Festuca arundinacea* (Fa) and *Lolium perenne* (Lp) measured at seven depths in the 0-70 cm soil layer on a sandy soil. (Trial codes indicated in table 7.1) (Significance codes: ***: $p < 0.001$, **: $p < 0.01$, *: $p < 0.05$, NS: $p > 0.05$).

Trial		0-10 cm	10-20 cm	20-30 cm	30-40 cm	40-50 cm	50-60 cm	60-70 cm	0-70 cm
2 A	Fa	669.1	83.6	42.6	44.7	15.8	11.8	10.0	877.6
	Lp	1039.2	91.1	76.4	63.4	14.6	8.9	5.1	1298.7
		NS	NS	NS	NS	NS	NS	NS	NS
2 B	Fa	821.2	195.6	120.5	101.8	42.2	55.1	45.6	1382.0
	Lp	1263.5	165.6	135.6	55.6	40.2	18.7	7.9	1687.1
		NS	NS	NS	NS	NS	NS	NS	NS

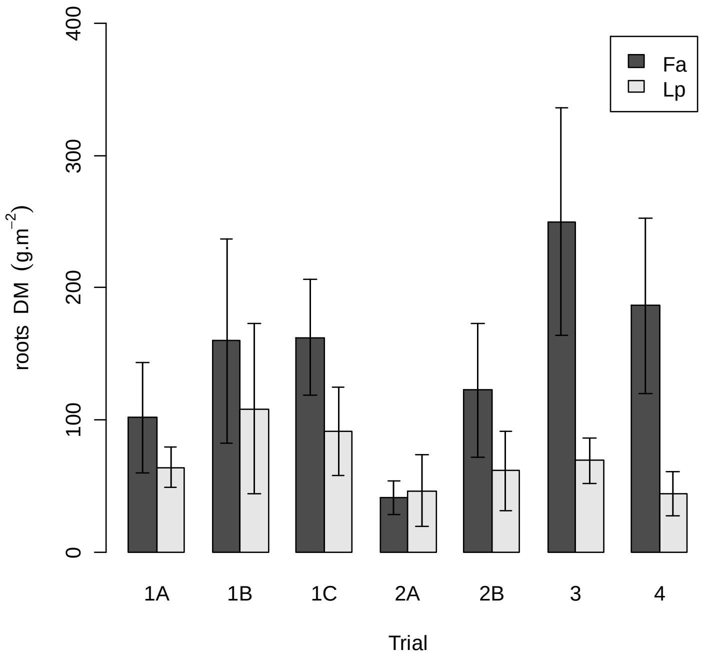


Figure 7.1 Root dry matter (DM) biomass of *Festuca arundinacea* (Fa) and *Lolium perenne* (Lp) measured below 30 cm soil depth in different trials (trial codes indicated in table 7.1)

7.4 Discussion

The result found for total root biomass, 1121 g m^{-2} averaged over all trials, was in line with the results found by other authors. Averaged over a period of five years on grassland with different grazing managements and fertilizations, Hejduk and Hrabe (2003) found an average grass root biomass of 976 g m^{-2} in a sandy loam soil in the Czech Republic in the 0-200 cm soil layer. Eekeren *et al.* (2010) found root biomasses of 1034 g m^{-2}, 217 g m^{-2} and 135 g m^{-2} for the 0-10cm, 10-20cm and 20-30cm layers respectively. These values are averages over five different grass species and grass species mixtures under a cutting regime on a sandy soil in the Netherlands.

Except for one location, no consistent differences in the root biomass over the whole soil profile between Fa and Lp were found: the roots present in the upper layer (0-30 cm) of the soil dominated the root biomass in the total profile and the root biomass seemed to be similar for both species. In addition, the presence of organic material in the upper layers complicated the washing of the roots resulting in high variations for root biomass in the upper layer.

Below 30 cm, root biomass of Fa was consistenly higher than that of Lp, particularly on the deep, fertile soils found in Merelbeke and in Poperinge. The difference in root biomass below 30 cm between the species was least pronounced on the sandy soil in Helvoirt. A part of this difference could be attributed to differences in sampling depth: 70 cm in Helvoirt compared to 90 cm in the other trials. The effect of this different sampling depth is expected to be small as few roots were growing in the pure sand found below 70 cm in Helvoirt.

At the end of the growing season root biomass was higher, and the difference between the species was larger compared to spring. Also from the practical point of view, sampling was easier in autumn compared to spring. The high soil water content in spring made it difficult to extract the deepest soil cores.

The results did not allow to draw conclusions on the effect of management (grazing or cutting) on the root biomass: the locations differed both in soil as in management. The largest differences between Fa and Lp however were observed on good soils under an intensive cutting management.

No distinction was made between living and dead roots. Although most of the roots remaining on the sieve were living roots, it is not excluded that presence of dead root material biased the results: the breakdown of the coarser Fa roots might be slower than that of the fine Lp roots, resulting in a higher proportion of dead roots measured in Fa compared to Lp.

7.5 Conclusions

Regarding our research hypotheses, we can conclude that:

1. When measured in autumn or late summer, root biomass of Fa below 30 cm was consistently higher compared to Lp. Fa root biomass was between 1.47 and 4.2 times higher than Lp root biomass. The root biomass below 30 cm represented 8 % and 17 % of the total root biomass for Fa and Lp respectively.
2. Root biomass was higher in late summer/autumn compared to spring. The highest difference in root biomass between Fa and Lp was found in autumn, on a sandy loam soil under intensive cutting management. The lowest difference was found on a sandy soil under intensive cutting management.

Chapter 8

Factors influencing preference of tall fescue genotypes for grazing sheep

Adapted from: Cougnon M., De Koker J., Fievez V. and Reheul D., 2013. Factors influencing animal preference of tall fescue genotypes. Accepted to be published in “Quantitative traits breeding for multifunctional grasslands and turf”, Proceedings of the 30th meeting of the EUCARPIA Fodder Crops and Amenity Grasses Section.

8.1 Introduction

The low voluntary intake and digestibility of tall fescue (*Festuca arundinacea* Schreb.; Fa) compared to ryegrasses (*Lolium* sp.) are limiting the use of Fa in grassland based production systems found in North-West Europe (**Chapter 2**). Particularly under grazing or when fed fresh, Fa voluntary intake is suboptimal. The intake of ensiled or hayed Fa is better than that of fresh Fa (Luten and Remmelink, 1984; Opitz von Boberfeld *et al.*, 2004), probably because ensiling decreases the leaf harshness of Fa (Peratoner *et al.*, 2011). As soft leaved genotypes proved to have a higher animal preference in the past (Gillet and Jadas-Hecart, 1965; Jadas-Hecart, 1982; Rognli *et al.*, 2010), the softness of the leaf blades, determined by touching the plants, is used in breeding to select genotypes with a higher preference. Scoring for leaf softness is highly subjective and demands a lot of experience. Moreover leaf softness is a very complex trait influenced by e.g. leaf dimensions and plant architecture. Repeatable, stable and quantifiable methods producing results matching with animal preference may advance the breeding of varieties with improved animal preference.

Total grass intake by ruminants is influenced by pre- and post-ingestion factors. Pre-ingestion factors influence the short term intake of grass. Many authors called this short term intake “palatability” (Tava *et al.*, 1995; Aderibigbe *et al.*, 1982; Kendal and Sherwood, 1975; Buckner and Burrus, 1962; Buckner and Fergus, 1960; Petersen *et al.*, 1958). Palatability however is subject to various interpretations, can be confounded with preference and is therefore not a recommended term (Allen *et al.*, 2011). Examples of factors affecting short term intake are leaf blade length and sward height (Barre *et al.*, 2006). Once the rumen of ruminants is filled, the retention time of the grass limits the further intake of grass. Digestibility and shear strength of grasses are examples of factors influencing post-ingestion intake (Inoué *et al.*, 1994a). The term “preference” is a measure of relative intake of different forages, where access to forage is unrestricted (Allen *et al.*, 2011). Preference integrates the effects of both pre-and post-ingestive factors on the intake; it describes an animal’s response but makes no assumptions about mechanisms determining the response (Hodgson, 1979).

The early work of Buckner and Burrus (1962) and Buckner and Fergus (1960) showed that it was possible to improve intake and quality of Fa through breeding. The progress in the intake and quality of Fa varieties developed in France with different breeding strategies was quantified by Emile *et al.* (1992, 1997): they compared the varieties ‘Lubrette’, a variety selected for softer leaves and improved intake and ‘Clarine’, a reference variety. In a three years grazing trial comparing both varieties, cows grazing ‘Lubrette’ produced on average 11

% more milk than cows grazing ‘Clarine’ (Emile *et al.*, 1992). Averaged over different trials, the DOM was 5 % points higher in ‘Lubrette’ compared to ‘Clarine’. The most straightforward method to improve intake of Fa is to use grazing ruminants in the breeding process (Petersen *et al.*, 1958; Jadas-Hecart, 1982). Preference of the grazed varieties (or genotypes) is determined by subjective scoring, by observing grazing time of the animals or by dry matter yield determination before and after grazing (so called difference-method). This last method is from a theoretical point of view the method that gives the best idea about preference, but it is often subject to very high coefficients of variation (Petersen *et al.*, 1958; Shewmaker *et al.*, 1997). Jadas-Hecart (1982) found correlation coefficients (r) between 65 % and 92 % for correlation between preference scoring on a 0-10 scale and the difference method in a trial comparing the preference of 15 Fa varieties grazed by sheep on 6 occasions in two successive years.

The use of a so called “through cafeteria” (Gillet *et al.*, 1983), in which cut grass of different varieties (or genotypes) is presented to sheep, allows to quantify the preference of different varieties very precisely. A practical advantage of the use of a cafeteria trial compared to a grazing trial is that the DMY of the compared genotypes/varieties can be determined first. A drawback of the use of the cafeteria trials compared to grazing trials is that the effect of the canopy/sward structure on the preference of the different genotypes is eliminated.

For practical reasons most preference trials have been performed with sheep; there is however limited evidence that cattle and sheep have the same preference. In a study comparing the preference by sheep, goats and steers among hay of eight tall fescue varieties (Burns *et al.*, 2001) preference between the different animals was similar.

Animal trials are not easy to manage and have a low repeatability as the preference of the animals is difficult to quantify exactly. For these reasons, both breeders and researchers have been looking for (quantifiable) methods that correlate well with grazing preference by animals.

Leaf softness, as determined by touching plants or swards, was found to correlate well with preference: in a two years trial Jadas-Hecart (1982) found correlation coefficients close to 50 %. In soft leaved varieties of tall fescue, silicium teeth prevalent on the borders of the leaves were reduced to bulb like structures (Rognli *et al.*, 2010). The presence of silicified dentations on leaf margins in cocksfoot (*Dactilys glomerata*) was found to be a dominant character (Van Dijk, 1964), and it is likely that also in tall fescue this is the case. Silicified teeth are only one aspect of leaf softness in tall fescue: the highly appreciated and soft leaved Italian ryegrass

(*Lolium multiflorum*) also has silicified teeth on its leaf margins (own observations). Breeders observed that in elite material, the correlation between leaf softness and animal preference is decreasing (Rognli *et al.*, 2010). Suter *et al.* (2009) found that in tall fescue variety trials, the varieties with the highest digestibility were not necessarily those with the softest leaves.

Grass leaf mechanical properties, tear and shear strength, have been related to animal production since a long time (Evans, 1967; Kneebone, 1960). The hypothesis is that mechanical properties reflect the ease with which the forage can be ingested by the animal, and can be reduced to a particle size that facilitates passage through the rumen (Mackinnon *et al.*, 1988; Henry *et al.*, 1997). Sclerenchyma, the main load bearing component in grass leaf blades (Vincent, 1982), is mainly made out of cellulose, hence tensile strength of the leaves is a good measure for fibre content and influences digestibility. Mackinnon *et al.* (1988) selected perennial ryegrass for low and high shear strength. The low shear strength population had a leaf shear force that was *circa* 41 % lower than that of the population with the high shear strength. Inoué *et al.* (1994a) estimated that the masticatory load to break down the leaves to particles smaller than 1 mm was 27 % lower in the population with the low shear strength, but no significant differences were found in the rate of particle breakdown, voluntary intake or animal performance in a trial where sheep were fed with grass of the two populations (Inoué *et al.*, 1994b).

Methods used to measure the mechanical properties of grass differ a lot between studies and often have been copied or were adopted from food or fibre industry. Easton *et al.* (1989) and Inoué *et al.* (1994a) measured shear force with a Warner-Bratzler shear machine developed for assessing tenderness of meat. Bryant *et al.* (2008) developed a punch and die apparatus that allowed to measure the shear force of a great number of leaves simultaneously. Henry *et al.* (1997) point that shear strength measurements (force applied perpendicularly on leaf length direction) are superior in rapidity and repeatability of measurements compared to tear strength (force applied in leaf length direction) measurements in which the clamping of the leaves often leads to breaking at the clamp.

Macadam and Mayland (2003) studied the relationship of leaf strength to cattle preference in eight Fa varieties. Shear strength was found to be negatively correlated with preference. A weakness of this study was that leaf mechanical properties and animal preference were not tested simultaneously: animal preferences used by Macadam and Mayland *et al.* (2003) were derived from Shewmaker *et al.* (1997). Moreover, part of the measurements was performed on single clones grown in a growth chamber.

Morphological characteristics of grass leaves and the sward canopy structure influence the ease with which grass can be grazed. Daily herbage intake of the grazing ruminants closely reflects the weight of the material harvested per bite. In studies with sheep in grazing cages on grass swards with different heights and densities, Burlinson *et al.* (1991) found that mean bite weight was positively correlated to surface height. Also in grazed perennial ryegrass, leaf blade length explained most of the variation present in short term intake rates between different varieties: the longest varieties had the highest intake rate (Barre *et al.*, 2006).

Chemical composition has an important influence on intake. Generally, it is accepted that fiber content and digestibility are negatively correlated with preference and intake (Barre *et al.*, 2006; Macadam and Mayland, 2003; Tas *et al.*, 2005; Wilman *et al.*, 1996). Evidence for this relation however is scarce for tall fescue. Moreover, in elite material of perennial ryegrass the correlation between digestibility, intake and animal performance was found to be low (Tas *et al.*, 2005; Orr *et al.*, 2003). Tas *et al.* (2005) found that the progress in dry matter digestibility (DMD) and, water soluble carbohydrate (WSC) concentration and neutral detergent fibre (NDF) content in Lp varieties did not lead to a significantly higher DM intake nor milk production in dairy cows fed with cut grass. In a study of Orr *et al.* (2003) sheep intake of different perennial ryegrass varieties was not correlated with green leaf mass, tiller density, or WSC concentration. A significant correlation between intake and digestibility was found in only one of the two experimental years.

Mayland *et al.* (2000) found a significant positive relation between non-structural carbohydrate content and cattle grazing preference in eight tall fescue varieties. In an Italian study relating differences of primary metabolites in Fa varieties to animal preference, water soluble carbohydrate content was highly related to preference (Tava *et al.*, 1995). A weakness of this study was that the animal preferences of the different varieties were not tested in this study, but were estimated from leaf flexibility.

Also secondary metabolites, were related to preference and animal intake of forages. Kendall and Sherwood (1975) found a negative correlation between preference of meadow voles (*Microtus pennsylvanicus*) and alkaloid levels in reed canarygrass. Relationship between volatile components content and animal intake was suggested in perennial ryegrass (Aderibigbe *et al.*, 1982) and tall fescue (Tava *et al.*, 1995), but clear evidence is lacking. Scehovic (1986) on the other hand proved in a cafeteria trial with sheep that volatile components of Fa had a repellent action. Tall fescue juice was sprayed on cut Italian ryegrass, and the other way around. The sheep preference of the Italian ryegrass, sprayed with the Fa juice, was clearly decreased compared to an Italian ryegrass control, whereas the effect of the Italian ryegrass juice on the Fa preference was neutral.

None of the above mentioned factors alone could predict the preference of grazing animals. Whereas most of the studies focused on the relationship between Fa preference and one factor, the aim of our trial was to compare preference with different quantifiable factors simultaneously. Only factors that could be easily assessed in a breeding programme were included.

Our research hypotheses were that:

1. Sheep can be used to distinguish between clones of Fa in a consistent way.
2. Grazing preference is positively correlated with leaf blade length, pre-grazing sward height and DOM and negatively correlated with leaf harshness, leaf shear strength, leaf shear force and dry matter content.
3. Rabbits and sheep have a similar preference.

8.2 Material and Methods

8.2.1 Trial establishment

A grazing trial was established in March 2011 on a sandy loam soil at the experimental farm of Ghent University in Melle, Belgium.

The plant material for this trial was selected in a clonal nursery of the Fa breeding programme (**Chapter 10**). The nursery contained 423 clones originating both from ecotypes and varieties. This clonal nursery was planted in the autumn of 2009: per clone ten plants were planted with a space of 0.5 m between and within the rows. Throughout the year 2010 the clones were scored for rust resistance, (re)heading, leaf harshness and vigour. In the autumn of 2010, sixteen clones with good rust resistance were selected for the establishment of the grazing trial (**Table 8.1**). Attention was paid to select clones with contrasting morphogenetic traits: broad *versus* fine leaves, dark green *versus* light green leaves, soft *versus* hard leaves. The plants were dug out in the spring of 2011 and divided into at least 240 ramets of 3-5 tillers per selected clone.

The plots were planted with the ramets of a single clone spaced 0.20 m between and within the rows, resulting in plots of 1.2 x 2.0 m with genetically identical plants. The trial was planted as a randomized complete block design with four replicates. Between blocks, and between blocks and fences, 2 m wide corridors were sown with an amenity type of tall fescue (4 g m^{-2}). In the establishment year 2011, plots were mown *circa* every month during the growing season with a lawn mower at a height of 5 cm. Fertilization in 2011 was 50 kg N ha^{-1}, 42.5 kg K ha^{-1} and 21.5 kg P ha^{-1}. The plots were kept free of weeds using herbicides (clopyralid + MCPA + fluroxypyr). No measurements were performed in the year 2011. Adjacent to the trial, a tall fescue pasture was established. Half of this area was sown with the variety Callina, the other half with the variety Castagne. (**Photograph 8.1**)

Photograph 8.1 View of the trial plots. (April 2012)

In addition, 12 ramets of each of the 16 clones were planted in a spaced plants trial with four blocks. In each block, three ramets of each of the 16 clones were planted randomly with 0.5 m between and within the rows. To prevent weeds in the blocks, plants were planted upon root cloth. Between the blocks, and between the blocks and the fence, 2 m wide corridors were sown with an amenity type of tall fescue (4 g m^{-2}). The spaced plants trial was fenced to allow grazing by rabbits (**Photograph 8.2**).

Table 8.1 Origin and characteristics of tall fescue clones selected for grazing trial. Meaning of the scores: Rust susceptibility: 0 no rust-5 very susceptible; Re-heading: 0: no reheading, h little reheading, H full reheading. Leaf harshness: 1:very soft and flexible leaves, 5 very hard and harsh leave blades

Clone	Type	Variety/Origin	Rust susceptbility	Re-heading	Leaf harshness	Remarks
1	Ecotype	Ihringen, Germany	0	H	4.5	Very broad leaves
2	Variety	Barolex	0	0	2.2	Fine leaves, light green colour
3	Variety	Barolex	0	0	2.0	Fine leaves
4	Variety	Lekora	0	H	2.75	
5	Variety	Barolex	1	H	2.0	
6	Variety	Elodie	0	0	1.75	Light green colour, shiny
7	Variety	Barolex	0	0	1.5	Very fine, short leaves
8	Variety	Barolex	0	0	1.5	Fine leaves
9	Variety	Barolex	0	0	2.25	Erect growth habitus
10	Variety	Barolex	0	0	1.25	
11	Variety	Barolex	0	0	1.5	Very fine leaves
12	Ecotype	Hamme, Belgium	1	0	3.0	Erect growth habitus, broad leaves
13	Ecotype	Melle, Belgium	0	0	2.5	
14	Ecotype	Melle, Belgium	0	0	2.3	
15	Ecotype	Drongen, Belgium	0	0	4	Erect growth habitus, harsh leaves
16	Ecotype	Oppem, Belgium	0	H	4	Short, broad leaves; dark green colour

Photograph 8.2 View of the spaced plants trial grazed by rabbits (July 2012)

8.2.2 Measurements

Between April and September 2012 morphological variables, quality variables and grazing preference by sheep were measured in 4 experimental periods (23 April, 9 July, 13 August, 28 September). On each occasion pre-grazing sward height, leaf blade length, leaf blade width, leaf blade shear force, leaf blade shear strength and leaf harshness were measured. These measurements took place the day before sheep were allowed to graze the trial.

Pre-grazing sward height was measured with a falling plate meter on four randomly selected spots per plot (Bransby *et al.*, 1977). The plate measured 20 cm x 20 cm and was made of 1 mm thick aluminium. In the middle of the plate, there was a hole with a diameter of 1 cm. To measure sward height, the plate was laid gently on the sward, and a plastic ruler through the hole indicated the distance between the plate and the ground.

Ten well developed tillers per clone were harvested randomly from the four plots the day before grazing started. Leaf blade length, leaf blade width and leaf blade shear strength were measured on the youngest adult leaf of each tiller. The leaf blades were clipped with scissors at the collar and the length from the ligule to the tip was measured using a ruler. Leaf blade width was measured with vernier callipers at 1/3 of the distance between the ligule and the tip. Macadam and Mayland (2003) found that leaf width was constant in this part of the leaf blade. Shear force, the maximum load needed to cause breakage at a 90° breaking angle to the length of the leaf, was measured using a texture analyser (Lloyd Instruments Ltd, Leicester, UK) equipped with a square cutting blade with a thickness of 1.02 mm. The leaf was mounted on the slotted testing table with paper clips so that the leaf was sheared at 1/3 of the distance between ligule and tip. The blade was moving through the leaf at a rate of 50 mm min^{-1}. Leaf shear strength [N mm^{-1}] was calculated by dividing leaf shear force [N] by leaf width [mm].

Leaf harshness was scored by two plant breeders on an ordinal scale from 1 (very soft and flexible leaf blades) to 5 (very hard and rough leave blades). Leaf colour was scored only in the first and the last growing period on an ordinal scale from 1 (very light green) to 5 (very dark green).

One sample of 500 g of fresh material per clone was collected by clipping *circa* 125 g of grass from each plot (clipping height 5 cm) and by pooling the material over the replicates. This pooling was necessary because the plots were too small to harvest the amount of grass needed for a reliable determination of the dry matter content (DMC) and the digestibility of the organic matter on every plot without jeopardizing the further observations. The samples were dried for 16 h at 75°C. The dried samples were milled to pass through a 1 mm sieve and the apparently rumen degraded organic matter (RDOM) (Demeyer, 1991) was determined from the samples of experimental periods 1, 2 and 4.

Before sheep were allowed to graze the plots, the area between and around the blocks was mown with a lawn mower at 5 cm height. The trial was stocked with sheep that had been grazing for at least two weeks on the tall fescue pastures adjacent to the trial. Stocking density was regulated to allow complete grazing of the trial in *circa* one week. The number of sheep varied between 4 ewes with two lambs each in the first experimental period to four ewes and one ram on the last grazing occasion. The sheep were either from the Flemish sheep breed either ‘Bleu du Maine’ crossbred sheep. In accordance with Jadas-Hecart (1982) and Shewmaker *et al.* (1997), grazing preference was scored every morning on an ordinal scale from 0 (no grazing at all), 1 (between 0 and 10 % of standing biomass eaten) to 9 (between 80 and 90 % of standing biomass eaten). As soon as one clone had reached a score of 9, measurements were stopped and the post-grazing sward height was measured, in the same way as the pre-grazing sward height. The values of the first scorings were called “preference begin” (after *circa* 24 h grazing) the final scorings were called “preference end” (at least one plot with score 9). At the end of a growing period sheep were removed from the trial and the whole trial area was mown with a lawn mower at 5 cm height to remove the non-grazed herbage. Prior to the first grazing, a fertilization of 50 kg ha^{-1} N, 82 kg ha^{-1} K and 35 kg ha^{-1} P was applied. After each grazing, 50 kg ha^{-1} N and 25 kg ha^{-1} K were applied.

Parallel with the sheep grazing, four female rabbits of a local breed were allowed to graze the spaced plants trial. During each experimental period all individual plants were scored twice on an ordinal scale from 0 (no grazing at all), 1 (between 0 and 10 % of standing biomass eaten) to 9 (between 80 and 90 % of standing biomass eaten). The observation that discriminated most between the clones, was withheld.

8.2.3 Data analysis

The effect of the different clones and different experimental periods on leaf blade length, width, shear force, shear strength and pre-grazing sward height were tested using two-way analysis of variance (anova) using the *aov()* function in R (R development core team, 2011). In case of significant *period:clone* interaction, data were split per experimental period and analysed with one-way anova.

Leaf harshness, sheep and rabbit preference and leaf colour were scored on an ordinal scale and were not normally distributed, neither homoscedastic. Hence, the effect of the different clones on these variables was tested using nonparametric one-way anova (Kruskall-Wallis analysis). The Kruskall-Wallis test and the multiple comparison of treatment means was performed in SPSS version 19 (IBM Corp., New York, USA).

The absence of replicates in the DMC and RDOM data, implicated that no anova for these variables could be performed.

Regression between different variables was performed with the *lm()* function. To cope with the different units of the different variables, regression between the preference of the sheep and other variables was done after standardisation of the variables using the *scale()* function.

All variables that were measured in all plots in every period were averaged and brought together in a multivariate dataset, resulting in one observation per variable for each clone in each experimental period. The relationship between all variables was analysed by principal component analysis, using the *princomp()* function. A biplot in the first two principal components was drawn using the *biplot()* function.

A multiple linear regression model was fitted to this dataset to find out which of the measured morphological variables predicted the sheep preference best. The minimum adequate model was obtained by minimizing the Akaike’s information criterion (AIC) using the *step()* function.

8.3 Results

In the summer of 2011, parts of the swards of clones 10 and 14 were infected by bacterial wilt (*Xanthomanas translucens* pv. *graminis*) and died partially. No observations were performed on the plots of these clones. No extreme weather events that could have hampered the grass growth or influenced the grazing behaviour of the sheep occurred in the growing season of 2012 (**Appendix 1**)

8.3.1 Animal preference

Sheep preferences for the clones in the clones trial at the beginning (preference_begin) and the end (preference_end) of each experimental period are given in **table 8.2**. In each period, the effect of the clones on the preference scores was significant. Significant differences between the clones preference scores were found at the beginning of the experimental periods 2, 3 and 4 and at the end of the experimental periods 3 and 4. In all periods the clones 3,7 and 16 were the among the most preferred and clone 1, 2 and 15 among the least preferred.

Table 8.2 Preference scores for fourteen tall fescue clones grazed by sheep in four experimental periods in 2012 either at the begin or either at the end of the experimental period. 0 (no grazing at all), 1 (between 0 and 10 % of standing biomass eaten) to 9 (between 80 and 90 % of standing biomass eaten).

	Preference_begin				Preference_end			
	Experimental period				Experimental period			
	1	2	3	4	1	2	3	4
Clone 1	0.3^{ab}	0.3^{c}	0.3^{b}	1.3^{b}	7.3^{ab}	7.3	2.3^{c}	4.3^{c}
2	0^{b}	0.8b^{c}	0^{b}	1.3^{b}	4.3^{b}	7.8	3^{bc}	4.8^{c}
3	0.5^{ab}	7.8^{a}	7.0^{a}	6.3^{ab}	7^{ab}	9	8.4^{a}	8.1^{ab}
4	0.8^{ab}	3^{abc}	2.5^{ab}	5.3^{ab}	6^{ab}	8.6	7.3^{ab}	7.6^{abc}
5	0.8^{ab}	2.8^{abc}	2.0^{ab}	3.8^{ab}	5.5^{ab}	8.9	5.8^{abc}	6.5^{abc}
6	3.8^{a}	4.5^{abc}	4.0^{ab}	4.5^{ab}	7.3^{ab}	8.5	6.3^{abc}	7.3^{abc}
7	3.8^{a}	4.5^{abc}	5.0^{ab}	6.8^{a}	8^{a}	8.8	7.5^{ab}	8.4^{a}
8	1.8^{ab}	1.5^{abc}	1.8^{ab}	2.3^{ab}	5.5^{ab}	8.1	4.8^{abc}	5.8^{abc}
9	3.5^{ab}	5^{abc}	4.0^{ab}	4.8^{ab}	7.5^{ab}	8.9	6^{abc}	7.5^{abc}
11	1^{ab}	2.3^{abc}	2.8^{ab}	4.8^{ab}	6.5^{ab}	9	5.3^{abc}	7.6^{abc}
12	1^{ab}	1.8^{abc}	1.3^{ab}	3.5^{ab}	7^{ab}	7.4	4.3^{abc}	6.0^{abc}
13	0.5^{ab}	2^{abc}	0.3^{b}	2.0^{ab}	6.8^{ab}	8.1	4^{abc}	6.5^{abc}
15	0.3^{ab}	0.8^{bc}	0.8^{ab}	1.8^{ab}	6.5^{ab}	7.5	4^{abc}	6.0^{abc}
16	1^{ab}	6.5^{ab}	4.0^{ab}	6.3^{a}	6.8^{ab}	9	7.8^{a}	8.4^{a}
Significance^{A}	***	***	***	***	**	*	***	***

A : Significance codes: ***: $p < 0.001$, **: $p < 0.01$, *: $p < 0.05$, NS: $p > 0.05$. Values in the same column with a different letter are significantly different ($p = 0.05$)

A significant correlation was found between the standardized preference scores at the beginning of each period and at the end of each period (Y = 0.62X; $R^2 = 0.37$, $p < 0.001$) (**Figure 8.1**). At the end of the experimental periods differences in preference between the clones decreased. Obviously, this was a result of the decreasing availability of forage of the preferred clones. Therefore, preference at the beginning of the experimental period was used as reference parameter to compare sheep preference in the remainder of this chapter.

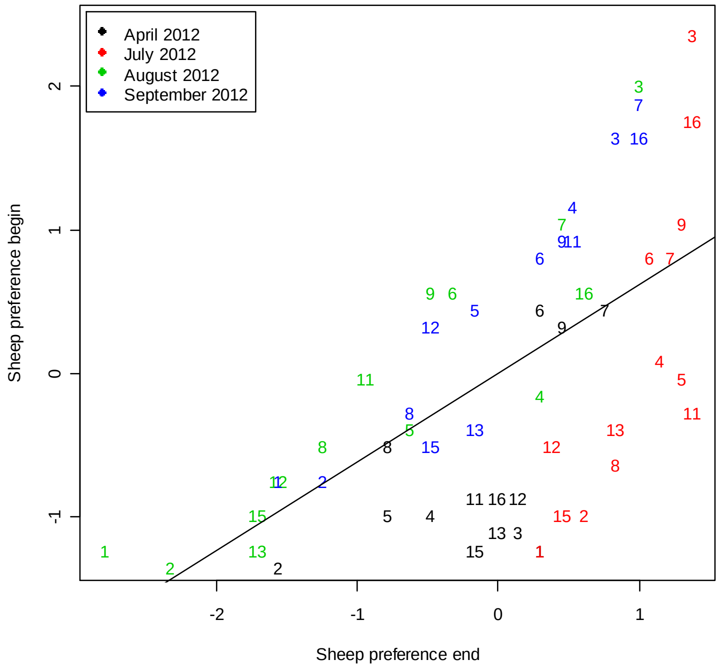


Figure 8.1 Relationship between standardized preference scores of sheep grazing fourteen tall fescue (1-9, 11-13, 15, 16) clones in four experimental periods in 2012 measured either at the beginning of an experimental period or either at the end of an experimental period (Y = 0.62X; $p < 0.001$, $R^2 = 0.37$)

Significant differences in rabbit preferences were found in the experimental periods 2,3 and 4 (**Table 8.3**). The clones 3,7 and 16 were among the most preferred, clone 1,2 and 8 were the least preferred.

Table 8.3 Preference scores for fourteen tall fescue clones grazed by rabbits in four experimental periods in 2012. 0 (no grazing at all), 1 (between 0 and 10 % of standing biomass eaten) to 9 (between 80 and 90 % of standing biomass eaten).

	Period			
	1	2	3	4
Clone 1	2.9	2.3^{c}	2.7^{b}	2.4^{c}
2	1.4	1.8^{c}	0.8^{b}	1.8^{c}
3	2.5	3.3^{bc}	5.2^{a}	6.7^{ab}
4	1.9	3.8^{abc}	4.8^{a}	5. 7^{ab}
5	1.9	3.5^{abc}	3.2^{b}	3.2^{bc}
6	2.5	3.3^{abc}	4.0^{a}	3.8^{abc}
7	2.7	5.2^{ab}	5.2^{a}	5.9^{ab}
8	2.1	3.6^{abc}	2.1^{b}	2.6^{c}
9	1.9	3.5^{abc}	3.8^{ab}	4.1^{abc}
11	2.2	3.0^{bc}	3.2^{b}	3.4^{bc}
12	3	2.9^{bc}	4.3^{a}	4.3^{abc}
13	2.3	3.3^{abc}	4.0^{ab}	4.3^{abc}
15	2.5	3.5^{abc}	3.3^{ab}	4.3^{abc}
16	2.9	8.2^{a}	7.1^{a}	7.9^{a}
Significance^{A}	NS	**	***	***

A : Significance codes: ***: $p < 0.001$, **: $p < 0.01$, *: $p < 0.05$, NS: $p > 0.05$. Values in the same column with a different letter are significantly different ($p = 0.05$).

8.3.2 Leaf blade morphology

Significant *period:clone* interactions were found for leaf blade length, width, shear force and shear strength (**Table 8.4**). When separated per period, the effect of *clone* was highly significant in every growing period for every measured parameter (**Table 8.4**). Clone 1 and 15 had the longest and widest leaves in the four periods and clone 11 the shortest and smallest leaves in the four periods. Clone 1 had the highest leaf shear force in the four periods. The lowest leaf shear forces were found in clone 8 in period 1, clone 11 in periods 2 and 3 and clone 7 in period 4. The highest leaf shear strengths were found for clone 4 in period 1 and clone 1 in periods 2, 3, 4. The lowest shear strengths were found in clone 8 in period 1 and for clone 11 in periods 2, 3 and 4. Notwithstanding the significant period:clone interactions clone 1 and 15 had the longest and the widest leaf blades with the highest leaf shear forces over all periods (**Figure 8.2**). The highest leaf blade shear strengths were found for the clones 1 and

12. Clones 7 and 11 had the shortest and smallest leaves with the lowest shear forces and shear strengths (**Figure 8.2**).

Table 8.4 *p*-values for ANOVA for the effect of clone and experimental period on leaf blade morphological variables of fourteen tall fescue clones in four experimental periods in 2012. Significance codes: ***: $p < 0.001$, **: $p < 0.01$, *: $p < 0.05$, NS: $p > 0.05$.

	Length	Width	Shear force	Shear strength
Main effects + interaction				
Clone	***	***	***	***
Period	***	***	***	***
Clone x Period	***	***	***	***
Main effect of Clone per period				
Clone Period 1	***	***	***	***
Clone Period 2	***	***	***	***
Clone Period 3	***	***	***	***
Clone Period 4	***	***	***	***

Significant correlation was found between shear force and leaf width (Y = 0.87X; $p < 0.001$, $R^2 = 0.76$) (**Figure 8.3a**) and between leaf shear force and leaf shear strength (Y = 0.90X; $p < 0.01$, $R^2 = 0.81$) (**Figure 8.3b**).

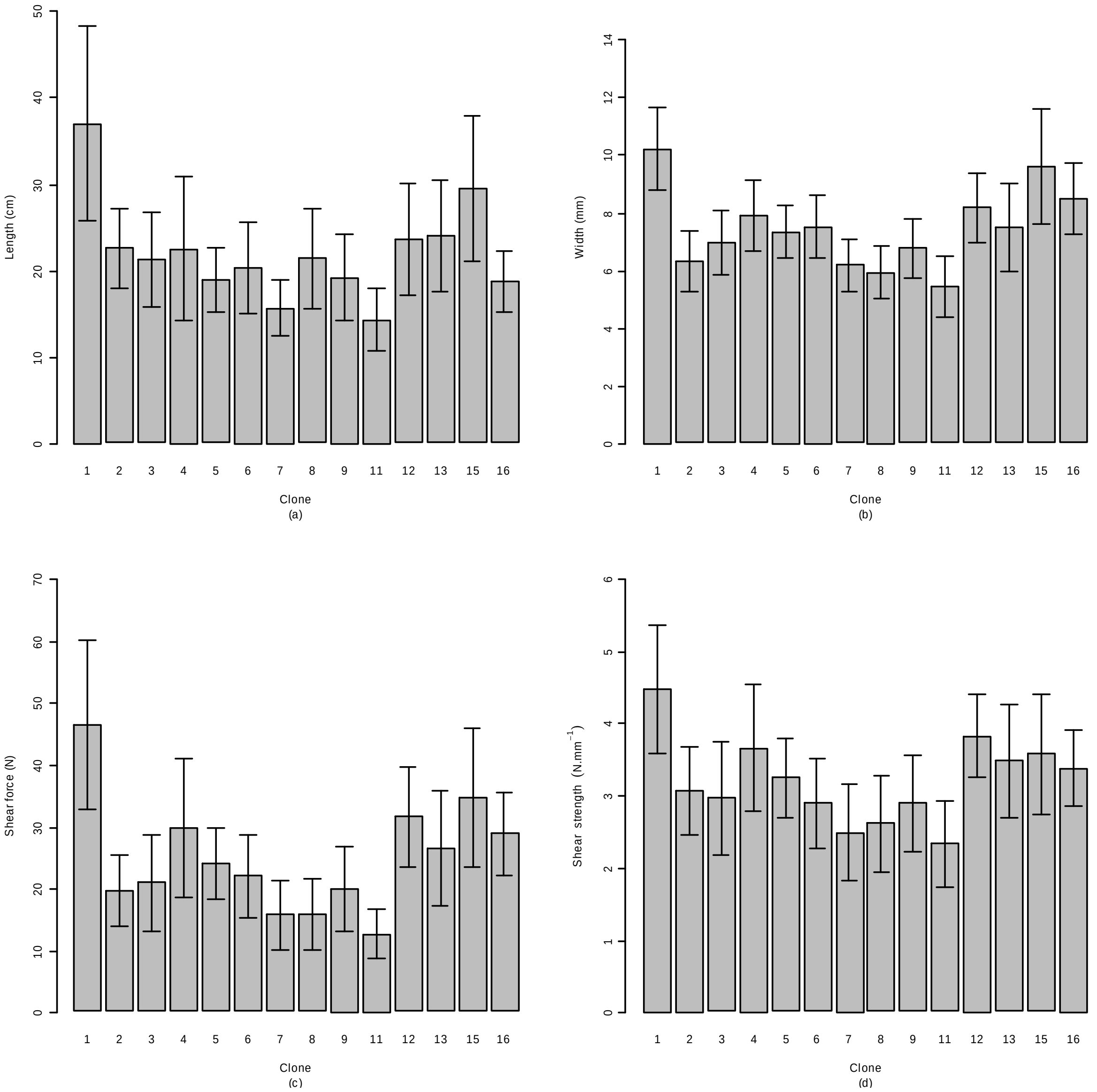


Figure 8.2 Leaf blade length (a), width (b), shear force (c) and shear strength (shear force/width) (d) for fourteen tall fescue clones in a grazing trial with sheep. Data are averages over four experimental periods in 2012. Error bars: ± standard deviation.

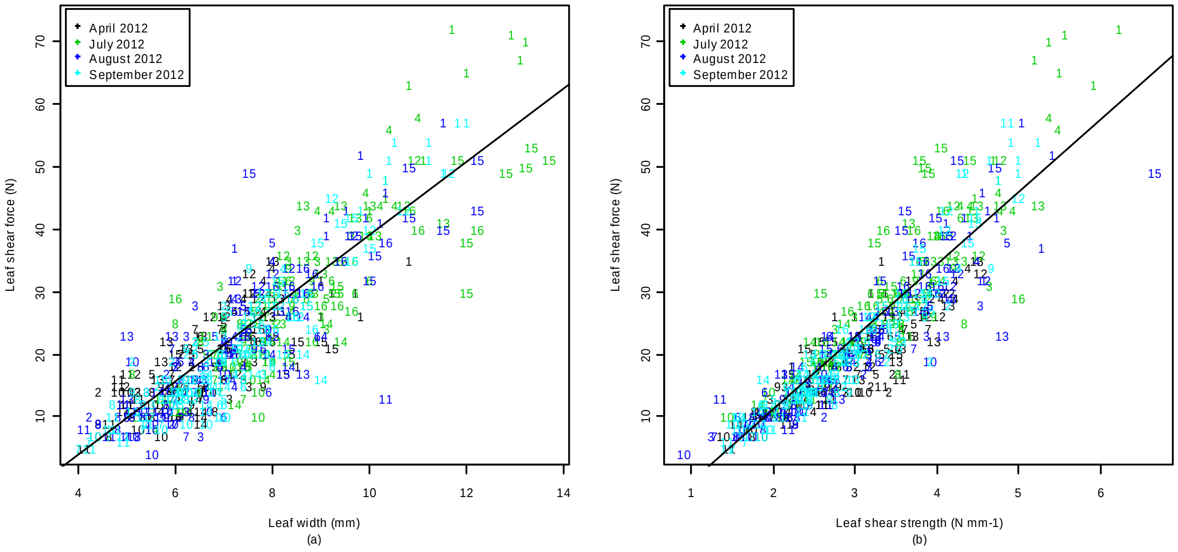


Figure 8.3 (a) Regression of normalized leaf shear force on normalized leaf blade width (Y = 0.87X; $p < 0.01$, $R^2 = 0.76$) (b) regression of normalized leaf shear force on normalized leaf shear strength (Y = 0.90X; $p < 0.001$, $R^2 = 0.81$) for fourteen tall fescue clones (1-9, 11-13, 15, 16) in four experimental periods in 2012

8.3.3 Sward characteristics

In every period, highly significant differences ($p < 0.001$) were found in the leaf harshness of the clones. Averaged over all periods, clones 1 and 15 had the hardest leaves, clone 3, 8 and 11 the softest leaves (**Figure 8.4a**).

A significant period:clone interaction was found in the pre-grazing heights ($p < 0.001$). When separated per period, the effect of clone on pre-grazing sward height was highly significant ($p < 0.001$) in every growing period. The highest pre-grazing sward heights were for clone 15 in period 1 and period 2; clone 1 in period 3 and clones 2 and 13 in period 4 with heights of 22.3 cm, 14.3 cm, 14.4 cm and 14.3 cm respectively. The lowest pre-grazing sward heights were for clone 7 in period 1; clone 5 in period 2; clone 11 in period 3 and clone 7 period 4 with heights of 11.9 cm, 9.1 cm, 10.8 cm and 9.5 cm respectively. Averaged over all periods the clones 1, 2 and 15 had the highest and clones 5, 7, 16 the lowest pre-grazing sward heights (**Figure 8.4b**).

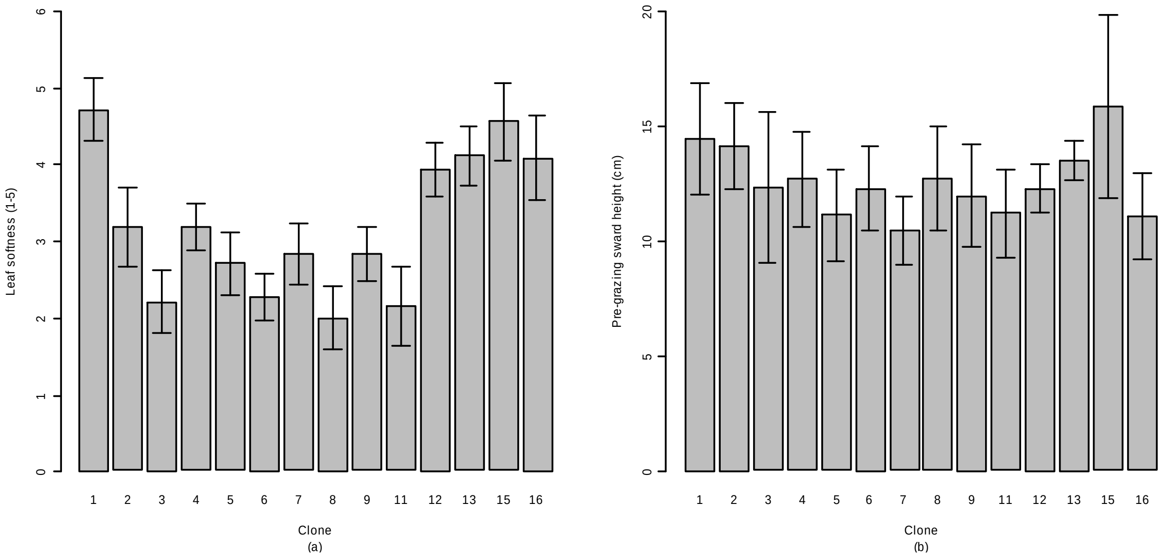


Figure 8.4 Characteristics for fourteen tall fescue clones in four experimental periods in 2012: (a) ordinal scores for leaf harshness (1:very soft – 5:very hard) (b) Pre-grazing sward height. Error bars: ± standard deviation.

8.3.4 Quality variables

Significant differences in sward colour were found in the first experimental period ($p < 0.001$) and in the fourth growing period ($p < 0.001$). Averaged over both periods, clones 2 and 6 were the lightest green and clone 13 and 16 the darkest green (**Figure 8.5a**).

No clear differences were found in the DMC of the clones, except in clone 6 that had a DMC that was consistently below that of the other clones (**Figure 8.5b**). Averaged over the three growing periods in which RDOM was measured, clones 4 (533 mg (g DM)$^{-1}$), 6 (502 mg (g DM)$^{-1}$) and 3 (482 mg (g DM)$^{-1}$) had the highest RDOM and clones 1 (455 mg (g DM)$^{-1}$), 13 (454 mg (g DM)$^{-1}$) and 16 (445 mg (g DM)$^{-1}$) the lowest RDOM (**Figure 8.5c**).

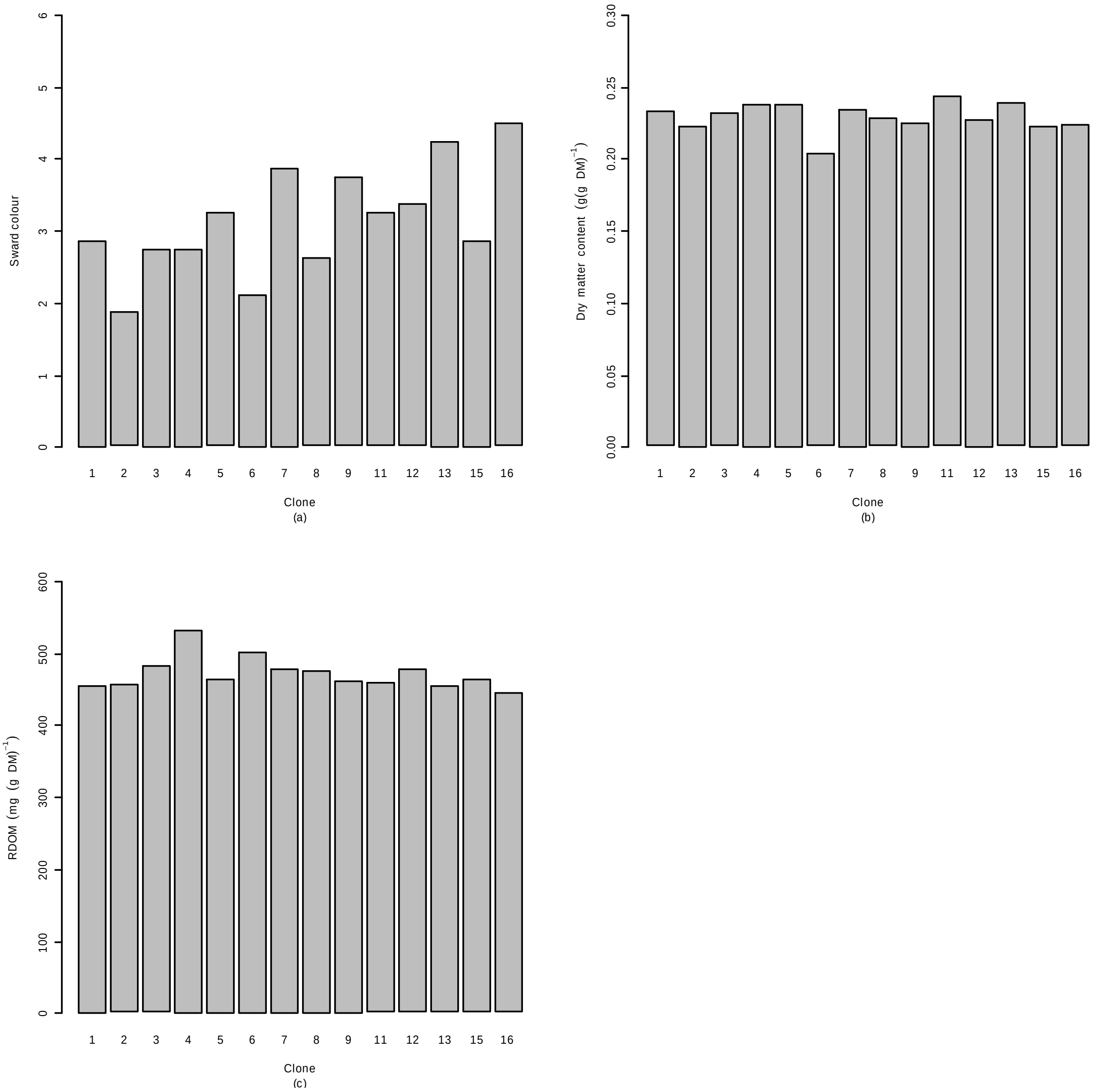


Figure 8.5 Sward characteristics for fourteen tall fescue clones in four experimental periods in 2012: (a) ordinal scores for sward colour(1:very light green – 5:very dark green) averaged over the first and the fourth experimental periods, (b) dry matter content of the harvested grass averaged over the first, second and third experimental periods, (c) apparently rumen degraded organic matter content of the harvested grass averaged over the first second and fourth experimental periods. Error bars: ± standard deviation.

8.3.5 Factors affecting preference

Regression was performed to elucidate the effect of individual variables on preference. Leaf harshness influenced sheep preference negatively (Y = -0.36X; p = 0.007, R^2 = 0.11). The preference was higher for clones 3 and 16 and lower for clones 2 and 8 than expected from their leaf harshness (**Figure 8.6**).

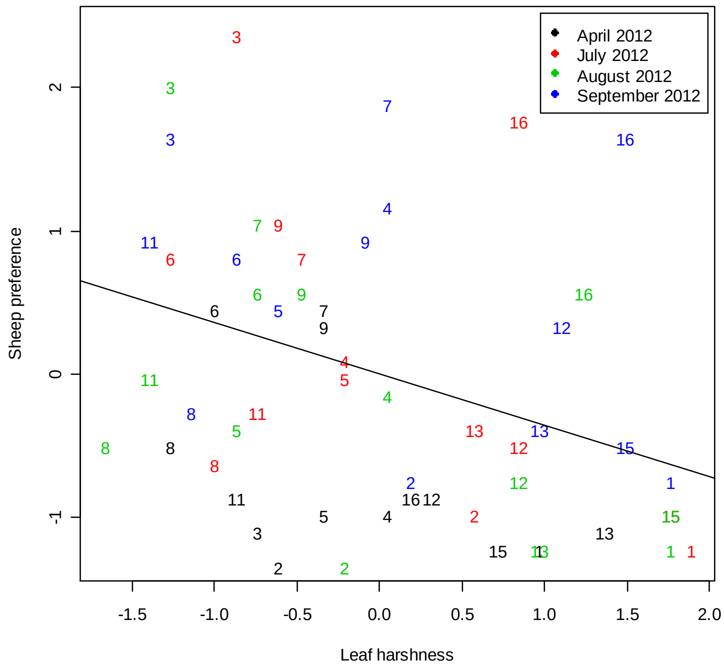


Figure 8.6 Relationship between standardized sheep preference scores and standardized leaf harshness for fourteen tall fescue clones (1-9,11-13, 15,16) grazed in four experimental periods in 2012 (Y = -0.36X; p = 0.007, R^2 = 0.11).

The negative effect of pre-grazing sward height on sheep preference (Y = -0.66X; $p < 0.001$, $R^2 = 0.43$) was consistent for most clones. Except for experimental period 1, clone 3 had a preference that was higher than expected from its sward height (**Figure 8.7**). Obviously, there was negative correlation between sheep preference and post-grazing sward height (Y = -0.74X; $p < 0.001$, $R^2 = 0.54$).

Rabbit and sheep grazing preference corresponded well (Y = 0.69X; $p < 0.001$, $R^2 = 0.47$), but in the second experimental period sheep preference for clone 3 was higher than what was expected from rabbit preference (**Figure 8.8**).

There was only a weak relationship between the leaf blade morphological variables and sheep preference: R^2 values were below 0.10 for all variables. Preference was negatively correlated with leaf blade length (Y = -0.27X; $p = 0.047$, $R^2 = 0.07$) (**Figure 8.9a**) and leaf blade shear strength (Y = -0.30X; $p = 0.025$, $R^2 = 0.09$) (**Figure 8.9d**). Slopes were not significantly different from 0 for leaf blade width ($p = 0.24$) (**Figure 8.9b**) and leaf shear force ($p = 0.073$) (**Figure 8.9c**). Surprisingly, leaf blade length had no significant relationship with pre-grazing sward height ($p = 0.64$).

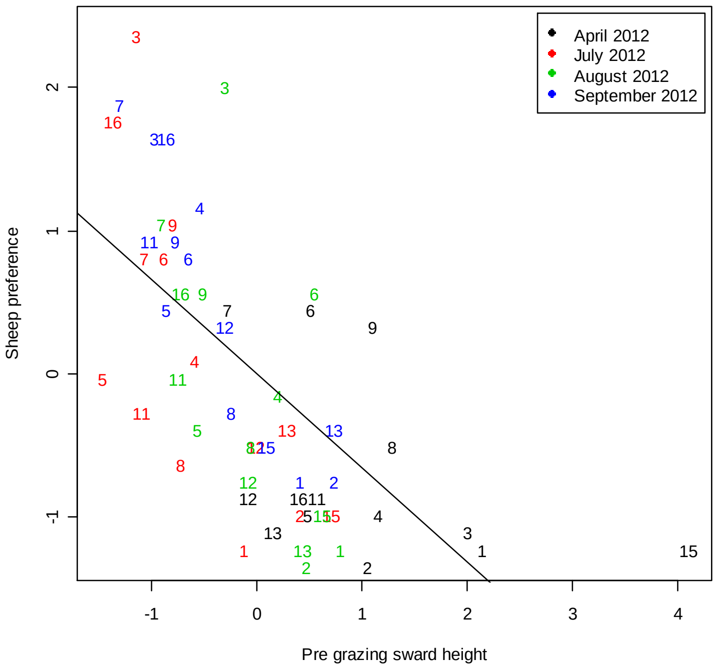


Figure 8.7 Relationship between standardized sheep preference scores and standardized pre-grazing sward height for fourteen tall fescue clones (1-9,11-13, 15,16) grazed in four experimental periods in 2012 (Y = -0.66X; p < 0.001, *R²* = 0.43).

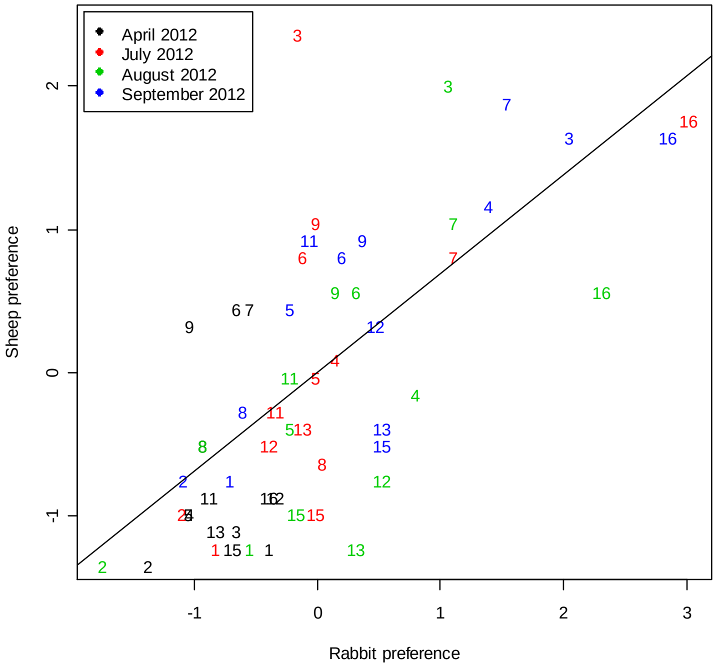


Figure 8.8 Relationship between standardized sheep preference scores and standardized rabbit preference for fourteen tall fescue clones (1-9,11-13, 15,16) grazed in four experimental periods in 2012 (Y = 0.69X; *p* < 0.001, *R²* = 0.47).

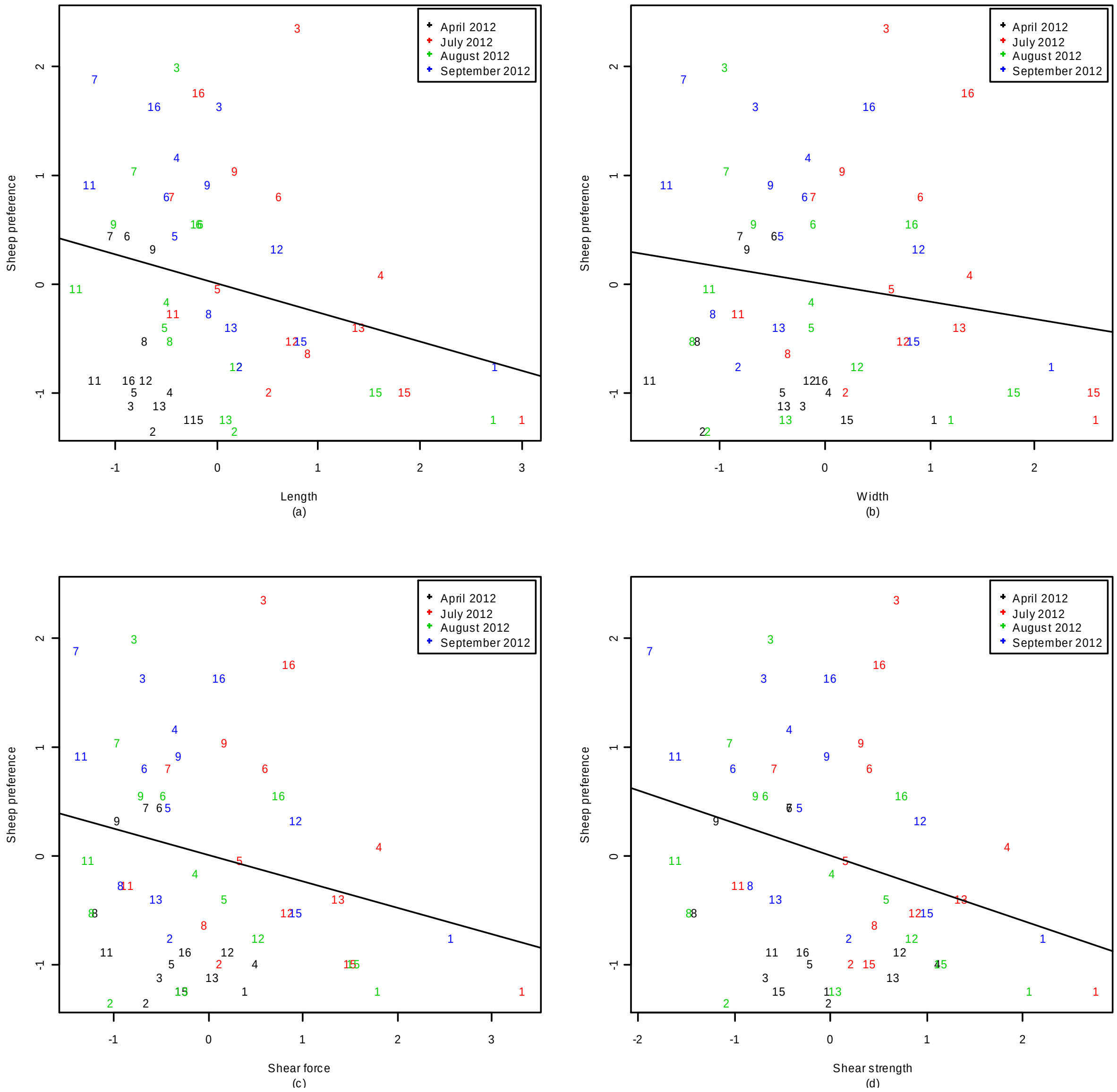


Figure 8.9 Relationship between standardized sheep preference scores and (a) standardized leaf blade length (Y = -0.27X; *p* = 0.047, *R²* = 0.05), (b) standardized leaf blade width (Y = -0.16X; *p* = 0.24, *R²* = 0.02), (c) standardized leaf shear force (Y = -0.24X; *p* = 0.073, *R²* = 0.06) and (d) standardized leaf blade shear strength (Y = -0.30X; *p* = 0.025, *R²* = 0.09) for fourteen tall fescue clones (1-9,11-13, 15,16) grazed in four experimental periods in 2012.

Although clone 16, one of the most preferred clones, and clone 2, one of the least preferred clones, were respectively dark and light green, there was no significant (p = 0.26) relationship between colour and sheep preference (**Figure 8.10**).

DMC had a significant (Y = -0.38X; p = 0.014, R^2 = 0.14) negative influence on preference (**Figure 8.11**), but no clear clone effect appeared. The experimental period on the other hand seemed to influence the preference: the DMC in the first period was clearly higher compared to the other periods.

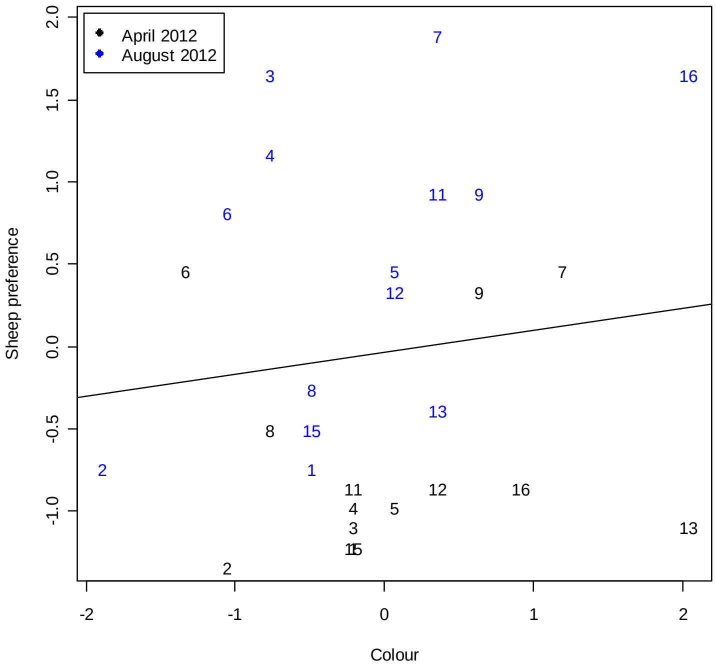


Figure 8.10 Relationship between standardized sheep preference scores and standardized sward colour for fourteen tall fescue clones (1-9,11-13, 15,16) grazed in four experimental periods in 2012 (Y = 0.15X; *p* = 0.26, R^2 = 0.005).

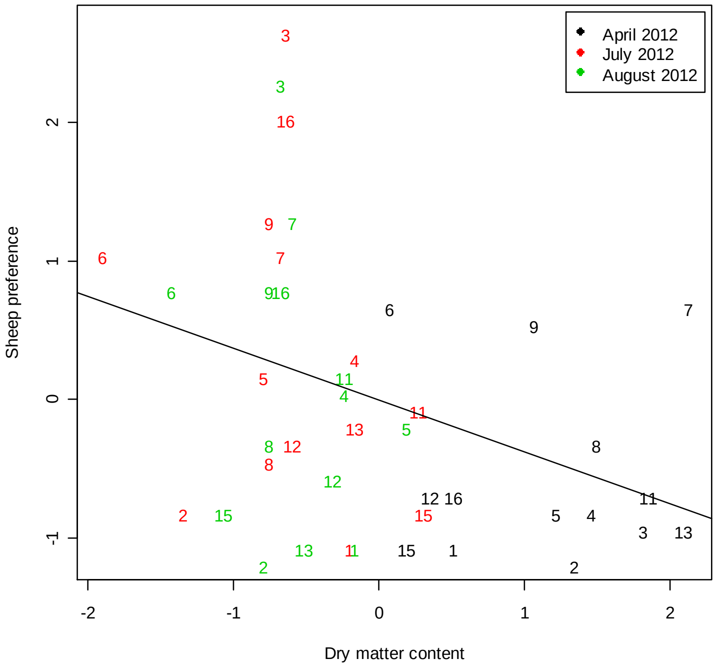


Figure 8.11 Relationship between standardized sheep preference scores and standardized dry matter content for fourteen tall fescue clones (1-9,11-13, 15,16) grazed in 3 experimental periods in 2012 (Y = -0.38X; *p* = 0.014, R^2 = 0.14)

There was no significant effect of RDOM on sheep preference ($p = 0.43$). Clone 16, one of the most preferred, was among the clones with the lowest RDOM (**Figure 8.12**).

The regression coefficients of all the variables that were measured in every experimental period on every plot were summarized in a correlation matrix (**Table 8.5**). Leaf blade length, width, shear force and shear strength were all positively inter-correlated (**Table 8.5**).

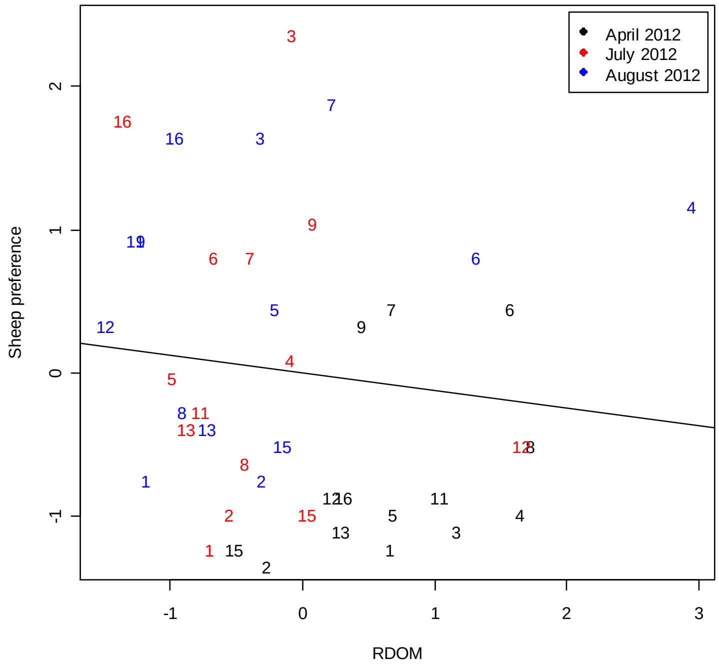


Figure 8.12 Relationship between standardized sheep preference scores and standardized apparently rumen degraded organic matter (RDOM) content for fourteen tall fescue clones (1-9,11-13, 15,16) grazed in 3 experimental periods in 2012 (Y = -0.13X; $p = 0.43$, $R^2 = 0.02$)

The first two components of the principal component analysis explained 77 % of the variance in the multivariate dataset, where PC 1contributed for 48 % and PC 2 for 29 %. **Figure 8.13** shows a biplot of the first two principal components: the points represent the scores of each clone in the four experimental periods and the vectors represent the loadings of each variable in the first two principal components. The loadings of the first principal component can be interpreted as a contrast between clones with fine, soft leaves (clones 3, 7 and 11) on one hand, and clones, with long, wide, harsh leave blades with a high leaf shear force and a high shear strength (clones 1 and 15) on the other hand (**Figure 8.13**). The loadings of the second principal component can be interpreted as a contrast between clones that are disliked both by rabbits as by sheep (Clone 2, 15) with a high pre-grazing sward height and clones that are liked by both animals with a low pre-grazing sward height (Clone 3, 7, 16) (**Figure 8.13**).

Over all experimental periods, rabbit preference and pre-grazing sward height were the variables that correlated best with sheep preference (**Table 8.5**). This was reflected in the multiple linear regression model. In the minimum adequate model the effects of pre-grazing sward height, leaf blade harshness and rabbit preference were significant (**Table 8.6**).

Table 8.5 Correlation matrix for variables measured on fourteen tall fescue clones grazed by sheep in four experimental periods in 2012. PB: sheep preference begin period, PE: sheep preference end period, LL: leaf blade length, LW: leaf blade width, SF: shear force, SS: shear strength, H0:pre-grazing sward height, H1: post-grazing sward height, HAR: leaf harshness, RP: rabbit preference

	PB	PE	LL	LW	SF	SS	H0	H1	HAR	RP
PB	1	0.62	-0.27	-0.16	-0.24	-0.30	-0.66	-0.74	-0.36	0.69
PE		1	-0.17	0.07	-0.04	-0.11	-0.44	-0.63	-0.22	0.46
LL			1	0.80	0.86	0.79	0.06	0.44	0.58	-0.10
LW				1	0.94	0.81	0.06	0.23	0.72	0.14
SF					1	0.95	0.04	0.31	0.72	0.03
SS						1	0.04	0.33	0.66	-0.04
H0							1	0.57	0.25	-0.51
H1								1	0.27	-0.55
HAR									1	0.13
RP										1

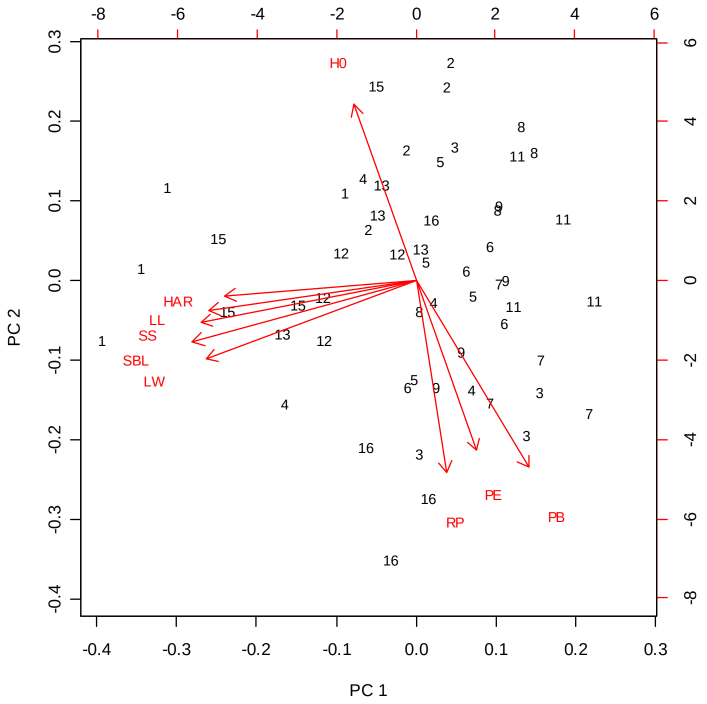


Figure 8.13 Biplot of the first two principal components (PC) for nine variables measured on fourteen tall fescue clones grazed by sheep in four experimental periods in 2012. PB: preference begin period, PE: preference end period, LL: leaf blade length, LW: leaf blade width, SBL: leaf shear force, SS: leaf shear strength, H0:pre-grazing sward height, HAR: leaf harshness, RP: rabbit preference.

Table 8.6 Minimal adequate multiple linear regression model to predict sheep preference from morphological variables, sward characteristics and grazing preference of rabbits measured on fourteen tall fescue clones grazed by sheep in four experimental periods in 2012. Significance codes: *: *p* < 0.001, **: *p* < 0.01, *: *p* < 0.05, NS: *p* > 0.05.**

	Coefficients	p-value	
Intercept	5.19	$8.9\text{x}10^{-5}$	***
Pre-grazing sward height	-0.22	0.011	*
Harshness	-0.84	$1.4\ \text{x}10^{-5}$	**
Rabbit preference	0.84	$8.2\text{x}10^{-9}$	***
	$R^2 = 0.71$		

8.4 Discussion

Scoring for the mass of grazed biomass allowed discriminating between preference of clones in a significant and consistent way. With the exception from the first experimental period, the preference ranks of the clones were similar in every experimental period. Seasonal differences in sheep preference for tall fescue varieties were found in Jadas-Hecart (1982). Earliness of the clones might have influenced these preference rankings. Preference differences were not only found between clones with a different origin, but also between clones originating from the same variety. Clone 2 and clone 3 for example originated from the same soft leaved variety, the former was one of the least preferred, while the latter was one of the most preferred.

Shewmaker *et al.* (1997), found a correlation coefficient of 92 % between the preference scores of eight varieties 30 h and 48 h after the start of a experimental period. In our trial, the correlation coefficient between preference score at the beginning and at the end of the experimental period was lower (37 %), but we found no great influence of time when scoring took place on the sheep preference ranks.

Scores for grazing preference were more discriminating at the start compared to the end of the experimental period. In the trials of Jadas-Hecart (1982) preference was also scored several times during each experimental period. The scoring that discriminated most, was used to compare the varieties. Averaged over all experimental periods, preference scores for 15 Fa varieties were between 1.6 and 5.3 on a scale that was equal to the scale used in our research. The values found in our trial, averaged over the 4 periods, were in the same range (0.5 - 5.4). The faster decrease in herbage mass of the most preferred material in the beginning of the trials, favours the grazing of the less preferred material later, resulting in smaller differences. Differences in preference in our trial were largest when roughly half of the biomass of the most preferred clones was grazed.

The leaf characteristic most positively associated with preference across the experiments of MacAdam and Mayland (2003) was leaf width ($r = 0.51$). This trait was negatively correlated with preference in our trial ($r = -0.16$). The smaller variation in leaf blade width and length and different leaf morphology in the study of MacAdam and Mayland (2003) compared to our trial might explain this difference. In our study, average leaf blade widths and lengths varied between 5.5 mm - 10.2 mm and 144 mm - 370 mm respectively, whereas average leaf widths and lengths in the study of MacAdam and Mayland (2003) varied between 4.7 mm - 7.3 mm and 405 mm – 487 mm respectively. So, the varieties in the study of MacAdam and Mayland (2003) were all relatively similar in leaf morphology compared to the clones in our study. MacAdam and Mayland (2003) explained the positive relationship between leaf width and cattle preference by a higher ratio of mesophyll to structural tissue in the wide leaved varieties. This hypothesis was not valid in our trial, as leaf width was positively correlated with shear strength (r = +0.81), which implicates that fiber strength or number is increasing with leaf width.

The correlation found between leaf shear strength and sheep preference found by MacAdam and Maryland was also negative (r = -0.16), but the correlation was stronger in the present study (r = -0.30). These differences can be explained by several factors: the different leaf morphology of the plant material used in both trials, the use of sheep *vs.* cattle, the use of clones *vs.* varieties.

Although the leaf harshness of the clones in our trial varied widely, the correlation between preference and leaf harshness (r = -0.36), was weaker than the value found in Jadas-Hecart (1982) (r = -0.54). The effect of leaf colour on preference was not significant in our trial. Jadas-Hecart (1982) on the other hand found a significant positive correlation between leaf darkness and sheep preference, but this correlation was not consistent over years and seasons. Vigour was negatively correlated with preference in the trials of Jadas-Hecart (1982): the slowest growing varieties were most preferred. Similar observations were found in our trial: the rather hard leaved but dark green and slow growing clone 16 was consistently among the most preferred clones.

Pre-grazing sward height had an important negative effect on sheep preference (r = -0.66). Surprisingly leaf length was not correlated with pre-grazing sward height. Hence, sward height was merely a result of plant habitus. Clones with an erect growth had a high pre-grazing sward height and were disliked (clone 15). The reason for this could be that erect growing clones stings the muzzle of the grazing animal. Leaf length was negatively correlated (r = -0.25) with sheep preference. Our sheep preferred clones with rather short leaves or long leaved with flexible (non-erect) leaves, that produced short swards. This finding contrasts with what is found in intake trials with cows grazing on perennial ryegrass, where sward height has a positive effect on intake rates (Barre *et al.*, 2006; Peyraud *et al.*, 1996).

Post-grazing sward height had an even higher correlation (r = +0.74) with sheep preference compared to pre-grazing sward height, but this was obvious and was of limited practical importance: this variable cannot be measured without sheep grazing.

The use of animals that select forage on an lower spatial scale than sheep may be useful in plant breeding, particularly in the early stages of a breeding programme when plant material is scarce and replicates are not obvious. But little is known about the correlation of preference of small herbivores and grazing ruminants. Preference of tall fescue varieties by meadow voles and by sheep did not correspond in trials performed by Kendall and Sherwood (1975). In our trial on the other hand, rabbit and sheep preference correlated well (r = 0.70). We expected that the sward characteristics would have less importance for rabbit preference compared to sheep preference, as rabbits grazed individual leaves rather than swards, but this could not be confirmed in this trial.

Digestibility measurements and dry matter measurements were no more than indicative in this trial, as replicates were absent for these variables. There seemed to be no direct effect of both variables on the preference in our study. An interesting observation was that the three clones with the best digestibility originated from varieties, whereas the clones with the worst digestibility originated from ecotypes, which indicates that breeding led to a better digestibility indeed. Also in other studies, clear effects were absent: Gillet *et al.* (1983) found a good correlation between sheep preference measured in the trough cafeteria and digestibility between species; but within species the relationship was less clear.

The large morphological differences in our trial might have masked the more subtle effect of digestibility. The effect of digestibility, on preference of grazed tall fescue should be studied in more detail, with clones with a similar morphology. Given the positive relationship found between water soluble carbohydrate content and preference in tall fescue varieties found by Mayland *et al.* (2000), it would be interesting to include also this factor in further studies.

The parameters with the highest correlation with sheep preference were rabbit preference, leaf harshness and pre-grazing sward height, but overall the predictive value of the measured prameters was low. A model including these three parameters, explained 71 % of the variation found in sheep preference. The results suggested that the preferred clones were soft, fine leaved and low growing, but that not all clones with these properties were preferred (eg. clone 11). With the knowledge we have now, animal preference trials remain the best method to make progress in animal preference for tall fescue.

8.5 Conclusions

Most of our research hypotheses were confirmed by this trial:

1. Significant differences in preference occurred, the most preferred and least preferred clones were consistently the same throughout the different experimental periods
2. Preference was negatively correlated with leaf blade length and shear strength, pre-grazing sward height and leaf harshness. Effects of leaf blade width, shear force and colour were not significant. There seemed to be no effect of dry matter content and digestibility.
3. Sheep and rabbit preferences were positively correlated.

Further conclusions of this trial that are useful in tall fescue breeding:

- The genotypes preferred by grazing sheep are generally soft and fine leaved and are low growing. The inverse is not necessarily true: soft and fine leaved types are not necessarily preferred.
- During animal trials, discrimination between the clones was best when the most preferred clones were eaten *circa* 50 %.
- Rabbit preference correlates well to sheep preference. This can be useful in the early stages of breeding, when the available plant material is scarce.

For practical use in breeding however some questions remain to be answered:

- What is the minimal plot surface that allows reliable preference scoring? The plot size and number of replicates in our trial were large enough to see preference. In breeding however, where plant material and labour are mostly scare, even smaller plot size and a reduced number of replicates would be preferable.
- How good is correlation between sheep preference and cattle preference?
- Which is the best growth stage for comparing Fa genotypes?

8.6 Acknowledgements

The author is grateful to Marc Seynaeve for providing the sheep, to the Laboratory for Animal Nutrition and Animal Product Quality (LANUPRO) for the RDOM analyses and the use of the texture analyser and to Johan De Koker, Franky van Peteghem and Jean-Pierre Vermaerke for their techical assistance during the experiments.

Chapter 9

Quantifying early vigour and ground cover using digital image analysis

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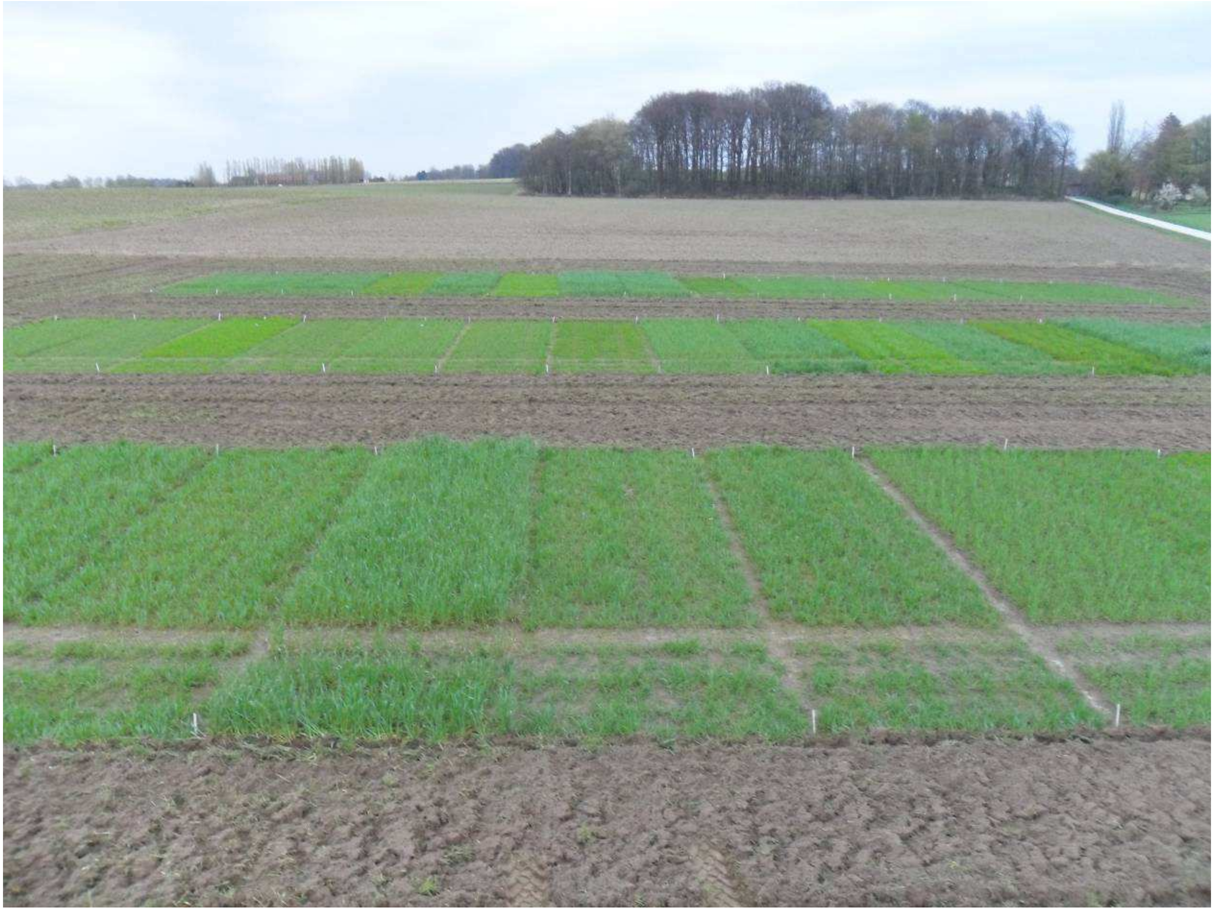


9.1 Introduction

Plant breeders use ground cover as a parameter to measure early vigour or density of grass swards. Especially in tall fescue (*Festuca arundinacea* Schreb.; **Fa**) selection for faster development and ground cover is useful as tall fescue is characterised by a weak early vigour often resulting in a delicate establishment with low yields in the first production season (**Chapter 2**). Lewis and Garcia (1979) suggested that early vigour of tall fescue could be improved by selecting for the presence of coleoptile tillers in the seedling stage. Hayes (1976) on the other hand, suggested to select for high seminal root growth rate to improve early vigour of tall fescue. Whatever the underlying reason may be, from a selection point of view, the reliable identification of plants, families or populations possessing a strong early vigour is a prerequisite for further progress.

Discontinuous scores of ground cover are currently used to assess this trait qualitatively, but this scoring is subjective and has a low repeatability. The use of digital image analysis could allow quantification of the covered ground area in a repeatable way. The method to quantify ground cover is basically simple. The number of green pixels in a digital image of the crop are counted and expressed as a proportion of the pixels in the whole image. The operator has to set limits to define which pixels are green, and thus covered by plant material, and which are not. The use of digital images to analyse ground cover was successfully used in several crops thus far. Purcell (2000) measured soybean canopy coverage and light interception with digital imagery. Behrens and Diepenbrock (2006) used digital image analysis to describe canopies of oilseed rape during the vegetative stage. They developed a regression that allowed to predict the leaf area index of oilseed rape fields from ground cover. Richardson *et al.* (2001) used digital image analysis and a commercial software package to quantify ground cover in turfgrass swards. Ground cover obtained with digital image analysis was compared with ground cover as scored by five trained evaluators. Image analysis produced a much lower variance than the subjective scores, resulting in more significant differences. A macro was developed (Karcher and Richardson, 2005) to perform ground cover analysis on batches of digital images, minimizing the time needed to analyse a high number of similar digital images.

Also Lock *et al.* (2004) tested the accuracy of digital image processing for quantification of ground cover in turf grass. In addition to the hue, saturation and lightness (HSL) colour space, they improved robustness of the method by using geometric classifiers: objects below a certain size or shape were not seen as grass leaves and neglected.

Although previous research suggested that digital image analysis can be used by breeders/crop scientists to distinguish between ground cover of different genotypes, varieties or crops in practise, this was proved nor illustrated so far. Furthermore, there is no information available on the correlation between the ground cover and standing biomass. The first objective of this study was to test whether analysis of digital images can quantify ground cover in a repeatable way in variety trials. The method was tested both under controlled environment as under field conditions. Secondly, correlation between ground cover and aboveground biomass production of establishing grass swards was investigated. Our research hypotheses were that:

1. Digital image analysis can be used in a breeding programme with Fa to measure ground cover in a repeatable and precise way both under controlled as well as under field conditions.
2. There is a good correlation between ground cover and biomass production.

9.2 Material and Methods

9.2.1 Plant material

The method of ground cover quantification using digital image analysis was tested in three distinct experiments. In two of these three experiments the relation between biomass and ground cover was studied. As the aim of the experiments was to study the performance of a method rather than comparing the early vigour of varieties, the varieties are identified by numbers.

The first experiment compared the early vigour of nine different Fa varieties (Variety 1 – Variety 9) from different European breeding companies. To widen the differences in early vigour two perennial ryegrass (*Lolium perenne* L., **Lp**) varieties, one diploid variety (Lp2x) and one tetraploid variety (Lp4x), were also included in the trial resulting in the comparison of eleven grass varieties. The first experiment was conducted in a controlled environment in order to minimize the effect of environmental variation on the early vigour.

On three occasions in 2009 (31 July, 3 September and 18 November) three plastic containers-measuring 42 cm long 39 cm wide and 8 cm deep-per variety were filled with a mixture of steamed sandy-loam soil and peat in a 1:1 ratio and planted with grass seedlings. Grass seedlings were planted instead of sown in the containers in order to have an equal number of uniformly developed plants in each container. Germinated seeds were planted in the containers at a density of 1000 plants m^{-2} in rows 3.3 cm apart and with a distance of 3 cm within the rows. Five days prior to planting, the seeds were germinated on moist paper in Petri dishes in a room with a temperature of 20°C and a light regime of 12h dark-12h light. Only uniformly germinated seeds were transplanted in the containers. All containers were planted on the same day. After planting, the containers were transferred to an unheated greenhouse on three flood benches; no supplementary light was provided. On each flood bench one container of each variety was placed in a random order. As soon as the first true leaf of the fastest growing seedlings was visible, the containers were photographed in a room with artificial light on regular time intervals. Each container was put under a camera (CANON eos 50D with lens EF-S 17-85 mm) which was mounted on a stand with a horizontal arm stretching 75 cm above the soil level in the containers. When the canopy of the fastest growing varieties was closed the experiment was terminated and the aboveground dry matter yield (DMY) of each container was determined by cutting the grass with shears at *circa* 1 cm height and drying the material for 16h at 75°C.

Through this chapter, this experiment that was replicated three times in 2009 will be called "greenhouse experiment". In the early summer replication, digital images were taken on 6 occasions (10/8, 18/8, 21/8, 24/8, 28/8 and 31/8); in the late summer replication on 9 occasions (from 9/9 till 30/9 with three day intervals) and in the autumn replication on 7 occasions (26/11, 3/12, 9/12, 18/12, 22/1, 26/2 and 15/3). In the autumn experiment, the measurements were terminated before closure of the canopies since a part of the plants succumbed to frost damage.

In the second experiment, pictures were taken in a field trial. The trial compared the yield of two varieties of Fa (variety 1 and variety 2) and a diploid and a tetraploid variety of Lp (Lp2x and Lp4x). The trial was established on 15/4/2009 on a sandy loam soil in Merelbeke, Belgium. Seeds were sown with a plot seeder with a row distance of 12.5 cm at a density of 1500 germinable seeds m^{-1}. The trial design was a randomized complete block with three replications; individual plot size was 7.8 m². Digital images were taken on three occasions from 20/5 till 17/6 with two weeks intervals. A camera (CANON eos 50D with lens EF-S 17-85 mm) was mounted on a stand with a horizontal arm stretching 1 m above the ground. The photographed area was *circa* 30 cm x 45 cm and contained two rows. As no protection against natural light was provided, digital images were taken when it was cloudy or when no direct sunlight was shining on the plots. Through this chapter, this experiment will be called “field experiment I”

In a third experiment, digital images were taken and biomass was harvested simultaneously on a trial comparing cover crops. Six winter rye (*Secale cereale* L.) varieties (Variety 1-Variety 6), one variety of Italian ryegrass (*Lolium multiflorum* L.) and one variety of lopsided oat (*Avena strigosa* Schreb.) were sown on two different dates : 22/9/2010 and 14/10/2010 on a sandy soil in Kruishoutem, Belgium. Although this trial did not include Fa, we collected data in this trial to validate the method in an experimental setting with autumn sowing and with a management close to agricultural practice (presence of weeds and crop residues). Given the very similar growing pattern of Fa and the monocot cover crops, we supposed that the outcome of this trial would also apply to Fa. The experimental design was a split plot design with three replications. Main plot factor was the sowing date and subplot factor the species/varieties. Plots were sown with a 3 m wide seed drill (row distance 12.5 cm) at a density of 300 seeds m^{-2} for the rye and oat and 2000 seeds m^{-2} for the Italian ryegrass. Individual plot size was 30 m². On six occasions from 24/10/2010 plots were photographed with an interval of *circa* 20 days (24/10, 12/11, 11/12, 31/12, 16/01 7/02) using a portable stand with a horizontal arm on which a camera (FUJIFILM FinePix F470) was mounted 1.5 m above the ground. Each photograph covered an area of approximately 2.5 m². The photographs were taken on cloudy days with no direct sunlight. On two occasions (20/1/2011 and 12/2/2011) standing aboveground biomass was determined, by cutting all plantlets just above the ground, on a surface of 1 m² per plot. Through this chapter, this experiment will be called “field experiment II”.

9.2.2 Image analysis

All digital images were saved in the JPEG (Joint Photographic Experts Group) format with a resolution of 3456 x 2304 pixels in the first and the second experiment, and with a resolution of 2048 x 1536 pixels in the third experiment. Image analysis was done with Image J, a public domain, Java-based image processing program (Image J, 2013), using the threshold_colour plugin (Landini, 2013). The threshold_colour plugin allows the user to search a digital image for a specific color or a range of color tones. Using this plugin, the original colour images were transformed into binary images where all pixels covered by plant material in the original image (green) had value 1 and all other pixels (ground, litter) had value 0. Digital images were processed in the Hue (H), Saturation (S), Brightness (B) colour space. In this colour space, each pixel in a digital image has a value for each of the three dimensions ranging from 0 to 255. Pixels with different colours can be divided in groups by putting limits on the values of H, S and B. These limits are commonly called threshold values. Ground cover was calculated as the number of pixels that had values for H, S and B within these limits, divided by the total number of pixels.

Classification of pixels was further improved by taking into account the geometry of the groups of selected pixels. In order to reduce the number of pixels representing soil and wrongly classified as plant material, groups of pixels below a certain size could not be classified as plant material. Distinction of groups of pixels representing developing dicot weeds and developing grass plants, was based on the higher circularity (4 x π x (area/perimeter^2)) of the former compared to the latter.

Threshold values for H, S and B that discriminated between green pixels and other pixels and minimum size and circularity of groups of pixels that represented grass plants, were searched by trial and error on a random subset of 10 % of all digital images taken on the same occasion (called “batch” hereafter). Once the right threshold values were found for a particular batch, it was analysed in an automated way using a macro. Inputs for this macro were a batch of digital images and threshold values; output was the ground cover for each digital image in the batch.

9.2.3 Data analysis

Analysis of variance for the ground cover was performed using the *aov()* function in R (R Development Core Team, 2011). Varieties and/or species were treated as a fixed factor, and block as a random factor. Treatment means were compared using the *TukeyHSD()* function. Regression was performed using the *lm()* function.

9.3 Results

9.3.1 Greenhouse experiment

As the plant material in the greenhouse experiments was photographed under controlled, artificial light, threshold values resulting in optimal discrimination between plant and soil were very similar for every batch of pictures in every experiment. Optimal distinction between the plant material and the bare soil was found with threshold values between 26 and 30 for H, 0 for S and 60 for B. At the end of the early summer and late summer trials, when the canopy was nearly closed, a slightly higher value for H was needed to include the shaded grass pixels lower in the sward. Exclusion of objects with a size smaller than 250 pixels and with circularity greater than 0.2, prevented small circular patches of algae growing on the soil surface to contribute to ground cover.

In the early summer and late summer experiments, differences in ground cover between the varieties were minimal on the first occasion that pictures were taken, but the differences in ground cover increased with time and were maximal when the fastest growing varieties reached a ground cover of around 80 %. Once this point was reached, the increase of ground cover slowed down and the differences between the varieties decreased (**Figure 9.1a** and **Figure 9.1b**). Due to frost damage, the autumn experiment was terminated before maximal differences in ground cover were reached. By that time ground covers for Lp2x and Lp4x were 35 % whereas variety 9, the slowest growing Fa variety, had a ground cover of only 15 % (**Figure 9.1c**).

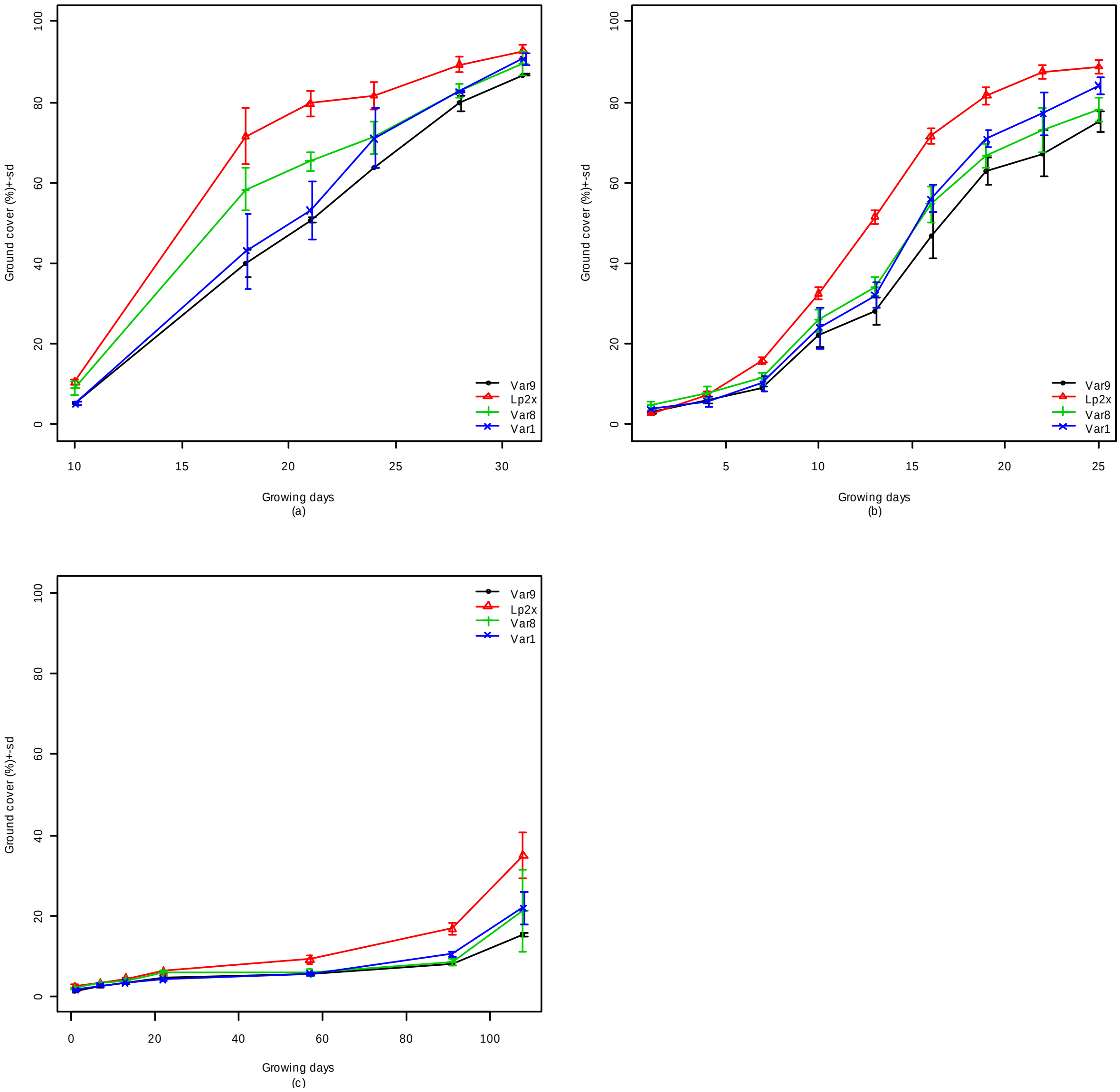


Figure 9.1 Evolution of mean ground cover in relation to sward age for three tall fescue varieties (Var1, Var8 and Var9) and one perennial ryegrass variety (Lp2x) in a greenhouse experiment that was repeated in (a) early summer (planting date: 31/7/2009), (b) late summer (planting date: 3/9/2009) and (c) in autumn (planting date: 18/11/2009). Error bars ± standard deviation (sd).

In the early summer experiment the largest differences in ground cover were measured after 21 growing days. At that moment, significant differences ($p < 0.001$) were found. Lp2x and variety 9 had the highest (80 %) respectively lowest (51 %) ground covers (**Figure 9.2a**). Significant differences occurred between Lp2x and most of the Fa varieties, but also between the Fa varieties (variety 8 *versus* variety 9). In the late summer experiment, the largest difference in ground cover was measured after 16 growing days: Lp4x had the highest ground cover (76 %) and variety 9 the lowest ground cover (47 %). Significant differences ($p <$

0.001) were found between the Fa varieties and Lp2x or Lp4x, but not among the Fa varieties (**Figure 9.2b**).

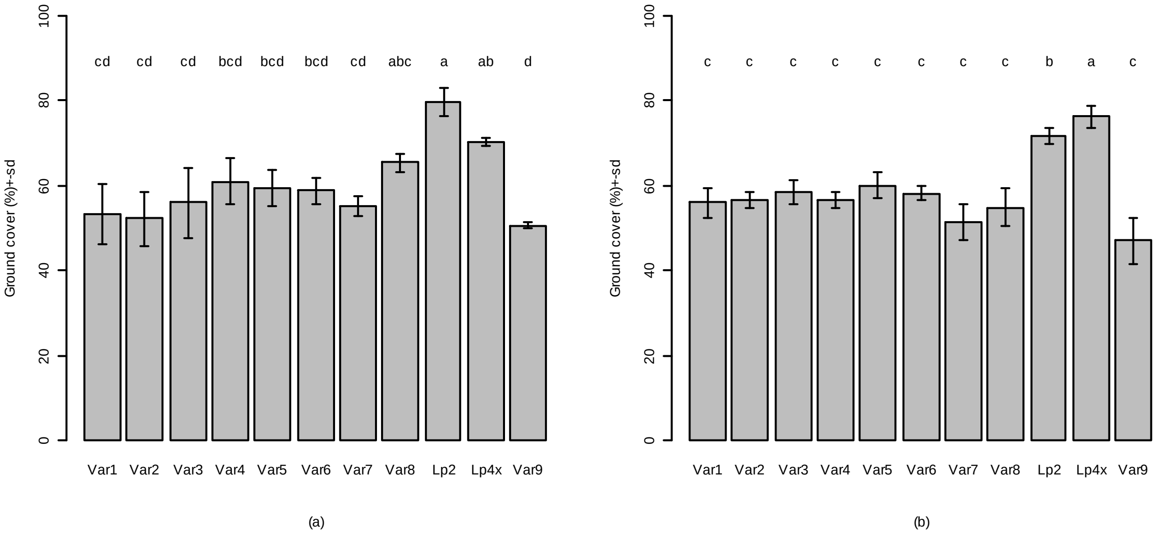


Figure 9.2 Mean ground cover for nine tall fescue varieties (Var 1-Var 9) and two perennial ryegrass varieties (Lp2x and Lp4x) at the moment of the largest differences in ground cover in a greenhouse experiment that was repeated in (a) early summer (planting date: 31/7/2009) and (b) in late summer (planting date: 3/9/2009) Error bars: ± standard deviation. Bars with a different letter are significantly different (Tukey, *p* = 0.05).

At the end of the early summer experiment, 31 days after planting, marginally significant differences in DMY between the varieties (*p* = 0.091) were found. Variety 8 and variety 7 had the highest respectively lowest DMY (**Figure 9.3a**). At the end of the late summer experiment on the other hand, significant differences in DMY were found (*p* < 0.001): the yield of variety 7 was significantly below that of variety 4, variety 8 and Lp4x (**Figure 9.3b**).

No significant correlation between ground cover and DMY was found at the end of the early summer (*p* = 0.29) experiment (**Figure 9.4a**). In the late summer experiments the correlation was marginally significant (*p* = 0.054) (**Figure 9.4b**). In both experiments, variety 8 combined a high DMY with an average ground cover whereas Lp4x combined a high ground cover with an average DMY.

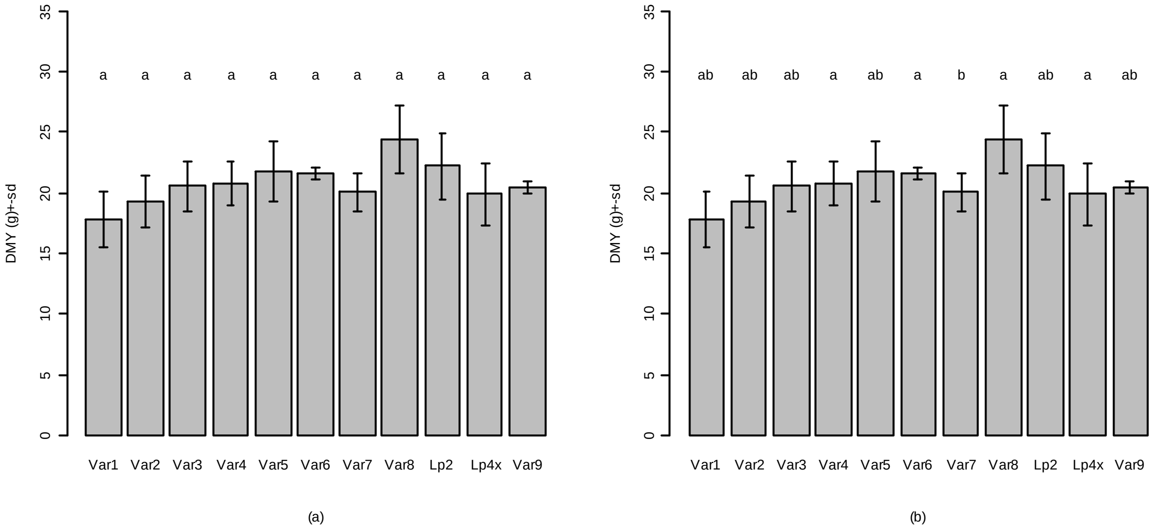


Figure 9.3 Mean dry mater yield (DMY) per container for nine tall fescue varieties (Var 1 – Var 9) and two perennial ryegrass varieties (Lp2x and Lp4x) at the end of a greenhouse experiment that was repeated in (a) early summer and (b) in late summer. Error bars ± standard deviation. Bars with a different letter are significantly different (Tukey, *p* = 0.05).

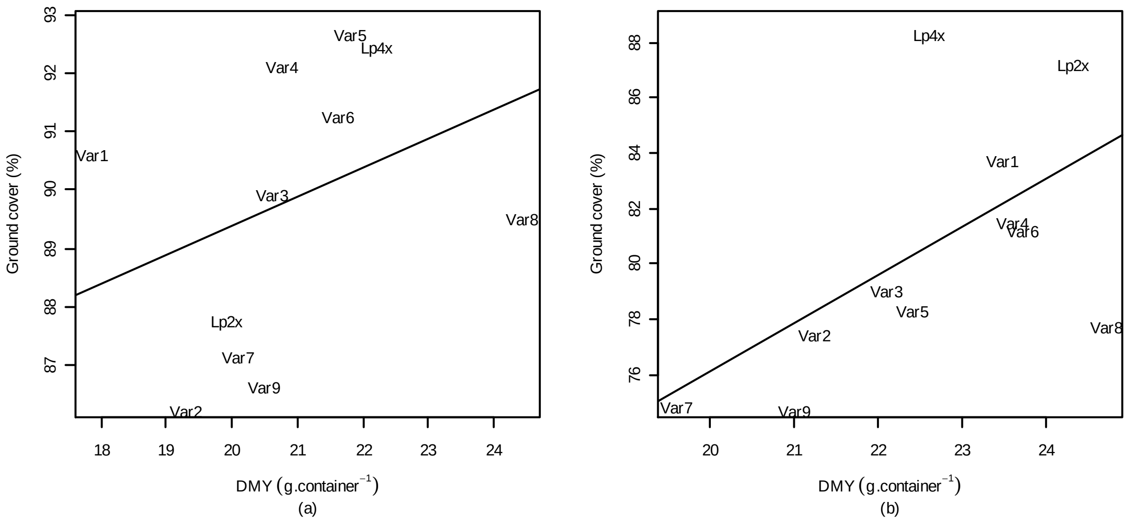


Figure 9.4 Relation between ground cover and dry matter yield (DMY) for nine tall fescue varieties (Var 1 – Var 9), one variety of diploid perennial ryegrass (Lp2x) and one variety of tetraploid perennial ryegrass (Lp4x) at the end of a greenhouse experiment that was repeated in (a) early summer (Y = 79.5+0.49X; *p* = 0.288, R^2 = 0.03) and (b) late summer (Y = 41.2+1.75X; *p* = 0.054, R^2 = 0.28).

9.3.2 Field experiment I

In field experiment I, optimal distinction between the plant material and the bare soil in the digital images was obtained with threshold values for H and S of 50 and 60 respectively (**Figure 9.5**). As the images were taken under natural light, threshold values for B varied between 100 for the digital images taken on 20/5/2009 and 50 for the images taken on 17/6/2009.

The largest differences in ground cover between the varieties were found in the digital images taken on 3/6/2009, 49 days after sowing (**Figure 9.6a**). At that moment, the highest ground cover was found for Lp4x (44 %) and the lowest ground cover for tall fescue variety 1 (15 %). The effect of the species/varieties on ground cover was significant ($p < 0.001$). Significant differences in ground cover were found between, perennial ryegrass and tall fescue, but also between the tall fescue varieties (**Figure 9.6b**).

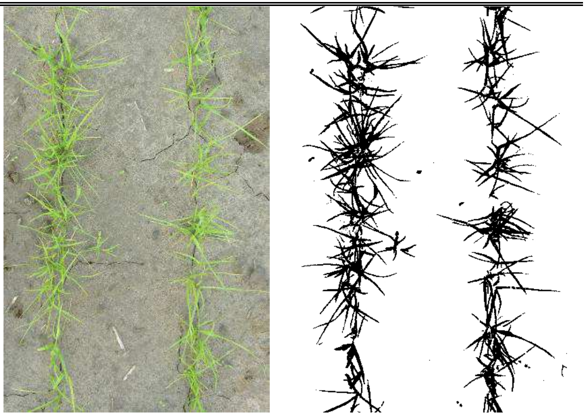
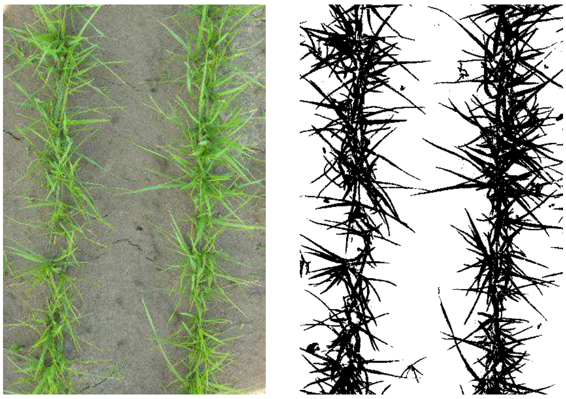
Fa variety 1 Ground cover: 13.5 %	
Fa variety 2 Ground cover: 29.9 %	
Lp2x Ground cover: 32.9 %	
Lp4x Ground cover: 46.3 %	

Figure 9.5 Image analysis of image taken on 3/6/2009 in field experiment I. Threshold values: hue 50, saturation 60 and brightness 100.

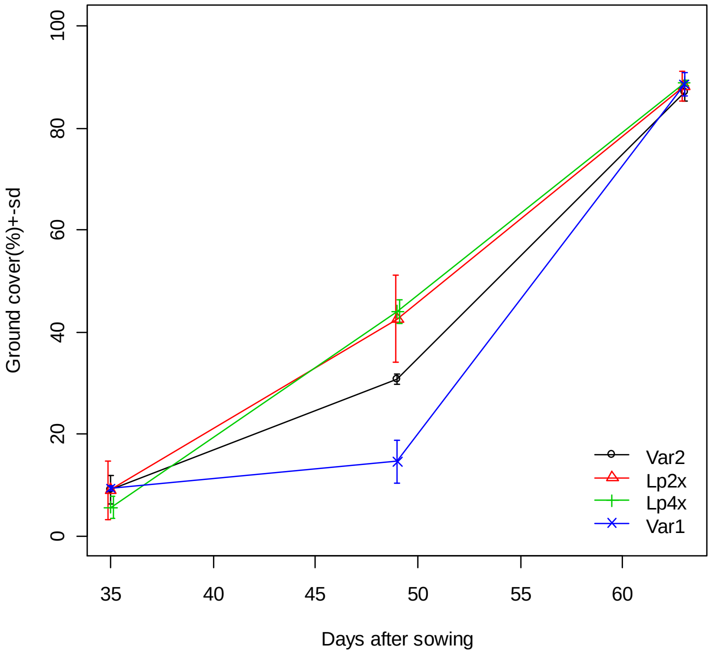


Figure 9.6 Ground cover for two tall fescue varieties (Var1 and Var2) and two perennial ryegrass varieties (Lp2x and Lp4x) sown on 15/4/2009 (a) Evolution of mean ground cover in relation to sward age (b) Mean ground cover 49 days after sowing, the moment of maximal difference in ground cover. Error bars: ± standard deviation. Bars with a different letter are significantly different (Tukey HSD, *p* = 0.05).

9.3.3 Field experiment II

In field experiment II, light was an uncontrollable factor and new threshold values had to be determined for each batch of digital images. Threshold values that discriminated between plant material and bare ground were between 30 and 120 for H, and between 50 and 110 for B. S had limited discriminating power and was not thresholded. Weeds were present in this field experiment. The most abundant species were *Stellaria media* L. and *Poa annua* L. In the early growth stages, the distinction between weeds and crops in the images based on size and circularity worked well (**Figure 9.7**), and most of the weeds could be removed from the images. In later stages it was no longer possible to distinguish between weeds and crops. But the contribution of the weeds to the ground cover was low as the rye and grass were far more competitive than the weeds. Only for lopsided oat, the least vigorous species, weeds were clearly present and thus contributing to the ground cover.

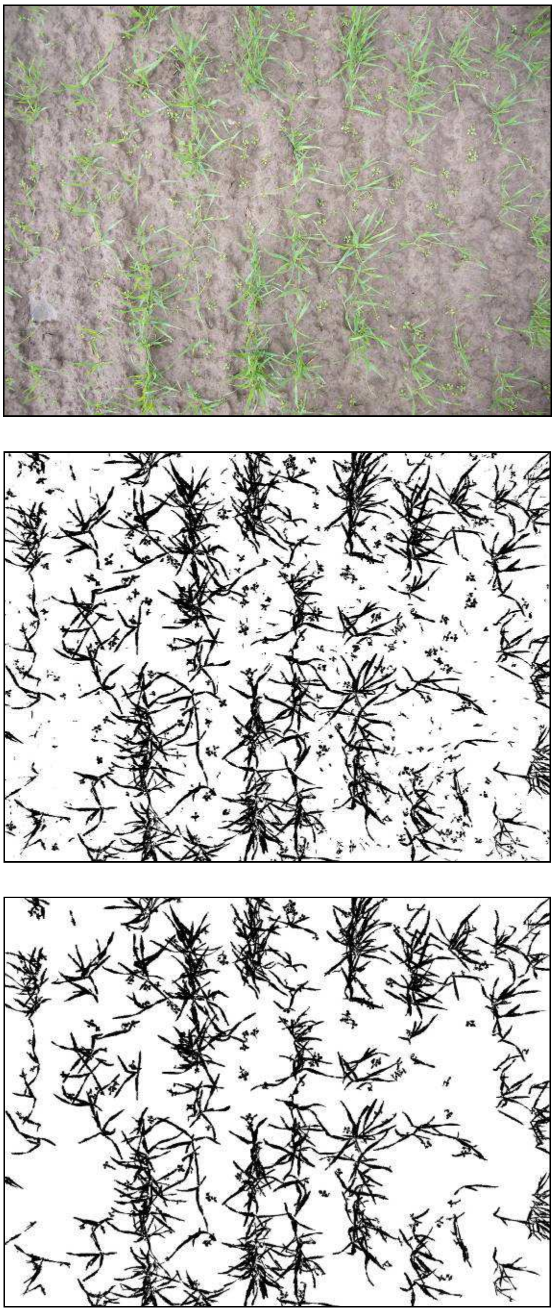


Figure 9.7 Above: Picture taken on 24/10/2010 of a rye plot sown on 22/09/2010; Middle: the same picture as above after thresholding, threshold values: Hue: 36, Saturation: 0, Brightness: 109. Ground cover = 20.3 %; Under: the same picture as in the middle, after removing particles with a size < 250 pixels and a circularity > 0.2; Ground cover = 18.6 %.

In the cover crops sown on 22/9/2012, maximal difference in ground cover occurred 41 days after sowing (**Figure 9.8a**). At that moment, rye variety 5 had the highest ground cover (70 %) and lopsided oat the lowest ground cover (35 %). Lopsided oat, Italian ryegrass and rye variety 1 had a significantly lower ($p < 0.001$) ground cover than the rye varieties 2 to 6 (**Figure 9.9a**).

In the cover crops sown on 14/10/2012, the maximal difference in ground cover occurred at the end of the experiment, 116 days after sowing (**Figure 9.8b**). At that moment maximal ground cover was only 38 %, and the difference in ground cover between the varieties was still increasing. The effect of the cover crop on the ground cover was significant ($p < 0.001$) and differences occurred both between the species as between the varieties: ground cover of the rye varieties 3, 4, 5 and 6 was significantly higher than that of rye variety 1 (**Figure 9.9b**)

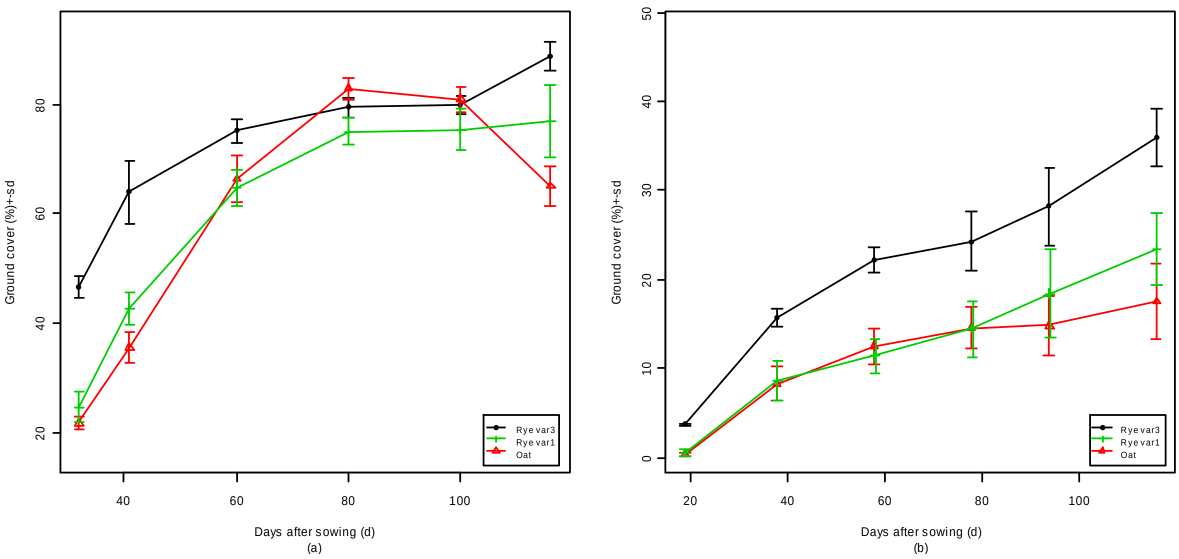
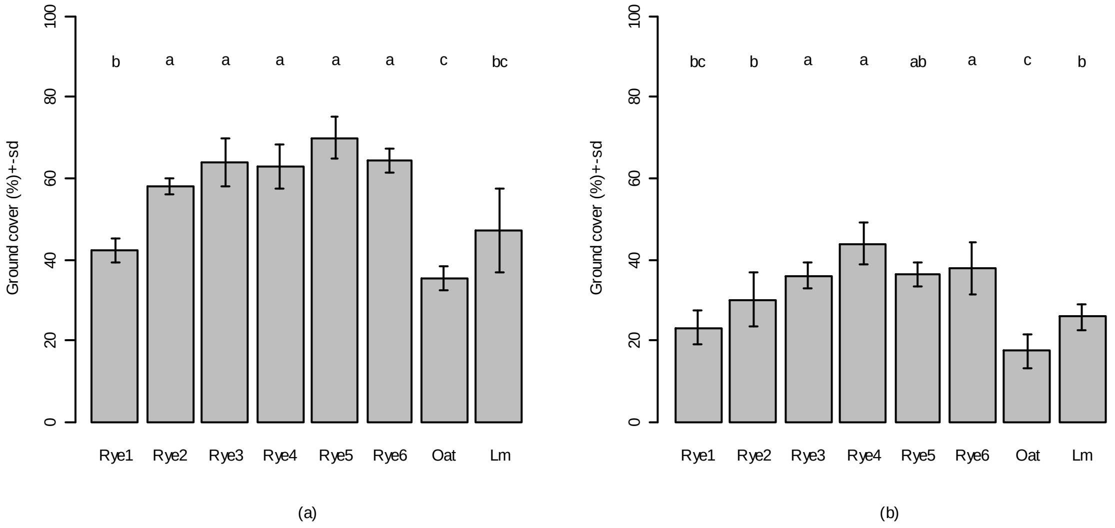


Figure 9.8 Evolution of ground cover in relation to sward age for two winter rye varieties and one lopsided oat variety sown on 22/9/2010 (a) or sown on 14/10/2010 (b) in field experiment II. Error bars: ± standard deviation (sd).

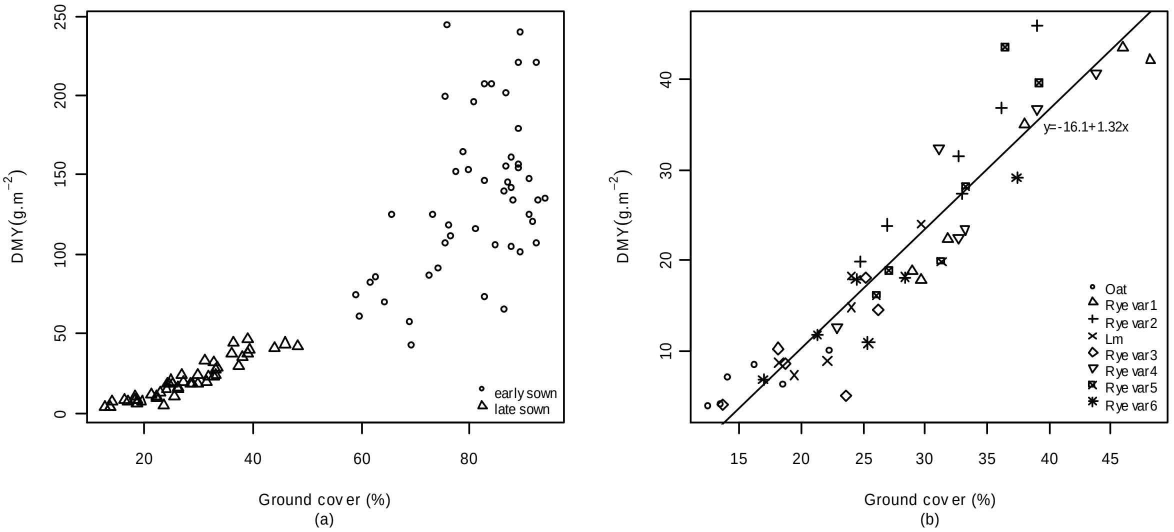
A good relationship was found between DMY and ground cover for the plots sown on 14/10/2010, but not for the plots sown on 21/9/2010 (**Figure 9.10a**). A linear model was fitted to the data of the plots sown on 14/10/2010. The regression ($Y = -16.1+1.32X$; $R^2 = 0.88$, $p < 0.001$) indicated that for a ground cover up to 50 %, an increase of 1 % in ground cover, corresponded with a DMY increase of 13 kg DM ha^{-1} (**Figure 9.10b**). In later growth stages (ground cover > 50 %) the relationship between ground cover and DMY was lost. Once a DMY of 150 g m^{-2} was reached, the ground cover of all varieties/species, was situated between 75 % and 90 %.



(a)

(b)

Figure 9.9 Mean ground cover for six winter rye varieties (Rye 1 – Rye 6), lopsided oat and Italian ryegrass (Lm) at the moment of maximal differences in ground cover. (a) 41 days after sowing for cover crops sown on 22/09/2010 and (b) 116 days after sowing for cover crops sown on 14/10/2010. Error bars: ± standard deviation. Bars with a different letter are significantly different (Tukey HSD, *p* = 0.05).



(a)

(b)

Figure 9.10 Relationship between dry matter yield (DMY) and ground cover for winter rye, lopsided oat and Italian ryegrass (a) contrast between early sown (21/9/2010) and late sown (12/10/2010) cover crops (b) regression between ground cover and DMY for the late sown cover crops sown on (12/10/2010) (Y = -16.1+1.3X; $p < 0.001$, $R^2 = 0.87$).

9.4 Discussion

The primary objective of this study was to test whether the use of digital image analysis for ground cover could be used in plant breeding to quantify the early growth of forage grass species in a fast and precise way. Image analysis proved to determine the ground cover by plants precisely: replicated measurements were close, leading to small standard deviations and significant differences between varieties. In the greenhouse trial under controlled light ground cover determinations were reproducible, but in the field trials on the other hand, the reproducibility was lower due to changing light conditions, as a consequence new threshold values were needed every time pictures were taken.

Both with the artificial light as with the natural light, some misclassification of soil pixels as plant pixels and *vice versa* was inevitable, but when comparing a series of digital images, this systematic error is not expected to affect the classifications when comparing different varieties/genotypes.

Provided processing the images was done in the HSB space, suitable threshold values both for the fastest as well as the slowest growing varieties were easily found within the same batch of images. Earlier attempts by us to use image analysis for ground cover prediction in the RGB (Red Green Blue) space gave unsatisfactory results as the threshold values were less stable (Verhelst, 2011). Purcell (2000), Richardson *et al.* (2001) and Lock *et al.* (2004) also worked in the HSB space. Behrens and Diepenbrock (2004) on the other hand worked successfully in the RGB space.

Significant differences were found both between species and between varieties within species in three different experimental settings. In the greenhouse experiment, the effects of plant density, weed presence and soil heterogeneity on ground cover were eliminated, but in the field experiments plant distribution was not as uniform as in the greenhouse trial. In field experiment II, the emergence of the seedlings was less homogenous compared to field experiment I. To overcome the effect of the field heterogeneity, images that covered a larger area were taken. The larger the area covered by a picture however, the more difficult it was to find threshold values to distinguish soil and plant material with high precision. A compromise between threshold precision and homogeneity of the photographed area has to be found.

Clear differences in ground cover occurred only in a restricted time interval during the development of the swards. Maximal differences in ground cover generally occurred when the ground cover of the fastest developing canopies was 70 % - 80 %. Differences decreased in later stages since newly formed leaves in the fastest developing canopies started overlapping with the existing leaves.

During early growth a good correlation between DMY and ground cover was found in field experiment II. Later on, correlation between DMY and ground cover disappeared, owing to differences in growth habitus of the different species/varieties. During early growth, plants are principally expanding in two dimensions. In later stages, plants also expand in height, and this dimension is not taken into account with digital image analysis for ground cover. In the greenhouse experiment the erect growing tall fescue variety 8 was among the highest yielding varieties but had only an average ground cover. The prostrate growing Lp2x combined a good ground cover with a high DMY. Digital image analysis techniques that take a three dimensional view of the growing plants might give a better idea of the biomass production. Biskup *et al.* (2007) developed an approach that is suited for three dimensional mapping of natural plant canopies in the field using two synchronised cameras.

We can conclude that the digital image analysis has limited application to asses emergence rate and seedling vigour of forage crops/grasses.

The technique may work well in greenhouse conditions to quantify differences of large numbers of breeding families or varieties of grass species provided the emergence is uniform. Indeed, using pre-germinated seed to install uniform mini-swards is too labour intensive to be used with huge amounts of breeding material. The highest discriminating power was observed under high light intensities (summer trials).

In field conditions there were more limitations: pictures had to be taken with indirect sunlight, and threshold values for the concerned parameters had to be searched according to the natural light conditions. Although it was possible to prevent the contributions of small dicot weeds (1-2 leaf stage) to the ground cover, this was no longer possible in later stages when the weeds and the crop started overlapping. The ground cover corresponding with the best discrimination between the species or varieties differed between the trials.

Both in the field as in the greenhouse trial, the relationship between DMY and ground cover was not consistent. As ground cover is a two dimensional parameter and yield reflects a three dimensional parameter, a technique using three dimensional mapping seems inevitable to predict DMY using digital image analysis.

9.5 Conclusion

Regarding our research hypotheses we can conclude that:

1. Both in a greenhouse trial as in a field trial, the method of digital image analysis proved to be able to determine ground cover in a repeatable and precise way. Applying the technique in the field is more cumbersome. The instability of the natural light, the presence of weeds and the heterogeneous seedling distribution under field conditions, limit the use of the method in practical breeding conditions.
2. An acceptable correlation between ground cover and DMY may be found up to a ground cover of 50 %. In later developmental stages, the correlation was lost due to differences in growth habitat of different cultivars/species. A three dimensional method seems inevitable to predict DMY with digital image analysis.

Chapter 10

The breeding of FEMELLE, the first Belgian tall fescue variety

10.1 Introduction

‘Femelle’ or the ‘Festuca from Melle’ might become the first (Belgian) tall fescue (*Festuca arundinacea*) variety on the national list. In 2008, a tall fescue breeding programme was started by the department of plant production of Ghent university at the experimental farm in Melle. ‘Femelle’ is the first synthetic variety resulting from this breeding programme that was applied for inclusion on the national list. This chapter explains how ‘Femelle’ was bred.

10.1.1 Breeding methods in forage grasses

Grasses of the *Poacea* family are mostly cross pollinating crops. Pollinisation is mediated by wind, and gamethophitic self incompatibility prevents self fertilization. Hence breeding of grasses follows the principles of open pollinated varieties: recombination of selected plants in order to increase the frequency of favourable genes in the population. This is a cyclic process (recurrent selection): after each generation of selection, gene frequencies are changing (Posselt, 2010). Breeding programmes differ in the way in which the plants that form the next generation are selected (Sleper and Poehlman, 2006), basically two groups of methods can be distinguished. In phenotypic selection, selection is performed on individual plants or clones. In genotypic selection, the performance of the progeny, generally half-sib families, determines which individuals are selected. Half-sib families can be obtained in several ways, but polycross and topcross mating are the most common. Both types of mating systems, assume a homogenous, common pollen cloud to pollinate the plants that are tested. In the topcross test, pollen comes from a common unrelated pollinator whereas in the polycross test the plants to be tested are planted in such a way that preferential mating between the tested plants is minimized. A perquisite for polycross testing is the possibility of vegetative propagation. An advantage of the phenotypic selection method is that the selection intensity is very high and that the method allows making the fastest possible progress. For traits with a low heritability however, progress is low if no progeny testing is performed (Posselt, 2010).

There are some particularities in forage grass breeding. Firstly, most forage grass species are perennial, thus plants can be maintained in a nursery while progeny testing is taking place. If progeny testing indicates that the progeny of a certain plant or combination of plants is very good, the parent plant(s) can be traced back in the nursery and be used in the next breeding cycle or to create a new variety. In addition, grass plants can be easily propagated vegetatively, which has multiple advantages (see lower).

Secondly, most forage grasses are ‘undomesticated’ crops: there are no genetic barriers nor is there a clear morphologic difference between the cultivated individuals and the wild material. This implies that every wild forage plant is potentially useful in breeding.

10.1.2 Genetic resources

Plant genetic resources (PGR) are the basis of every breeding programme. According to Boller and Greene (2010) there are three basic categories in forage crops breeding programmes:

1. Wild relatives: in forage grasses cultivated varieties are still very close to wild populations, hence wild material is a major source for genetic improvement.
2. Semi-natural populations: Grassland mostly exists only as a consequence of human activity in zones where forest would be the natural vegetation. Adapted native grasses originating from non-agricultural habitats settled in permanent grassland together with naturalized populations of the same species which may have spread from initial seeding. Such populations form a continuum of wild populations in non-agricultural habitats to populations of natural and semi-natural grasslands.
3. Landraces: Populations which have adapted to a specific region or location, such as a farm, by repeated harvest and human mediated reseeding in the same region or location.
4. Varieties: Any cultivated variety, whether freely available on the market, protected by plant breeder’s rights or having become obsolete and stored in gene banks can be used in breeding without any restriction. The right to freely use even protected varieties as PGR in breeding is called “breeder’s exemption” (UPOV, 1991).

The distinction between the categories 1 and 2 is not always clear. Therefore, we will call populations of both categories “**ecotypes**”: populations which have adapted to a known environment after many years of natural selection, usually involving natural reseeding but without deliberate human intervention such as selection, seed harvest or human-mediated seeding (Boller and Greene, 2010).

10.1.3 Breeding objectives in tall fescue

As indicated in **chapter 2**, the major disadvantage of tall fescue is the low voluntary animal intake and the low digestibility of this species. Progress in animal preference was made by selecting soft leaved genotypes (**Chapter 8**). Leaf softness is an important criterion in tall fescue breeding programmes. In elite material however, the correlation between leaf softness, digestibility and palatability is decreasing (Rognli *et al.*, 2010). Therefore, animal preference trials and digestibility determination of breeding material should be performed to make progress in these traits. There is however few (short term) interest for breeding companies to select strongly for animal preference and digestibility: in official variety testing, these traits are actually of limited or no importance. In addition, these traits are mostly negatively correlated with DMY, which jeopardize the future of varieties bred for improved digestibility/ animal preference as long as digestibility and animal preference trials are not included in value for cultivation and use (VCU) testing.

Although yield of Fa is generally higher than that of other cool season grasses (**Chapter 2**), yield remains an important selection criterion as a higher yield is a *conditio sine qua non* to pass value for cultivation and use testing. Biomass yield is most appropriately measured on sward plots, as the correlation between biomass yield measured or estimated in a spaced plants nursery and the yield measured in sward plots is low due to the absence of competition between the plants in spaced plant nurseries. (Casler and van Santen, 2010).

If a high DMY for new varieties is a prerequisite to be accepted on national lists, high seed yield is necessary for the commercial success of a new variety. Also for seed yield, phenotypic selection in spaced plants trials is of limited value to predict seed yield in drilled plots (Boelt and Studer, 2010).

Compared to perennial ryegrass, Fa is heading early (end April - mid-May). As soon as heading starts, quality of the grass falls dramatically (Minson, 1964). Late heading varieties offer the farmer more flexibility for grazing or cutting before quality decreases. In France, the emphasis was put on breeding Fa varieties with a higher “souplesse d’exploitation”, which is the period between the growing start and heading (Gillet, 1975). Early heading varieties on the other hand have a more even distribution of dry matter production, resulting eventually in a higher annual yield (Cassler and van Santen, 2010).

Bacterial wilt (*Xanthomonas translucens pv. graminis*) and crown rust (*Pucinnia coronata*) are the main diseases encountered in Fa grown in North-West Europe. Whereas rust leads to yield and quality losses of the grass sward in a restricted period of the growing season (mostly at the end of the summer), bacterial wilt infections can kill entire plants, leading in some cases to important yield losses. Where phenotypic selection in spaced plant nurseries for increased yield failed, important improvements in disease resistance were achieved with spaced plant selection for rust resistance in Lp. For a durable resistance, selection and observations in several locations and for several years are necessary (Reheul and Ghesquière, 1996; Schübinger *et al.*, 2011). Also for bacterial wilt resistance, phenotypic selection of artificially infected plants lead to improved resistance in *Lolium multiflorum*. Advance in resistance however stagnated after a few selection cycles, and even in advanced breeding populations, susceptible plants were found (Wichmann *et al.*, 2011).

10.2 Breeding of 'Femelle'

10.2.1 Genetic rescources

In 2008, we collected the plant genetic resources for the breeding programme. Throughout the summer of 2008, we collected seeds from *Festuca arundinacea* ecotype populations *in situ* in Belgium and different neighbouring countries. Part of the populations were scouted in the spring of 2008 on locations were we expected to find Fa. In June, July and August, we harvested seeds in these scouted and in newly found populations.

Within populations we only took seed of healthy vigorous plants. The number of individuals sampled in a population depended on how large and how diverse the populations was. Mature seeds were collected by cutting *circa* 10 ears per plant and by bagging the ears in a paper bag per plant. Each bag was given a unique identification number. The bags containing the ears were dried in a greenhouse, treshed and cleaned. Cleaned seeds were conserved in a separate bag for each sampled plant.

627 plants were sampled in July and August 2008 from 52 Belgian, 17 Dutch, 9 French, 5 German and 1 Spanish populations, resulting in an average of 7.5 sampled plants per sampled population. The number of sampled plants per population varied between 34 and 1. The habitats is which we found Fa were in order of importance: road verges, canal verges, fallow land and extensively managed grasslands. In addition, 48 ecotypes from different European countries were obtained from the IBERS gene bank (Aberystwyth University, Aberystwyth, UK), 28 ecotypes from Germany and Poland were obtained from Leibniz Institute of Plant Genetics and Crop Plant Research (IPK, Gatersleben, Germany), resulting in a total of 703 ecotypes. In addition we had seeds of the French varieties 'Bariane', 'Barolex', 'Dulcia', 'Elodie', 'Aprillia', 'Carmine' and 'Ondine'.

In August 2008, 20 seeds of each ecotype and 150 seeds of each variety were planted in trays (QP 150T, Quickpot, Ering am Inn, Germany) filled with potting substrate (Aura, Peltracom, Ghent, Belgium) and placed on an irrigated container field. On 17 March 2009, these plants were planted in a field nursery. Nine well developed plants of each ecotype were planted in a row. Ten rows of nine plants of each of the varieties were planted. 33 gene bank ecotypes had a very low or zero germination and were not planted, resulting in a nursery with 6030 ecotype plants and 630 plants from varieties. Space within the rows and between the rows was 50 cm. Just before planting, the nursery was oversown with an amenity type of *Lolium perenne* L. *cv.* 'Palmer' at a density of 30 kg ha^{-1}. This was done for two reasons. Firstly this prevented weeds between the Fa plants and secondly we conceived this as an indirect selection method for plant with a good early vigour and a good competitive ability (**Photograph 10.1**). N fertilization in the nursery was low (100 kg N ha^{-1}) in order to make the plants more susceptible to crown rust.

Photograph 10.1 Ecotypes of *Festuca arundinacea* in a sward of an amenity type of *Lolium perenne*. Picture taken in August 2009.

10.2.2 Selection

Throughout the summer of 2009, we scored families for vigour (plant biomass). In September 2009, we selected within the most vigorous families, the plants that were absolutely free of crown rust. 425 plants were selected from which 307 originated from ecotypes and 118 from varieties.

In October 2009, the selected plants (called ‘clones’ hereafter) were dug out and were propagated vegetatively and planted in a clonal nursery. A row of nine ramets of at least 3-5 tillers was planted per clone with a distance of 50 cm between and within the rows. One month after planting, the field was treated with herbicides (1000 g ha^{-1} ethofumesate + 75 g ha^{-1}isoxaben) to prevent weeds.

Throughout the year 2010, the clones we scored for vigour (on 4 occasions), leaf softness (on 3 occasions), leaf colour (on 2 occasions) and rust resistance (on 2 occasions). We took note of the heading dates in the spring and of re-heading and *Xanthomonas* infection in case of occurrence in summer. Fertilisation in 2010 was 200 kg N ha^{-1}, 123 kg K ha^{-1} and 36 kg P ha^{-1}. No more fertilisation was applied after the 15th of July, in order to make the clones more susceptible to rust infection. A severe natural rust infection took place in August 2010.

In mid-September 2010, 20 clones with excellent rust resistance, soft leaves and good vigour were selected and planted in five polycrosses of 4 clones (called “polycross components” hereafter) each. Each polycross combined plants that were morphologically similar and with similar heading dates. In each polycross, the components originated both from ecotypes and from varieties. The planting design of polycrosses matched a perfect Latin square. Polycrosses were separated 9 m from each other (**Photograph 10.2**). Winterrye was sown around and between the polycrosses in October 2010 to assure isolation of the polycrosses.

Photograph 10.2 Polycross with 4 components, isolated by rye (*Secale cereale*). Picture taken 11th May 2011.

In June 2011, Syn 1 (first generation from the synthetic variety) seeds were harvested. Seeds were dried, threshed and cleaned in July 2011. Seed weight was determined by weighing 2 samples of 500 seeds per seed lot, and germination of each seed lot was determined following the ISTA protocol (ISTA, 1993) (**Table 10.2**). Important differences were found in seed yield per plant: between 3 g per plant for PC4_2 (progeny of second polycross component of PC4) and 19.6 g per plant for PC3_1.

Table 10.2 Seed yield, 1000 seed weight and germination percentage of 20 polycross components harvested in June 2011.

Polycross component	Yield (g plant^{-1})	Seed weight (g 1000 seeds^{-1})	Germination (%)
PC1_1	7.5	3.44	81
PC1_2	14.6	3.58	90
PC1_3	7.0	3.68	85
PC1_4	6.3	2.6	95
PC2_1	11.6	3.24	86
PC2_2	4.6	3.18	81
PC2_3	16.0	2.9	87
PC2_4	4.1	3.16	71
PC3_1	19.6	3.02	95
PC3_2	4.0	2.36	92
PC3_3	10.7	2.96	92
PC3_4	14.8	288	79
PC4_1	14.0	3.42	91
PC4_2	3.0	3.32	75
PC4_3	9.9	3.42	78
PC4_4	8.6	3.18	57
PC5_1	5.5	3.02	99
PC5_2	4.6	3.36	91
PC5_3	12.5	3.32	92
PC5_4	3.4	2.94	87

The harvested seeds were used for progeny testing and for seed multiplication. Progeny testing was performed both on individual plants as in microswards.

For the progeny test of individual plants, 150 seeds of each polycross component were sown in trays (QP 150T, Quickpot, Ering am Inn, Germany) filled with potting substrate (Aura, Peltracom, Ghent, Belgium) and placed on an irrigated container field in the beginning of august 2011. At the end of September 2011, 100 progeny plants of each polycross component were planted in a spaced plants nursery. Four rows of 25 plants were planted per polycross component. Distance between and within the rows was 50 cm.

Progeny testing in swards was not possible for every polycross component, due to the limited amount of seed harvested for some polycross components. We created synthetic varieties (Syn 1) of each polycross by mixing an equal amounts of viable seeds of each of the four components for each polycross (called “PC1, PC2, PC3, PC4, PC5” hereafter). In addition, we created 2 mixtures containing only a restricted number polycross offsprings. The mixture called “PC1_23” and “PC3_14” contained an equal amount of viable seeds of components 2 and 3 of polycross 1 and components 1 and 4 of polycross 3 respectively. Two single polycross component progenies were also included in the trial (PC3_4 and PC4_1). The seed mixtures were sown in a yield trial at the institute for agricultural and fisheries research (ILVO) in Merelbeke, Belgium (See **section 10.2.3**).

As the polycross components in PC1 proved to have good vigour, soft leaves and a good rust resistance, seed multiplication was initiated (See **section 10.2.4**).

10.2.3 Progeny test in swards

In September 2011, a yield trial comparing the DMY of PC1, PC2, PC3, PC4, PC5, PC1_23, PC3_14, PC3_4 and PC4_1 with that of the reference varieties: ‘Barolex’, ‘Callina’ and ‘Otaria’ was established at ILVO. The trial was a split plot design with three replications. N fertilization was the main plot factor (High N or Low N: 300 and 190 kg ha^{-1}yr^{-1} respectively), the twelve varieties and PC progenies were the subplot factors. Five cuts were harvested in 2012. Anova was performed to test the effect of ‘variety’ (the three reference varieties and the polycross progenies) and ‘fertilization’ (high or low) on the DMY. Both the effects of fertilization (p = 0.006) as variety (p < 0.001) were significant, there was no significant interaction. Under high N-fertilisation, the highest yield was obtained with PC3_14, the lowest yield with the variety ‘Otaria’, with yields of 16184 kg DM ha^{-1} and 14297 kg DM ha^{-1} respectively. The yield of all polycross progenies, except PC1_23 was higher than that of the average yield of the reference varieties (14814 kg DM ha^{-1}). Under low N-fertilisation, the highest yield was obtained with PC3, the lowest yield with the variety ‘Otaria’ with yields of 13126 kg DM ha^{-1} and 10495 kg DM ha^{-1} respectively. The yield of all polycross progenies, except PC1_23 was again higher than that of the average yield of the reference varieties (11246 kg DM ha^{-1}).

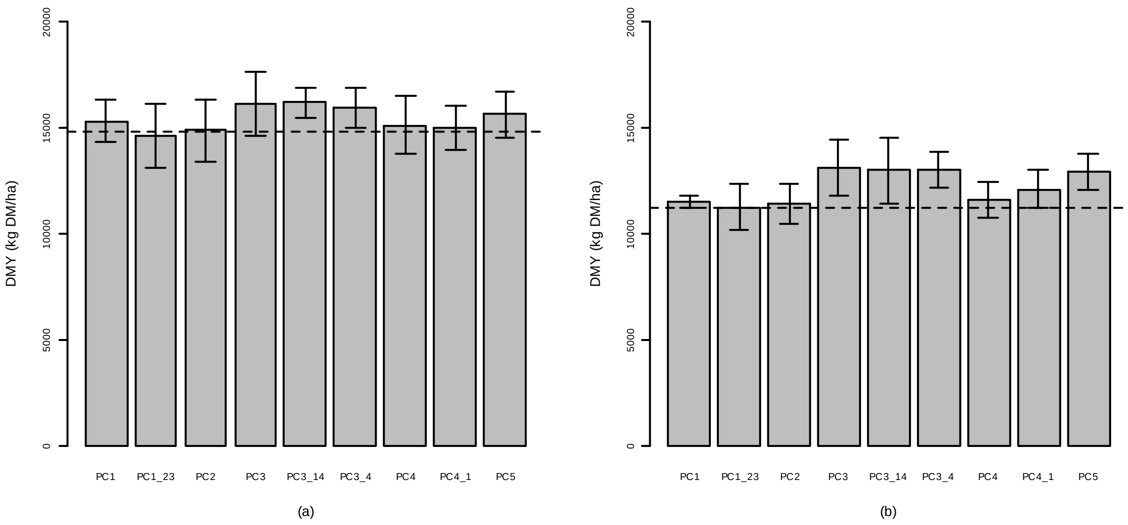


Figure 10.1 Dry matter yield (DMY) of nine polycross progenies over five cuts harvested in 2012 under (a) high fertilization (300 kg N ha^{-1}) or (b) low fertilization (190 kg N ha^{-1}). The dashed line indicates the average yield of the varieties 'Barolex', 'Callina' and 'Otaria'. Error bars: ± standard deviation.

The yield of PC1, the PC with the best rust resistance and the softest leaves was compared in more detail with the reference varieties. The high standard deviations in the first cut were caused by the (heterogeneous) presence of weeds in the plots. In the high N treatment, PC1 was overyielding the reference varieties in the first and fourth cut (**Figure 10.2a**). In the low N treatment, PC1 was overyielding 'Otaria' in every cut. Barolex was overyielding PC1 in every cut except the first (**Figure 10.2b**). Over all cuts PC1 was yielding more than 'Callina' and 'Otaria' but it was overyielded by 'Barolex' both in the high as in the low N treatment. All varieties survived well the winter of 2012-2013.

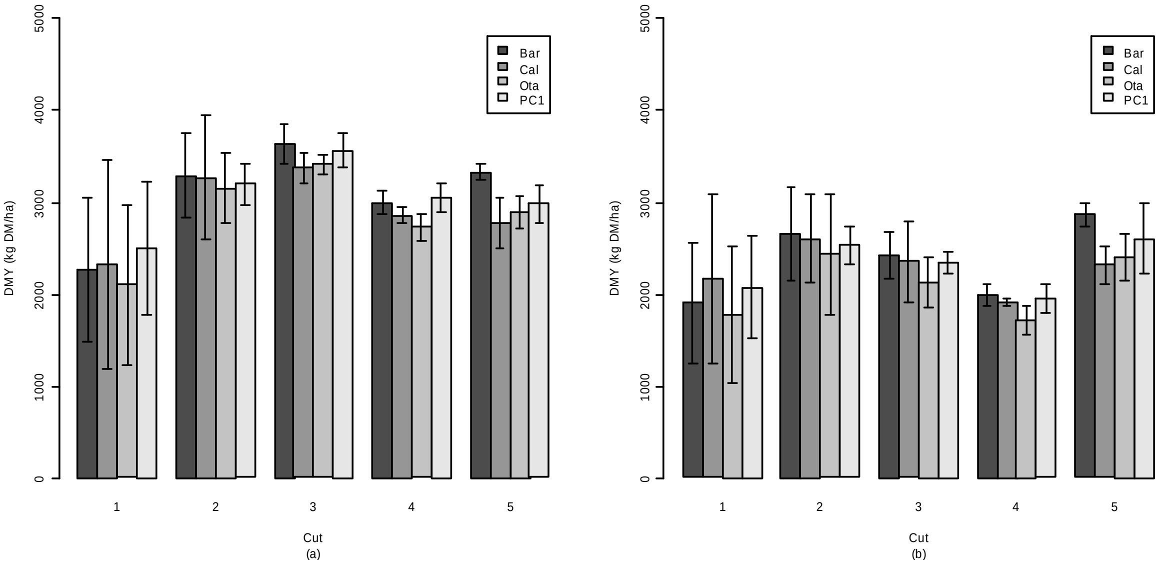


Figure 10.2 Dry matter yield (DMY) of PC1, 'Barolex' (Bar), 'Callina' (Cal) and 'Otaria' (Ota) in five successive cuts harvested in 2012 under (a) high fertilization (300 kg N ha^{-1}) or (b) low fertilization (190 kg N ha^{-1}). Error bars: ± standard deviation.

10.2.4 Seed multiplication PC1

In the beginning of August 2011, seeds of each polycross component were sown in trays (QP 96T, Quickpot, Ering am Inn, Germany) filled with potting substrate (Aura, Peltracom, Ghent, Belgium) and placed on flood tables in a greenhouse. In September 2011, seedlings were planted in the field in a design matching a perfect Latin square. The seed multiplication field was kept free of weeds by hand weeding. In May 2012, a negative selection was applied to discard off types. In July 2012 the seed was harvested in bulk, threshed and cleaned. This seed is the breeder's seed of 'Femelle'. An overview of the breeding process of 'Femelle' is given in **figure 10.3**.

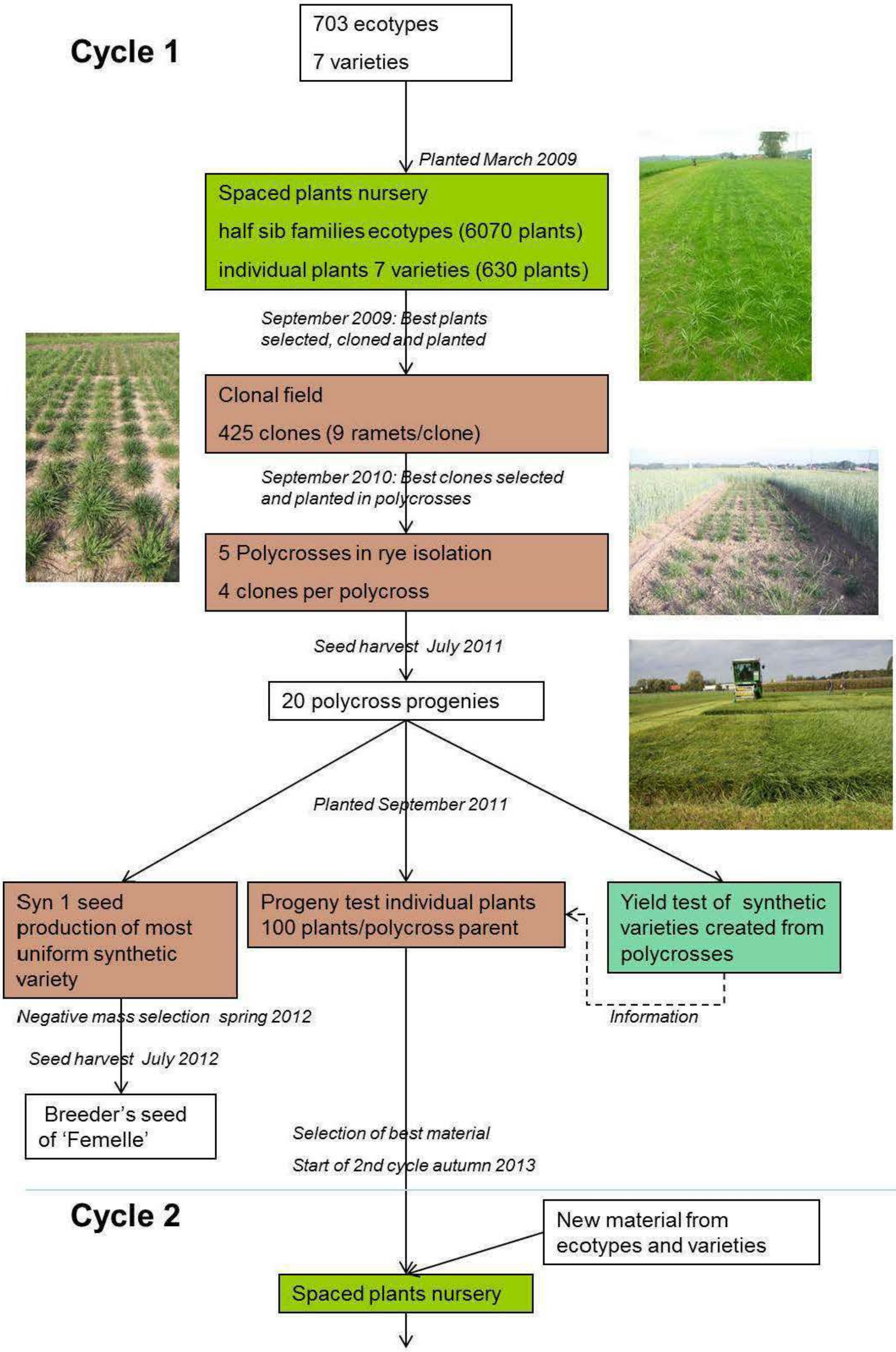


Figure 10.3 Scheme representing the *Festuca arundinacea* breeding programme at the department of plant production of Ghent University. Squares without background colour represent seeds; squares with coloured background represents field trials: Squares with light green background represent spaced plant nurseries undersown with *Lolium perenne*; squares with brown background represent nurseries with naked soil; squares with dark green background colour represent swards.

10.2.5 Further seed multiplication ‘Femelle’

Syn 2 seed production was started in September 2012. Heading dates were recorded in May 2013 (**Figure 10.4**). Off types were removed in due time.

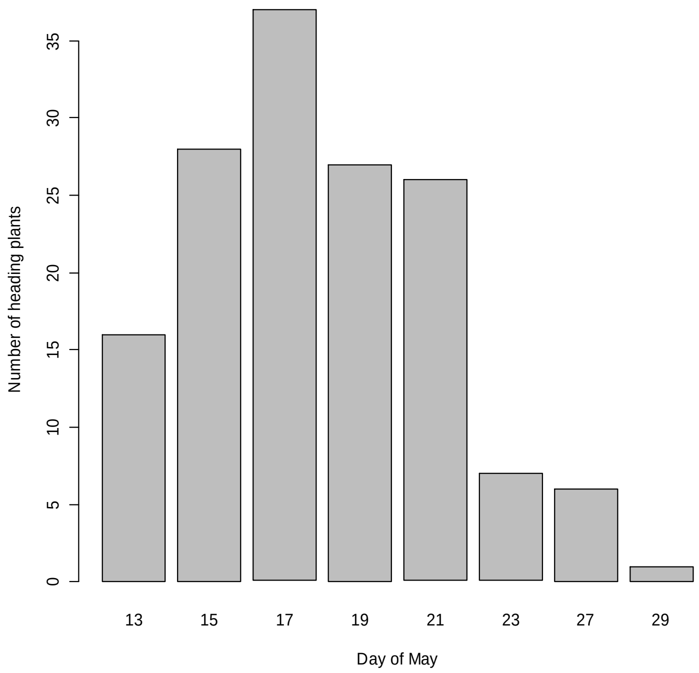


Figure 10.4 Date of inflorescence emergence of 148 ‘Femelle’ plants in Melle in May 2013.

10.3 Application procedure

In the autumn of 2012, the procedures were started to obtain plant breeder’s rights for ‘Femelle’ and to start the value for cultivation and use trials for the Belgian National list (**Appendix 3**).

Chapter 11 Feedback to hypotheses and research questions, discussion and outlook

“La fétuque élevée qui a la plus large adaptation géographique (sécheresse, humidité, chaleur, froid), la plus longue saison de végétation (fin d’hiver, été, arrière saison), ce qui lui donne le plus fort rendement annuel; elle a aussi la plus longue pérennité…Par contre c’est sans doute l’espèce la plus délicate à utiliser: si elle peut être conseillée presque partout, elle ne peut l’être à n’importe qui.” (Jadas-Hécart et Gillet, 1964)

11.1 Feedback to hypotheses and research questions

11.1.1 Hypothesis 1 and research questions 1.1-1.4

Fa is more productive, particularly under drought stress, but has a lower digestibility and animal preference than Lp.

RQ1.1 *How large is the yield difference between Fa and Lp under favourable growing conditions?*
Based on literature research and analysis of (unpublished) results of trials, we found that the yield potential of Fa is on average around 20 % higher compared to Lp under favourable conditions (high N input, no drought). Under very favourable conditions without any limitation in a temperate climate, the yield potential of Lp proved to be as high as that of Fa. In our own trials comparing the yield of Fa and Lp under a cutting regime representative for Belgian agricultural practise (**Chapter 3**), we found yield advantages of 13 %, 22 %, 35 % for Fa compared to Lp in 2010, 2011 and 2012 respectively.

RQ1.2 *How large is the yield difference between Fa and Lp under drought conditions?*
Based on literature research and analysis of (unpublished) results of trials, we found that the yield advantage of Fa compared to Lp was higher under drought conditions compared to favourable conditions. The magnitude of this yield advantage depended on the severity of the drought, but up to 65 % higher yields per year were found for Fa compared to Lp. In our trials (**chapter 3**), we found yields that were up to 2.5 times higher for Fa compared to Lp in cuts preceded by drought.

RQ1.3 *How large is the initial yield loss due to the slow establishment of Fa compared to Lp?*
Based on (unpublished) results from Belgian trials we found, up to10 % lower yields for Fa compared to Lp in the first production year after September sowing. After April sowing, the difference is expected to be even larger. The results of the trial presented in **chapter 4** suggest that the low yield of Fa in the first cuts after sowing can be tackled by sowing Fa in a mixture with a low proportion of Lm seeds. The Lm in the mixture acts as a cover crop for Fa: it provides a high yield in the first cuts, but gradually it disappears leaving a pure Fa sward. The substitution of 1/8 Fa seeds by Lm seeds was sufficient to obtain the desired effect.

RQ1.4 *How large is the difference in digestibility and intake between Fa and Lp?*
Based on literature research and analysis of (unpublished) results of trials, we found that the digestibility of the organic matter (DOM) of Fa was generally lower compared to Lp. Up to

20 % points lower DOM was found for Fa compared to Lp in individual cuts. Annual mean DOM was found to be 5-10 % points lower for Fa compared to Lp. In our own trials (**chapter 3**) the annual mean digestibility of Fa was between 6 % and 8 % points lower compared to Lp2. Especially in the first cuts, differences between Fa and Lp2 were high: we found up to 18 % points lower digestibility for Fa compared to Lp. Taking the first cut of Fa earlier in the season will produce more digestible forage and is likely to improve animal intake. According to literature references, the intake of cut Fa compared to Lp was approximately 5-10 % lower.

Conclusion: H1 is accepted: Fa is more productive than Lp (except in the first production year) but it has a lower digestibility and lower animal preference than Lp. Therefore, improving digestibility and animal preference should be the main goals in breeding programmes with Fa.

11.1.2 Hypothesis 2 and research questions 2.1 - 2.8

There is transgressive overyielding with mixtures of Fa and Lp or Fa and Lm: mixtures can yield more than the monospecific swards of these species.

RQ2.1 *How large is the yield gain due to transgressive overyielding in mixtures of Fa and Lp or Lm?*

No significant transgressive overyielding was found in swards sown with mixtures of Fa and Lp nor in mixtures of Fa and Lm. The yield of swards sown with mixtures of Fa and Lp was mostly between that of monospecific swards of Fa and Lp: a linear relationship was found between the yield of the species mixtures and the Fa content in the species mixtures indicating the absence of a positive interaction between both species. Even without a transgressive overyielding of mixed swards, there was some interest in mixing both species. In dry periods, the Fa in the mixtures compensated the low yield of the Lp component resulting in a yield of the mixed swards that was close to that of the pure Fa swards, whereas under favourable conditions the presence of the Lp increased the digestibility of the mixed swards compared to that of pure Fa swards.

Swards sown with mixtures of Fa and Lm had a higher yield than monospecific Fa and Lm swards, but the yield differences were never significant. Especially in the first production year, when the yield of Fa swards was low owing to its slow establishment, mixtures were advantageous. Swards sown with mixtures of Fa and Lm combined the high yield of Lm in the first cuts after sowing and the good yield potential of Fa in the late summer and autumn.

Mixing Fa with (a low proportion of Lm) can be used as a strategy to tackle the slow early establishment of Fa.

RQ2.2 *Which proportion of Fa and Lm or Lp in the mixtures is leading to the highest transgressive overyielding?*

As indicated in **RQ1.1**, no significant transgressive overyielding was found in swards sown with mixtures of Fa and Lp or Lm. Both in the swards sown with mixtures of Fa and Lp as well in mixtures with Fa and Lm, the Fa content in the DMY in the first year was much lower than the sown proportion. Although the Fa content in all mixtures was increasing steadily over time, the effect of the Fa proportion in the seed mixture on the Fa content in the harvested grass continued to be significant after three growing seasons.

In the mixtures with Fa and Lp, all mixtures were dominated by Fa from the second year on. In the third year of the trial, the content of Fa was above 80 % in the mixtures with 1/8 of Lp in the seed mixture and between 60 % and 80 % in the mixtures with 1/4 of Lp. The results suggest that a Lp proportion higher than 1/4 is recommended to obtain an evenly distributed sward in the mid-long term. A more exact proportion however can not be recommended based on the results of this study.

A proportion of 1/8 Lm in the seed mixture, proved to be sufficient to obtain a yield close to that of a pure Lm sward in the first cuts after sward establishment and evolved to a pure Fa sward in the third growing season. Swards sown with mixtures containing 1/4 Lm, continued to be dominated by Lm in the first cuts of the third growing season. Hence we conclude that mixtures between Fa and Lm should not contain more than 1/8 Lm if the final goal of sowing mixtures is to obtain eventually a uniform sward of Fa.

The effect the Fa variety on the botanical composition, yield and quality of the mixtures can not be estimated from the studies presented in Chapters 3 and 4. The limited effect of the ryegrass varieties included in the studies however suggest that no huge varietal Fa effect is to be expected.

RQ2.3 *What is the effect of the ploidy of the ryegrass component in the mixtures under cutting management?*

In contrast to what we expected, Lp4 competed better with Fa compared to Lp2. Also in the swards sown with mixtures of Fa and Lm, Lm4 suppressed Fa more compared to the Lm2, particularly in the first three cuts of every year. In later cuts, the FaC was increasing steeper in mixtures with Lm4 compared to Lm2, resulting in comparable FaC in the later cuts.

RQ2.4 *How large is the difference in feed quality of the mixtures compared to the monospecific swards?*

The CPC and DOM of the grass species mixtures was intermediate to that of the monospecific swards. In the Fa and Lp mixtures, the effect of the FaC in the mixtures on the weighted average annual CPC and DOM was linear. The effect of the FaC on the CPC was linear in mixtures between Fa and Lm too. For DOM the relation between the DOM of the mixtures and the FaC was convex in some years due to the seasonal pattern in DOM and in FaC: low digestibility of Fa in combination with high FaC in the first cut and smaller digestibility difference between Fa and Lm in combination with low FaC in later cuts.

RQ2.5 *Does adding clover to the mixtures alter the answer of RQ2.1, RQ2.2, RQ2.3 and RQ2.4?*

Basically the answer is no: the interaction of the two grass species was similar with or without clover, both in the mixtures of Fa and Lp as in the mixtures of Fa and Lm. In the mixtures of Fa and Lm with red clover, the competition of both Lm and Tp was too strong for Fa to establish well after three years. In accordance with other studies, adding clover, allowed to obtain higher DMY, CPC and NY with a lower N input compared to the same sward compositions without clover. The presence of clover in the mixtures influenced the DOM of the harvested material positively.

RQ2.6 *How large is the yield gain owing to transgressive overyielding in mixtures of Fa and Lp under grazing management?*

No transgressive overyielding was found in swards sown with mixtures of Fa and Lp in association with white clover under a management of mowing the first cut followed by grazing. The yield of the mixtures was intermediate to that of single grass species in association with white clover.

RQ2.7 *Which proportion of Fa and Lp in the mixtures is leading to the highest transgressive overyielding under grazing management?*

As indicated in **RQ 2.6**, no transgressive overyielding was found in swards sown with mixtures of Fa and Lp. The effect of the initial proportion of Fa in the seed mixture on the FaC in the harvested forage continued to be significant in the third year after sowing. Seed mixtures of Fa and Lp containing 1/4 of Lp seeds led to swards in which Lp and Fa were equally contributing to the DMY three years after sowing.

RQ2.8 *What is the effect of the ploidy of the ryegrass component in the mixtures under grazing management?*

Contrary to what we expected, the Fa content in the swards sown with seed mixtures of Fa and Lp4 was not significantly higher compared to swards sown with seed mixtures of Fa and Lp2.

Conclusion: H2 is rejected: We found no transgressive overyielding in swards sown with mixtures of Fa and Lp with or without white clover or sown with mixtures of Fa and Lm with or without red clover.

11.1.3 Hypothesis 3

Weed invasion is lower in Fa compared to Lp2 or Lp4, owing to the better persistence of Fa.

The weed abundance and the weed content in the dry matter yield in grazed Fa swards was generally lower compared to Lp swards but these differences were not significant.

Conclusion: H3 is rejected: We found no significant lower weed content nor weed abundance in Fa swards compared to Lp swards.

11.1.4 Hypothesis 4 and research questions 4.1 - 4.2

NIRS calibration can be used to develop an equation to predict the botanical composition of mixtures of Fa + Lp with white clover.

RQ4.1 *Which calibration strategy leads to the smallest prediction error?*

A calibration strategy based on hand sorted samples that contains as much environmental variation as possible gave the best results. Calibration strategies for the determination of the botanical composition of forage samples based on artificial samples (like in Coleman *et al.*, 1985) failed to predict the composition of real hand sorted validation samples. We found that the failure of equations based on artificial samples was due to a lack of spectral variation, originating from factors excluding the botanical composition, in the artificial samples. Alternatively, a strategy in which relevant spectral variation is added to spectra of artificial samples produced good results.

RQ4.2 *How large is the prediction error when the best calibration strategy found in RQ3.1 is applied to the samples of the trials described in chapter 3?*

The calibration strategy based on hand sorted samples was applied to the forage samples from our trials with the mixtures of Fa and Lp with white clover. We used 1/3 of the hand sorted samples from the 15 cuts for calibration. The resulting equation was validated with the remaining 2/3 of the samples. The Tr content in the DMY of the remaining samples was predicted with an RMSEP of 5 %. For Fa and Lp content, the prediction was unsatisfactory: RMSEP of 8 % and 10 % respectively were found.

Conclusion: H4 is accepted: NIRS calibration can be used to predict the botanical composition of mixtures of Fa and Lp with white clover given a calibration strategy based on diverse hand sorted samples is used.

11.1.5 Hypothesis 5 and research questions 5.1 - 5.2

Fa has a higher root biomass than Lp below 30 cm but the difference is season and soil dependent.

RQ 5.1 *How large is the effect of location (soil, management and fertilisation)?*

When measured in autumn or late summer, root biomass below 30 cm was consistently higher for Fa compared to Lp. Depending on the location, Fa root biomass was between 1.5 and 4.2 times higher than Lp root biomass. The largest differences between Fa and Lp in root biomass below 30 cm were found on locations with deep, fertile sandy loam or loam soils under cutting management. The smallest difference between Fa and Lp was found on a location with sandy soils under cutting management. Averaged over all the sampled locations, root biomass below 30 cm represented 15 % and 8 % of the total root biomass for Fa and Lp respectively.

RQ 5.2 *How large is the effect of sampling season (spring or autumn)?*

Root biomass below 30 cm was significantly higher in autumn or late summer compared to spring. Depending on the location, root biomass below 30 cm was up to 3.0 times higher in autumn compared to spring for Fa, and up to 1.7 times higher in autumn compared to spring for Lp.

Conclusion: H5 is accepted: Fa has a higher root biomass compared to Lp below 30 cm, but location and sampling season are influencing the magnitude of the difference between both species. The difference in root biomass below 30 cm was smaller on the location with a sandy soil. The higher root biomass of Fa below 30 cm can therefore be seen as a factor that can explain the better drought resistance of Fa compared to Lp.

11.1.6 Hypothesis 6 and research questions 6.1 - 6.3

Ruminant preference of Fa genotypes can be predicted without using ruminants.

RQ6.1 *How is the correlation between sheep preference and leaf morphological parameters?* Sheep preference was generally negatively correlated with leaf blade length and leaf blade shear strength, but the relation was weak due to the presence of genotypes with a preference that was the inverse from what was expected from leaf morphological parameters. There was no significant relationship between sheep preference and leaf width and shear force. Although there was a good relationship between the leaf morphological parameters of tall fescue and animal preference in different studies (for example in Macadam and Mayland, 2003), this was not the case in our study.

RQ6.2 *How is the correlation between sheep preference and sward characteristics?* Sheep preference was negatively correlated with leaf harshness, but also for this parameter genotypes occurred for which the preference was the inverse from what we expected from the leaf harshness.

Sheep preference was consistently negatively correlated with pre-grazing sward height. Pre-grazing sward height and leaf blade length were not correlated, hence pre-grazing sward height gave information on plant habitus. Erect growing genotypes with a high pre-grazing height were disliked; prostrate growing genotypes with flexible leaves were liked. No significant effect of digestibility and leaf colour was found on sheep preference.

RQ6.3 *How is the correlation between sheep preference and rabbit preference?* Rabbit preference correlated well with sheep preference in our study. This is an important finding: in the early stages of breeding programmes, when plant material is scarce, rabbits can be used as a proxy for ruminant preference.

Conclusion: H6 is rejected: Based on the parameters we measured it was not possible to predict sheep preference in a reliable way using single parameters. We found that pre-grazing sward height, leaf harshness and rabbit preference were the parameters with the highest correlation with sheep preference. We conclude that the sheep in our trial preferred genotypes with rather short or long leaves with flexible (non-erect) leave blades that produce short swards. With the knowledge we have now, the best method of determining animal preference is by monitoring grazing activity, but the logistics of doing this in a breeding programme is severely limited. This is because workloads preclude use at the early stages of a selection programme when numbers of breeding lines are very high.

11.1.7 Hypothesis 7 and research questions 7.1 - 7.2

Digital image analysis can be used to quantify ground cover and early vigour in a repeatable way in the field.

RQ7.1 *Does the method allow detecting of significant differences in ground cover between grass species or grass species varieties?*

Significant differences were found in ground cover both between species and between varieties within species in three different experimental settings.

RQ7.3 *In which developmental stage differences between the varieties are maximal?* Depending on the trial conditions, maximal differences in ground cover generally occurred when the ground cover of the fastest developing varieties was 70 % - 80 %. In later stages differences decreased since newly formed leaves in the fastest developing canopies started overlapping with the existing leaves.

Conclusion: H7 is accepted: Digital image analysis can be used to determine ground cover of developing grass swards in a repeatable way. The method however was cumbersome in the field: the right weather circumstances (no rain, no direct sunlight) were needed for repeatable measurements and the presence of weeds in the plots biased the results.

11.1.8 Hypothesis 8

There is a good correlation between ground cover measured by digital image analysis and biomass production.

Conclusion: H8 is rejected: In the early growth stage (ground cover below 50 %) there was a good correlation between dry matter yield and ground cover. In later stages, the correlation disappeared owing to differences in growth habitus of the different species/varieties. This means that digital image analysis has limited value to select tall fescue genotypes with an improved early vigour. Digital image analysis techniques that take a three dimensional view of the growing plants (like in Biskup *et al.*, 2007) might give a better idea of the biomass production.

11.1.9 Hypothesis 9

Recurrent selection offers opportunities to breed new Fa varieties with soft leaves and a good rust resistance under Belgian conditions.

Conclusion: H9 in accepted: ‘Femelle’ that was created by selecting and crossing the most rust resistance plants from a genetically very broad tall fescue population, showed to have an excellent rust resistance and clearly has soft leaves. The official VCU testing will give a decisive answer about the potential of ‘Femelle’ in Belgium.

11.2 General discussion and outlook

The research reported in this manuscript focused on the agronomy and breeding of tall fescue in Belgium, highlighting its advantages and weaknesses and quantifing them where possible.

Compared to the ryegrasses, the predominant forage grass species in Belgium, tall fescue lacks an early vigour and has a substantially lower digestibility; animals prefer ryegrasses over tall fescue, particularly under a grazing management. Tall fescue out yields perennial ryegrasses with about 20% in Belgium, is much more productive during and after a period of drought and takes up more nitrogen (Chapter 3). It does not out yield Italian ryegrass, but is more persistent and has a higher tiller density (Chapter 4).

The main farming reason for sowing tall fescue is the better persistence and drought tolerance compared to Italian ryegrass when used in temporary grassland. Farming reasons for sowing tall fescue in long lasting pastures are the substantially higher yield, summer yield, drought tolerance and the better nitrogen use efficiency compared to perennial ryegrass.

The weaker animal preference is not an important weakness when used in temporary grassland as this grassland is mainly used under a cutting management: literature mentions that differences in animal intake are less pronounced with wilted and conserved grass. The lower digestibility of the tall fescue does however jeopardize animal performances. I comment on this further on.

In order to limit the effects of the lower feed quality, one can mix perennial ryegrass with tall fescue, provided that one can succeed in creating swards that are not dominated by one of the two species. I hypothesized that a low proportion of perennial ryegrass in the seed mixture would offer opportunities to end with a botanically balanced sward. Due to the weak early vigour mixed swards were initially dominated by perennial ryegrass. However the proportion of tall fescue in the DM yield increased with time and in order to prevent the swards becoming dominated by perennial ryegrass, the proportion of perennial ryegrass seeds in the seed mixtures should not be above 25 %. Acting like this, tall fescue dominated the first cut owing to its earlier development in the spring. Its abundance decreased after the first cut in favourable growing conditions to increase again during summer and autumn. This means that the composition of the forage in these bispecies swards changes during the growing season, which is a disadvantage under grazing. Indeed changes in the botanical composition may increase fluctuations in milk production and quality accros the grazing season.

Studying more mixture compositions with low shares of the perennial ryegrass component and introducing a range of varieties with presumed additive values may help to find out how to keep these fluctuations under control. As it is expected that species mixtures always will continue to evolve owing to environmental effects, an equivalent consistency in sward quality compared to monocultures, is probably unattainable. On the other hand, the disadvantage of fluctuating quality, can to some extent, be compensated for by greater constancy in sward performance when periodic acute drought stresses can be expected.

Furtermore, as the exploitation of grassland is changing quickly in Western Europe, with an increased share of zero grazing, the precise composition of a sward becomes less important since cut grass is fed together with other forages and the weaker animal preference of tall fescue and its lower digestibility can be more easily overcome compared to grazing situations. Tall fescue even may be advantageous, since it can supply more structure in the feeding ration.

Mixing tall fescue with Italian ryegrass has very specific advantages during the establishment and early growth of reseeds. As the persistence of Italian ryegrass is much lower than the persistence of tall fescue, the “Italian” component in the mixture serves as a cover crop to the fescue component. Depending on the particular persistence of the variety of Italian ryegrass, the mixture is believed to evolve quite fast to a pure tall fescue sward. More research is needed to find out which type and which proportion of Italian ryegrass in the mixture should be used to reach a particular endpoint.

In my opinion, the most important question for future research should be: “How to improve digestibility in tall fescue?” When used under grazing voluntary intake is an equally important characteristic to improve. This work indicated that the relationship between both is not straightforward. Moreover the relationship between digestibility and intake is reported to decrease in elite material. Whether the low digestibility and intake of tall fescue is seen as one problem or as two separate problems, does not really matter: success in improving both the intake and the digestibility would really help tall fescue to become a viable alternative for ryegrasses for on-farm use.

The studies reported in this manuscript were not successful in identifying the real reasons for diverging animal preferences. Further research is needed to find out to what extent leaf morphology and the chemical composition are determining factors. Open research questions to be solved are: “What is the influence of the sclerenchyma proportion or silicium content on the harshness and shear strength of leave blades?”, “How does leaf blade geometry affect preference and voluntary intake?”. Such a research should be applied preferentially on phenotypes with a known animal preference, by studying leaf characteristics and grazing behaviour simultaneously. A collection of contrasting phenotypes with a moderate to good preference is a prerequisite to be relevant for farming conditions. Once determining morphological/chemical traits have been identified, their heritabilities should be studied in order to find out how large the selection response might be. Indeed, improving feed quality is mainly a matter of breeding. However a good management can help: our research indicated that the difference in digestibility between perennial ryegrass and tall fescue is the largest in the first cuts. These data have been produced in trials where both perennial ryegrass and tall fescue were cut at the same dates. Since tall fescue is becoming generative much earlier than perennial ryegrass, future comparisons should be harvested in the same developmental stage to allow proper comparison. Translated towards the practice of grassland management this means that by cutting tall fescue earlier than perennial ryegrass, one can harvest better digestible tall fescue forage. The impact of this on the relative yield potential of these species would need to be established as there may be tradeoffs between yield and quality that need to be considered.

In the longer run, breeding new tall fescue varieties with improved digestibility and animal intake is a more sustainable solution: if intake and digestibility come closer to perennial ryegrass the quality in monospecific or mixed swards will fluctuate less.

This brings along an important dilemma for breeders: “Should tall fescue predominantly be bred for cutting or for grazing ?” The natural growth habit of tall fescue makes the species intrinsically more suited for a cutting management and most of the current varieties are well adapted to a cutting management: low tiller density, erect growing and early heading. However, despite the increasing importance of zero grazing, it is believed that having animals out grazing will remain, particularly in beef production in the less intensive production regions. As the phenotypic diversity in tall fescue is very large, breeders should work on both options. Indeed some phenotypes of tall fescue are morphologically hard to distinguish from perennial ryegrass. That is why a breeding population composed of a diverse genetic variability offers realistic opportunities to create new genetic recombinations that can be directed both towards a grazing or towards a cutting management.

Using animals or not in the breeding programme is an old breeders’ dilemma. On top of this question comes the question in what breeding stages animals should be used. Theoretically as early as possible in order to allow an early (negative) selection. As spaced plants in the early stages of the breeding programme usually are present without replicates, results of grazing trials are not very reliable. Tandem selection, with the introduction of animals in the later stages of the breeding process, only can succeed when the heritability of the digestibility is high. As heritabilities in other species like *Lolium* and *Phleum* are not very large, it is not wise to have too high expectations. Nonetheless the large phenotype variability in Fa suggests that there is potential to make improvements.

Unfortunately animal trials remain cumbersome. Therefore there is a need for further research to find good markers or proxies for animal preference and voluntary intake. Small animals such as rabbits may be more manageable "markers" than sheep or cattle. It was demonstrated that rabbit and sheep preferences are positively correlated. Information regarding quantitative information on the magnitude of the correlation between sheep and cattle intake of tall fescue were not found (Chapter 8).

As intake is a multifactorial trait, it will be difficult to find a single trait that can replace the animal. It will be necessary to focus on a combination of traits, provided that these traits can be easily, quickly and effectively measured. The work of Decruyenaere *et al.* (2009) suggests that NIRS calibrations could be developed to predict animal intake of forages. Here is extra work to be done to validate this preliminary evidence.

Finally, Parsons *et al.* (2011) point that it is essential to find out how traits (digestibility and improved animal voluntary intake *in casu*) can be positively selected for during the development of varieties for improved animal performance related characteristics. As grass varieties and particularly mixtures of varieties or species are such a complicated communities with interactions above and under the ground, it is not at all completely clear that the gain found in breeding stages and experiments will be expressed in real farm situations.

As digestibility and animal intake of candidate tall fescue varieties are actually not assessed in VCU testing - at least in Belgium - , there is little (short term) incentive for breeders to select strongly for animal voluntary intake and/or digestibility. Therefore national list trials can offer important incentives to improve the quality of tall fescue. As the species is ± 20 % more productive than perennial ryegrass, it seems erroneous to reward further yield increases and to neglect quality. The available evidence presents a strong case for the improvement of the quality. This could in time lead to tall fescue coming closer to perennial ryegrass in morphology, growth habit and quality. If the robustness and good tolerance to extreme conditions are conserved, tall fescue could continue to offer important advantages.

Appendix 1

Meteorological data

Table A1.1 Rainfall (mm) measured in the Meteorological station of Melle, Belgium during the experimental period.

		Year				
Month	Day	2009	2010	2011	2012	Norm[a]
January	1-10	2.1	3.3	21.2	39.0	
	11-20	24.6	15.1	35.2	27.1	
	21-31	29.3	25.4	12.7	12.5	
	1-31	56	43.8	69.1	78.6	71.0
February	1-10	39.9	13.9	9.0	1.1	
	11-20	14.0	1.1	9.4	23.7	
	21-28	3.0	55.2	23.7	1.3	
	1-28	56.9	70.2	42.1	26.1	56.0
March	1-10	19.4	4.4	0.4	48.6	
	11-20	1.3	24.8	9.3	0.9	
	21-31	49.7	35.7	5.6	0.0	
	1-31	70.4	64.9	15.3	49.5	63.0
April	1-10	6.4	15.0	2.3	20.5	
	11-20	17.3	0.0	2.9	22.0	
	21-30	7.9	1.5	5.7	60.5	
	1-30	31.6	16.5	10.9	103.0	49.0
May	1-10	1.0	10.0	4.3	38.3	
	11-20	34.0	6.8	3.3	14.3	
	21-31	40.3	21.7	25.8	2.6	
	1-31	75.3	38.5	33.4	55.2	59.0
June	1-10	45.1	25.0	26.5	56.3	
	11-20	14.9	3.1	32.8	38.9	
	21-30	5.9	0.0	16.0	20.4	
	1-30	65.9	28.1	75.3	115.6	75.0
July	1-10	20.2	16.2	2.3	75.7	
	11-20	23.2	45.1	98.6	52.7	
	21-31	34.3	11.0	26.8	3.8	
	1-31	77.7	72.3	127.7	132.2	76.0
August	1-10	13.7	40.2	23.0	10.5	
	11-20	7.4	132.4	34.3	0.0	
	21-31	5.0	60.2	41.2	38.6	
	1-31	26.1	232.8	98.5	49.1	80.0
September	1-10	15.9	65.7	51.2	0.3	
	11-20	10.2	14.2	27.0	17.7	
	21-30	1.2	47.8	0.0	44.0	
	1-30	27.3	127.7	78.2	62.0	70.0
October	1-10	71.5	20.1	19.7	46.5	
	11-20	9.6	43.6	13.1	40.0	
	21-31	6.0	25.8	9.5	29.4	
	1-31	87.1	89.5	42.3	115.9	79.0
November	1-10	35.9	35.7	4.0	19.2	
	11-20	27.1	81.3	0.2	8.6	
	21-30	79.2	19.4	4.0	10.3	
	1-30	142.2	136.4	8.2	38.1	78.0
December	1-10	54.1	14.6	26.1	63.8	
	11-20	9.2	25.6	132.2	32.9	
	21-31	37.0	3.3	20.9	107.1	
	1-31	100.3	43.5	179.2	203.8	80.0
Year		816.8	964.2	780.2	1029.1	836.0

[a] Norm: average rainfall in period 1981-2010.

Table A1.2 Average temperature (°C) ((minimal temperature + maximal temperature)/2) measured in the Meteorological station of Melle, Belgium during the experimental period.

		Year				
Month	Day	2009	2010	2011	2012	Norm[a]
January	1-10	-4.0	-3.0	4.0	8.3	
	11-20	3.3	1.6	7.7	4.4	
	21-31	1.8	1.1	1.6	4.2	
	1-31	0.4	0.0	4.3	5.6	3.4
February	1-10	2.3	2.5	6.1	-6.4	
	11-20	2.5	-0.9	5.8	2.1	
	21-28	5.8	6.8	4.4	6.6	
	1-28	3.4	2.5	5.5	0.6	3.8
March	1-10	5.3	1.7	4.0	6.6	
	11-20	7.2	7.7	8.4	8.7	
	21-31	6.4	10.5	8.7	9.9	
	1-31	6.3	6.8	7.1	8.4	6.7
April	1-10	10.7	9.0	13.0	6.8	
	11-20	12.9	8.7	11.5	7.0	
	21-30	10.7	11.5	15.4	10.9	
	1-30	11.5	9.7	13.3	8.2	9.4
May	1-10	12.2	8.9	14.8	12.6	
	11-20	13.8	9.4	14.0	11.6	
	21-31	15.7	14.2	15.1	17.9	
	1-31	14.0	11.0	14.6	14.1	13.3
June	1-10	13.8	16.7	16.6	14.0	
	11-20	15.9	15.1	15.1	15.3	
	21-30	18.0	18.6	18.0	18.2	
	1-30	15.9	16.8	16.6	15.8	15.9
July	1-10	18.5	21.5	16.2	14.5	
	11-20	18.1	20.2	16.2	18.3	
	21-31	18.3	18.0	15.9	16.5	
	1-31	18.3	19.8	16.1	18.3	18.1
August	1-10	18.4	17.2	17.6	17.9	
	11-20	19.1	16.9	18.0	21.1	
	21-31	18.0	17.1	16.7	17.9	
	1-31	18.5	17.1	17.4	18.9	17.9
September	1-10	16.8	14.5	17.2	16.6	
	11-20	15.6	14.5	15.1	14.1	
	21-30	14.6	13.2	16.6	13.2	
	1-30	15.6	14.0	16.3	14.6	14.9
October	1-10	13.4	15.6	15.5	12.0	
	11-20	8.5	8.4	10.5	11.6	
	21-31	11.2	8.3	11.1	9.9	
	1-31	11.0	10.7	12.4	11.1	11.2
November	1-10	8.9	9.9	12.3	8.4	
	11-20	11.1	7.6	5.1	6.2	
	21-30	9.8	1.4	6.8	7.1	
	1-30	9.9	6.3	8.1	7.3	7.0
December	1-10	7.4	-1.8	6.8		
	11-20	-1.1	0.0	5.1		
	21-31	1.9	-0.3	7.6		
	1-31	2.7	-0.7	6.6		4.1
Year		10.6	9.5	11.5		10.5

[a] Norm: average temperature average in period 1981-2010.

Appendix 2

Captions of the photos on cover and between chapters

Chapter 1: Ear of *Festuca arudinacea* in Bahai de La Concha, Donostia, Spain (July 2008).

Chapter 2: Plot of *Lolium perenne* (left) and *Festuca arudinacea* (right) after a drought period in September 2009 in Merelbeke.

Chapter 3: Overview of trials ‘fa09.01’ and ‘fa09.02’ at ILVO in Merelbeke (August 2011).

Chapter 4: Overview of trials ‘fa09.03’ and ‘fa09.04’ at ILVO in Merelbeke (May 2011).

Chapter 5 : Calves grazing a *Lolium perenne* and *Festuca arudinacea* mixture in Melle (May 2013).

Chapter 6: *Festuca arundinacea – Trifolium repens* sward in Melle (June 2013).

Chapter 7: Contrast between root biomass of *Lolium perenne* (Lp) left and *Festuca arundinacea* (Fa) right sampled until 90 cm deep in Merelbeke (October 2012).

Chapter 8: Overview of sheep preference trial with 16 *Festuca arundinacea* clones in Melle, (April 2012).

Chapter 9:Overview of trial comparing cover crops in Kruishoutem (March 2011).

Chapter 10: Seed multiplication field of ‘Femelle’ in Melle (April, 2013).

Chapter 11: Clonal nursery in Melle (April, 2012).

Appendix 3

Plant breeder’s right - VCU trials for ‘Femelle’

economie
FOD Economie, K.M.O., Middenstand en Energie

1000 Brussel, 11.01.2013

E3

Prof Dirk Reheul

Coupure links 653
9000 GENT
België

Betreft : *Uw aanvraag om Belgische Kwekersrecht*

Soort: *Festuca arundinacea* Schreb.
Voorgestelde rasbenaming: FEMELLE
Referentie van de kweker: -
Aanvraag nr.: 90868
Datum: 19/11/2012

uw berichten
25/01/2007
uw kenmerk
-
ons kenmerk
E3.PIIE/FDS/4.1/
813883
bijlagen
- Aanvraag

Geachte,

Ik heb de eer u hierbij één exemplaar van uw aanvraag om een kwekerscertificaat voor voormeld ras te laten geworden.

Deze aanvraag werd ingeschreven in het register der aanvragen onder het nummer en op de datum hierboven vermeld.

Voormeld aanvraagnummer wordt behouden tijdens de ganse behandelingsprocedure van uw aanvraag, tevens dient alle briefwisseling onder vermelding van dit nummer te geschieden.

Hoogachtend,

Françoise De Schutter

Ondernemingnr: 0314.595.348

Contactpersoon: Mevr. Françoise De Schutter, attaché
Algemene Directie Regulering en Organisatie van de Markt
Dienst voor de Intellectuele Eigendom

Koning Albert II-laan 16
1000 Brussel

+ 32 2 277 92 55
+ 32 2 277 52 62

francoise.deschutter@economie.fgov.be
http://economie.fgov.be

.be

economie
FOD Economie, K.M.O., Middenstand en Energie

1000 Brussel, 11.01.2013

E3

Prof Dirk Reheul
Coupure links 653
9000 GENT
België

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Hoogachtend,

De Schutter

Françoise De Schutter

Ondernemingnr.: 0314.595.348

Contactpersoon: Mevr. Françoise De Schutter, attaché
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http://economie.fgov.be

.be

economie

Dienst voor de
Intellectuele Eigendom

Aanvraag volgnummer:	90868
Officiële datum:	19 -11- 2012

AANVRAAG OM EEN KWEKERSCERTIFICAAT

1. Aanvrager(s): *naam, adres, tel, fax, e-mail, ondernemingsnummer* Universiteit Gent Sint-Pietersnieuwstraat 25 9000 Gent, België Nationaliteit(en) : Belgische	**2. Briefwisselingsadres:** *naam, adres, tel, fax, e-mail, ondernemingsnummer* **Prof Dirk Reheul** **Coupure links 653** **9000 Gent** **Tel 09 264 6096** **Fax 09 264 6224** **Email: dirk.reheul@UGent.be** Dit is het adres van de kweker

3. Soort: Festuca arundinacea

4. Voorgestelde benaming (*in blokletters*): FEMELLE
Referentie van de kweker:Fa.ME.V.2010/1

5. De kweker(s) is (zijn) de volgend(e) perso(on)(nen): Prof. Dr. ir. Dirk Reheul en ir. Mathias Cougnon

Geen ander persoon was bij het kweken of vinden betrokken.
Het ras werd overgedragen aan de aanvrager(s) door arbeidsovereenkomst
Het ras werd gekweekt of gevonden in (Staat / Staten): België

6. Vorige aanvragen	Neerlegging (Staat - datum)	Nummer van aanvraag	Toestand	Benaming of referentie van de kweker
Kwekersrecht	Dit is de eerste neerlegging			FEMELLE Fa.ME.V.2010/1
Officiële rassenlijst	geen		Zal worden aangemeld najaar 2012	FEMELLE Fa.ME.V.2010/1

7. ☐ Op de voorrang van de aanvraag neergelegd in (Staat) .... op (datum) .... wordt beroep ingediend.

8. Het ras werd niet voor verkoop aangeboden of verhandeld in België voor de eerste maal op (datum) 13 november 2012
Het ras werd in geen enkele Staat verhandeld of aangeboden voor verkoop

9. De aanvrager(s) machtig(t)(en) de Dienst met de bevoegde overheden van ieder ander UPOV Lidstaat, alle nuttige inlichtingen en materiaal betreffende het ras uit de wisselen, onder voorbehoud van het vrijwaren van de rechten van de kweker.

10. Andere bijgevoegde formulieren en documenten: nihil

Ondergetekende(n) vra(agt)(gen) de bescherming van kwekersrecht op het ras dat hij (zij) aanbied(t)(en). Ondergetekende(n) verkla(art)(ren) dat bij zijn (hun) weten de inlichtingen nodig voor het onderzoek van de aanvraag, gegeven in dit formulier en in de bijlagen, volledig en juist zijn.

Te GENT 13 /11 / 2012.

Handtekening(en), Prof. Dr. Luc MOENS
Vice-Rector

VAK VOORBEHOUDEN AAN DE ADMINISTRATIE

	Nummer	Datum	Uur
Ontvangstbewijs van de betaling van de betrokken rechten		19-11-2012	
Ontvangst van de aanvraag		19-11-2012	
Ontvangst van het geheel van voormelde stukken		19-11-2012	

STEMPEL VAN DE DIENST:

OFFICE DE LA PROPRIETE INTELLECTUELLE BELGIQUE
DIENST VOOR DE INTELLECTUELE EIGENDOM BELGIË

Opmerkingen:

GEVES

Address of the requesting authority:
Agentschap voor Landbouw en Visserij-
Produktkwaliteitsbeheer
Ellips, 4e verdieping
Koning Albert II laan 35, bus 41
1030 BRUSSEL
Belgique

20 130 1 - 1113

UPOV ANSWER TO THE REQUEST FOR EXAMINATION RESULT

Information on the variety :

Common name of taxon :	Tall fescue
Botanical name of taxon :	Festuca arundinacea Schreb.
Breeder's reference :	fa.me.v.2010/1
Variety denomination :	Femelle
Our reference number :	**4051904**
Reference number or requesting authority :	VG/A/013 /00004
Date of application in requesting state :	26/11/2012

The examination of the variety

☐ has already been completed

☐ has been in progress since/for

☐ will be undertaken as from
on the basis of an application or a request already submitted

☒ will be undertaken as from 01/02/2013
on the basis of your request

The examination report

☐ is enclosed. Please make a remittance of

☒ will be forwarded to you as soon as possible after 01/04/2016
The costs are expected to amount to 762 € / cycle

Remarks :
Material(s) to be delivered: already received
Before :
Delivering address :

Done at Beaucouzé, Wednesday, January 23, 2013

Graziella REKAB

Landbouw
en Visserij

Agentschap voor Landbouw en Visserij

Productkwaliteitsbeheer
Koning Albert II-laan 35 bus 41
1030 Brussel
Tel. 02 552 74 40 - Fax 02 552 74 01
els.debruyn@lv.vlaanderen.be

UNIVERSITEIT GENT - VESTIGING COUPURE
T.A.V. PROF. DIRK REHEUL
COUPURE LINKS 653
9000 GENT

uw bericht van	**uw kenmerk**	**ons kenmerk**	**bijlagen**
22/11/2012		ALV/KWA/RL/JI/201211-1143	1

vragen naar / e-mail	**telefoonnummer**	**datum**
Jo Ivens Jo.Ivens@lv.vlaanderen.be	02 552 74 51	29/11/2012

Betreft: Kennisgeving van aanmelding rietzwenkgras

Geachte

In bijlage vindt u een kopij van het formulier A, vervolledigd met het dossiernummer en de officiële aanmeldingsdatum.

Dossiernr.	Soort	Kwekersref.	OHB aanvraag
VG/A/013 /00004	Rietzwenkgras	Fa.ME.V.2010/1	Duitsland

Gelieve in alle toekomstige correspondentie betreffende het ras (de rassen) steeds op een duidelijke manier het dossiernummer te vermelden.

Dank bij voorbaat

Hoogachtend,

Gilbert Crauwels
Afdelingshoofd

KWA/RC/1/080729

AANVRAAG OM INSCHRIJVING VAN EEN RAS OP DE NATIONALE RASSENLIJST - Formulier A

Vlaamse Overheid
Agentschap voor Landbouw en Visserij
Productkwaliteitsbeheer
Ellips, 4^{e} verdieping
Koning Albert II laan 35, bus 41, 1030 Brussel
Tel : + 32 2 552.74.48 - Fax : + 32 2 552.74.01
mia.defrancq@lv.vlaanderen.be
http://www2.vlaanderen.be/ned/sites/landbouw/plant/rassen.html

In te vullen door de behandelende afdeling

ontvangstdatum
26 NOV. 2012

dossiernummer
VG/ 4|013|00004

1. Aanvrager(s)

Naam: Universiteit Gent
Adres: Sint Pietersnieuwstraat 25
9000 Gent
Tel: 09/264.99.54
Fax:
E-mail: octrooien@ugent.be
Nationaliteit(en): Belgische
☐ heeft geen volmacht verleend

2. Aanvraaggemachtigde [eventueel]

Naam:
Adres:
Tel:
Fax:
E-mail:

3. Briefwisselingsadres

Naam: Prof Dirk Reheul
Adres: Coupure links 653
9000 Gent
Tel: 09 264 6096
Fax: 09 264 62 24
E-mail: Dirk.reheul@UGent.be

Dit is het adres van de kweker

4. Facturatieadres

Naam: Dirk Reheul
Adres: Coupure links 653
9000 Gent
Tel: 09 264 6096
Fax: 09 264 6224
E-mail: Dirk.reheul@UGent.be
Rekeningnummer: BE59 3900 9658 0026
Dit is het adres opgegeven door de: ☐ aanvrager

5. Soort

Rietzwenkgras *Festuca arundinacea* Schreb

6. Ras

Kwekersreferentie: Fa.ME.V.2010/1 *[juiste aanduiding van spaties, punten, a.u.b.]*
Voorgestelde benaming: FEMELLE Type: ☐ fantasie
Het ras valt onder de reglementering van de genetisch gemodificeerde organismen (GGO) ☐ neen

7. De kweker(s)

Naam: Dirk Reheul en Mathias Cougnon
Adres: Coupure links 653 9000 Gent
Tel: 09 264 6096 Fax: 09 264 62 24 E-mail: Dirk.reheul@UGent.be

Geen ander persoon was bij het kweken betrokken.

Het ras werd gekweekt in het volgende land: België

Indien de aanvrager(s) verschillend is(zijn) van de kweker(s) werd het ras overgedragen **(bewijs in bijlage)** door de kweker aan de aanvrager(s), ondertekenaar(s) door:

☐ arbeidscontract

8. De verantwoordelijke(n) voor de instandhouding voor België

Naam: Prof. Reheul

Adres: Coupure links 653 9000 Gent

Tel: 09 264 6096 Fax: 09 264 6224 E-mail: Dirk.reheul@UGent.be

Oorsprong, wijze van instandhouding en beschrijving van het ras zijn vermeld in de technische fiche (Formulier B, specifiek per soort, ter beschikking op aanvraag)

9. Overige aanvragen voor inschrijving “Kwekersrecht en Officiële rassenlijst” inclusief België

Land	Aard	Aanvraag nummer	Aanvraag datum	Aanvraag status	Kwekers-referentie	Benaming	Benaming status
geen			/ /				
			/ /				
			/ /				
			/ /				

10. OHB onderzoek (niet in te vullen voor de suikerbieten)
Voor maïs ook betreffende de COMPONENTEN vermelden

10.1	Is het OHB onderzoek reeds **afgerond**?	☐ neen
10.2	Indien ja, in welk EU-Lidstaat of EU-Instelling?	
10.3	Indien neen, is het OHB onderzoek **opgestart (bezig) of zal het opgestart worden** in het kader van een andere aanmelding (Catalogus of Kwekersrecht) in een andere EU-Lidstaat of EU-Instelling?	☐ Ja
10.4	Indien ja, in welk EU-Lidstaat of EU-Instelling?	BSA in het kader van kwekersrecht, aangevraagd nov 2012

11. Machtiging

De aanvrager(s) machtigt(machtigen) de Afdeling om met de bevoegde overheden van elk ander land, alle nuttige inlichtingen en materiaal betreffende het ras uit te wisselen, onder voorbehoud van het vrijwaren van de rechten van de kweker. De proefresultaten van alle rassen betrokken in Belgische proefnemingen worden samengevat in één rapport met melding van de kwekersreferentie, de aanvrager(s) en in voorkomend geval, de aanvraaggemachtigde. Dit rapport wordt ter beschikking gesteld van de betrokkenen.

12. Andere bijgevoegde formulieren en documenten

Documenten opgesteld in het kader van aangevraagd kwekersrecht

13. Ondertekening

Ondergetekende(n) vraagt(vragen) de inschrijving van het ras op de nationale rassencatalogus voor landbouwgewassen **(Schrappen wat niet past).**

Ondergetekende(n) verklaart(verklaren) dat bij zijn (hun) weten de inlichtingen nodig voor het onderzoek van de aanvraag, gegeven in dit formulier en in de bijlagen, volledig en juist zijn.

Datum: 19/11/2012 Plaats: Gent

Naam en handtekening van de indiener van de aanvraag:

Prof. Dr. L. MOENS
Vice-rector

VAK VOORBEHOUDEN AAN DE ADMINISTRATIE		Datum	Stempel van de afdeling
Ontvangst van het verzoek	Referentie indicateur:	26 NOV. 2012	VLAAMSE GEMEENSCHAP
Datum van aanwezigheid van het **“volledig”** dossier (1)		26 NOV. 2012	
Datum van ontvangst van de betaling van de betrokken vergoeding (2)		22 NOV. 2012	
Officiële datum van het dossier [= de laatste datum van (1) en (2)]		26 NOV. 2012	

Opmerkingen :

Voorschriften voor het invullen van het formulier A

Algemene voorschriften

0.1 **Alle gegevens in blokletters a.u.b.**

0.2 De data als volgt aanduiden: **dag / maand / jaar**.

0.3 De landen dienen aangeduid te worden met de code die op hen toepasselijk is voor de inschrijving van de voertuigen (uitgezonderd het Verenigd Koninkrijk = UK).

0.4 Gelieve "nihil" te vermelden in de leeg gelaten rubrieken.

Rubrieken

Rubriek 1

1.1 Volledige gegevens van de aanvrager(s) (natuurlijk of rechtspersoon), met inbegrip van het land vermelden; enkel de nationaliteit aanduiden in geval van natuurlijke personen;
Indien er meer dan één aanvrager is de volledige gegevens van ieder van hen vermelden;
Indien de ruimte van rubriek 1 niet toelaat alle nodige inlichtingen te vermelden alleen de namen vermelden en de adressen in bijlage toevoegen.

Rubriek 2

2.1 De aanvrager kan een aanvraaggemachtigde aanduiden m.b.t. de inschrijvingsaanvraag. Indien de aanvrager(s) **geen onderdaan is(zijn) van een lidstaat van de EU** en/of **indien er meer dan één aanvrager is moet(en)** de aanvrager(s) een aanvraaggemachtigde aanduiden. Daartoe dient een volmacht, ingevuld en ondertekend door de aanvrager(s), bijgevoegd te worden. Deze rubriek mag **maximum één adres** bevatten.

Rubriek 3 en 4

Deze rubrieken mogen **maximum één adres** bevatten. Het briefwisselingsadres is het adres bij uitstek voor alle berichtgeving betreffende een bepaald dossier. De briefgeadresseerde dient eventuele andere betrokkenen op de hoogte te houden.

Rubriek 5

5.1 De naam van de soort, geslacht of ondersoort, dient dezelfde te zijn als deze die voorkomt in de Belgische wetgeving (Latijnse en Nederlandse namen).

Rubriek 6

6.1 De voorgestelde rasbenaming en de kwekersreferentie moeten dezelfde zijn in de verschillende landen.

6.2 De aanvrager moet aangeven of de voorgestelde rasbenaming een fantasienaam of een code is.
Wanneer de aanvrager niets meedeelt over het type van de voorgestelde benaming, wordt deze als fantasienaam aangemerkt.
Indien de aanvrager geen benaming voorstelt in dit formulier, dient hij naderhand tijdens het onderzoek van zijn ras een rasbenaming voor te stellen aan de hand van het specifieke formulier. **Er kan slecht één rasbenaming met tekeer worden voorgesteld.** Een ras kan niet op de nationale rassen catalogus worden opgenomen indien de benaming niet**, in België**, volgens de geëigende procedure is vastgesteld.

6.3 De aanvrager moet aangeven of het ras een GGO (Genetisch Gemodificeerd Organisme) is en de nodige autorisaties voor proefneming en in de handel brengen dienen bijgevoegd te worden.

Rubriek 7

7.1 Volledige gegevens van de kweker(s) (natuurlijk of rechtspersoon), met inbegrip van het land vermelden; enkel de nationaliteit aanduiden in geval van natuurlijke personen;
Indien er meer dan één kweker is volledige gegevens van ieder van hen vermelden;
Indien de ruimte van rubriek 7 niet toelaat alle nodige inlichtingen te vermelden alleen de namen vermelden en de adressen in bijlage toevoegen.

Rubriek 8

8.1 Het ras moet systematisch in stand worden gehouden. Deze instandhouding moet plaats vinden ofwel in België ofwel in een andere EG-lidstaat, waar het ras op de nationale rassencatalogus ingeschreven is of in procedure van inschrijving is. Een verantwoordelijke voor de instandhouding voor België **moet** vermeld worden.

8.2 De inlichtingen vermelden op het technische formulier ' rasbeschrijving'. De instandhoudingsmethode dient te worden vermeld.
Voor de hybriderassen, aanduiden of de genealogische componenten vertrouwelijk moeten blijven.
Voor de GGO rassen, de referentie van de toestemming voor de proeven aanduiden.

Rubriek 9

9.1 "Kwekersrecht" in het kader van de Verordening (EG) NR. 2100/94 van de Raad van 27/07/1994 inzake het communautaire kwekersrecht en van de Wet tot bescherming van kweekproducten van 20/05/1975.

9.2 "Officiële rassenlijst" betekent iedere lijst van rassen waarvan het verhandelen toegestaan is door de terzake bevoegde overheden in opvolging van de Richtlijn 2002/53/EG van de Raad van 13/06/2002 betreffende de gemeenschappelijke rassenlijst van landbouwgewassen en Richtlijn 2002/55/EG van de Raad van 13/06/2002 betreffende het in de handel brengen van groentezaad en het KB betreffende de nationale rassencatalogi voor landbouw en groentegewasssen van 8/07/2001.

9.3 Alle overige aanvragen dienen, zonder uitzondering en in chronologische volgorde, te worden vermeld, met inbegrip van deze neergelegd bij een andere Belgische instelling of bij landen, al dan niet lid van de Europese gemeenschappen en/of de Internationale Unie tot bescherming van kweekproducten (UPOV).

9.4 De volgende afkortingen gebruiken:

In de kolom "Aanvraag status":

A = aanvraag aanhangig
B = aanvraag afgewezen
C = aanvraag ingetrokken
D = verleende kwekersrechten of ras ingeschreven op de officiële rassenlijst

In de kolom "Benaming status"

A = rasbenaming aanhangig
B = afgewezen rasbenaming (negatief)
C = ingetrokken rasbenaming (gestopt)
D = aangenomen rasbenaming (positief)

9.5 De aanvrager is verplicht, zo gauw hij ze verneemt, alle bijkomende of nieuwe inlichtingen over de toestand van het ras in België of in andere landen mede te delen.

Rubriek 13

13.1 Terug te sturen in 3 exemplaren :

- de technische vragenlijst (Formulier B)
- de volmacht indien er een aanvraaggemachtigde m.b.t. de inschrijving wordt aangeduid
- in geval van GGO: de nodige toestemmingen
- de overdracht door de kweker aan de aanvrager door contract, successie of andere.

Naar volgend adres:

Vlaamse overheid
Agentschap voor Landbouw en Visserij, Productkwaliteitsbeheer
Ellips, 4e verdieping
Koning Albert II laan 35, bus 41
1030 BRUSSEL
Tel. +32(0)2 552 74 48 – Fax +32(0)2 552 74 01
e-mail: mia.defrancq@lv.vlaanderen.be

13.2 Aanvraagformulieren en andere nodige formulieren zijn op hierboven vermeld adres te verkrijgen of te vinden, in voorkomend geval op de website **Website : http://www2.vlaanderen.be/ned/sites/landbouw/plant**

References

Aderibigbe A.O., Church D.C., Frakes R.V. and Petersen R.G., 1982. Factors determining palatability of ryegrass to cattle. *Journal of Animal Science,* 54:164-172.

Allen V.G., Batello C., Berretta E.J., Hodgson J., Kothmann M., Li X., McIvor J., Milne J., Morris C., Peeters A. and Sanderson M., 2011. The forage and grazing terminology committee: An international terminology for grazing lands and grazing animals. *Grass and Forage Science,* 66:2-28.

AGIV, 2013.Agentschap voor geografische informatie Vlaanderen. Available at: http://geo-vlaanderen.gisvlaanderen.be/geo-vlaanderen/bodemkaart/. Last accessed 30 April 2013.

Annicchiarico P. and Proietti S., 2010. White clover selected for enhanced competitive ability widens the compatibility with grasses and favours the optimization of legume content and forage yield in mown clover-grass mixtures. *Grass and Forage Science*, 65:318-324.

Baert J., De Vliegher A., Van Hulle S., Van Waes C. and Muylle H., 2012. Biomass yield and composition from semi-extensively cultivated perennial fodder grasses. *Grassland Science in Europe,* 17:460-462.

Baert J., Vandewalle M., De Riek J., De Boever J., Fievez V. and Van Waes C., 2012. Variation and heritability of alpha-linolenic acid content and rumen escape protein fraction in fodder grass and clover. In Barth S. and Milbourne D. (eds) *Breeding Strategies for sustainable forage and turf grass improvement. Proceedings of the 29th Eucarpia Meeting, Dublin, Ireland,2012:* 181-188. Springer, Dordrecht, The Netherlands.

Barre J.P., Emile J.C., Betin M., Surault F., Ghequière M. and Hazard L., 2006. Morphological characteristics of perennial ryegrass leaves that influence short-term intake in dairy cows. *Agronomy Journal*, 98:978-985.

Behrens T. and Diepenbrock W., 2006. Using digital image analysis to describe canopies of winter oilseed rape (*Brassica napus* L.) during vegetative developmental stages. *Journal Agronomy and Crop Science,* 192:295-302.

Berg C.C., Webster G.T. and Jauhar P.P., 1979. Cytogenetics and genetics. In: R.C. Buckner & L.P. Bush (eds) *Agronomy monograph no 20: Tall Fescue:* 93–109. American Society of Agronomy, Madison, USA.

Biskup B., Scharr H., Schurr U. and Rascher U., 2007. A stereo imaging system for measuring structural parameters of plant canopies. *Plant, Cell & Environment,* 30:1299-1308.

Boelt B. and Studer B., 2010. Breeding for Grass Seed Yield. In: Boller, B., Posselt, U.K. & Veronesi, F. (eds) *Handbook of Plant Breeding Vol. 5 Fodder crops and amenity grasses.* 161-174. Springer, Dordrecht, The Netherlands.

Boller B.C. and Greene S.L., 2010. Genetic resources. In: Boller, B., Posselt, U.K. & Veronesi, F. (eds) *Handbook of Plant Breeding Vol. 5 Fodder crops and amenity grasses.* 13-38. Springer, Dordrecht, The Netherlands.

Boller B.C. and Nösberger J., 1987. Symbiotically fixed nitrogen from field-grown white and red clover mixed with ryegrasses at low level of ^{15}N-fertilization. *Plant and Soil,* 104:219-226.

Bommelé L., 2007. Growing potatoes and grass-clover after turned down grassland. Thesis submitted in fulfilment of the requirements for the degree of Doctor (PhD) in Applied Biological Sciences. Faculty of Bioscience Engineering, Universiteit Gent, Gent, Belgium.

Bouton J., 2007. The economic benefits of forage improvement in the United States. *Euphytica* 154: 263-270.

Bransby D.I., Matches A.G. and Krause G.F., 1977. Disk meter for rapid estimation of herbage yield in grazing trials. *Agronomy Journal* 69:393-396.

Bryant R.H., Nicol A.M., Wilson F.E. and Sedcole J.R., 2008. Between-cultivar variability in breaking force of components of perennial ryegrass (*Lolium perenne*). *Grass and Forage Science*, 63: 350-359.

Buckner R.C. and Burrus P., 1962. Comparison of techniques for evaluating palatability differences among tall fescue strains. *Crop Science,* 2:55-57.

Buckner R.C. and Bush L.P., 1979. Agronomy monograph nr 20: Tall Fescue. American society of agronomy, Madison, USA.

Buckner R.C. and Fergus E.N., 1960. Improvement of tall fescue for palatability by selection within inbred lines. *Agronomy Journal,* 52:173-174.

Bullock J.M., Clear Hill B., Silvertown J. and Sutton M., 1995. Gap colonization as a source of grassland community change – effects of gap size and grazing on the rate and mode of colonization by different species. *Oikos*, 72:273–282.

Burlinson A.J., Hodgson J. and Illius A.W., 1991. Sward canopy structure and the bite dimensions and bite weight of grazing sheep. *Grass and Forage Science,*46:29-38.

Burns G.A., 2012. Variation in nutritive quality of *Lolium* as assessed by Near Infrared Reflectance Spectroscopy. Thesis submitted for the degree of Doctor, Queens University Belfast, UK.

Burns J.C. and Chamblee D.S., 1979. Adaptation. In: In: R.C. Buckner & L.P. Bush (eds) *Agronomy monograph no 20: Tall Fescue:* 9-30. American Society of Agronomy Madison, USA.

Burns J.C., Fisher D.S. and Mayland H.F., 2001. Preference by sheep and goats among hay of eight tall fescue cultivars. *Journal of Animal Science,* 79:213-224.

Carlsson G. and Huss-Danell K., 2003. Nitrogen fixation in perennial forage legumes in the field. *Plant and Soil,* 253:353-372.

Casler M.D. and van Santen E., 2010. Breeding objectives in forages. In: Boller, B., Posselt, U.K. & Veronesi, F. (eds) *Handbook of Plant Breeding Vol. 5 Fodder crops and amenity grasses.* 115-136. Springer, Dordrecht, The Netherlands.

Chataigner F., Surault F., Huyghe C. and Jullier B., 2010. Determination of botanical composition in multispecies forage mixtures by near infrared reflectance spectroscopy. In: Huyghe C (ed) *Sustainable use of genetic diversity in forage and turf breeding. Proceedings of the 29th Eucarpia Meeting, La Rochelle, France,* 199-203. Springer, Dordrecht, The Netherlands.

Chaves B., De Vliegher A., Van Waes J., Carlier L. and Marynissen B., 2009. Change in agronomic performance of *Lolium perenne* and *Lolium multiflorum* varieties in the past 40 years based on data from Belgian VCU trials. *Plant Breeding,* 128:680-690.

Coleman S. W., Barton F. E. and Meyer R. D., 1985. The use of near-infrared reflectance spectroscopy to predict species composition of forage mixtures. *Crop Science,* 25:834-837.

Coleman S. W., Christiansen S. and Shenk J. S., 1990. Prediction of botanical composition using nirs calibrations developed from botanically pure samples. *Crop Science,* 30:202-207.

Cougnon M., Van Waes C., Baert J. and Reheul D., 2012. NIRS calibration strategies for the species composition of grass clover mixtures. In Barth S. and Milbourne D. (eds) *Breeding Strategies for sustainable forage and turf grass improvement. Proceedings of the 29th Eucarpia Meeting, Dublin, Ireland,* 371-375, Springer, Dordrecht, The Netherlands.

Crawley J., 2007. The R book. Wiley, Chichester, UK.

CSAR, 2013. Commissie samenstelling aanbevelende rassenlijst landbouwgewassen. Rassenlijst 2013 Veehouderij. Available on: http://www.rassenlijst.info/rassenlijsten. Last accessed 7 May 2013.

Decruyenaere V., Lecomte Ph., Demarquilly C., Aufrere J., Dardenne P., Stilmant D., Buldgen A., 2009. Evaluation of green forage intake and digestibility in ruminants using near infrared reflectance spectroscopy (NIRS): Developing a global calibration. *Animal Feed Science and Technology*, 148: 138-156.

De Dauw K., 2009. Breeding of Tall fescue (*Festuca arundinacea* Schreb.). Master Thesis Ghent University, Gent, Belgium

De Santis G. and Chiaravalle E., 2001. Heritabilities of nutritive quality factors and interrelationships with yield in selected progenies of tall fescue. *Plant Breeding*, 120:337-343.

Demeyer, D.I., 1991. Quantitative aspects of microbial metabolism in the rumen and hindgut. In Jouany J.P. (ed) *Rumen microbial metabolism and ruminant digestion*. 217-237. INRA editions, Paris, France.

Deprez B., Lambert R., Decamps C. and Peeters A., 2004a. Nitrogen fixation by red clover (*Trifolium pratense*) and lucerne (*Medicago sativa*) in Belgian leys. In: Lüscher A., Jeangros B., Kessler W., Huguenin O., Lobsiger M., Millar N. and Suter D. (eds) *Land Use Systems in Grassland Dominated Regions: Proceedings of the 20th general meeting of the European Grassland Federation, Luzern, Switserland,* 2004: 469-471.

Deprez B., Lambert R., Decamps C. and Peeters A, 2004b. Production and quality of red clover (*Trifolium pratense*) and lucerne (*Medicago sativa*) in pure stand or in grass mixtures in Belgium. In: Lüscher A., Jeangros B., Kessler W., Huguenin O., Lobsiger M., Millar N. and Suter D. (eds) *Land Use Systems in Grassland Dominated Regions: Proceedings of the 20th general meeting of the European Grassland Federation, Luzern, Switserland,* 2004: 498-500.

Deru J., Van Eekeren N. and De Boer H., 2012. Rooting density of three grass species and eight *Lolium perenne* cultivars. In: Goliński P., Warda M. and Stypiński P. (eds) *Grassland – a European Resource? Proceedings of the 24th General Meeting of the European Grassland Federation Lublin, Poland, 2012*: 604-606.

Drake J., 2003. Why does grassland productivity increase with species richness? Disentangling species richness and composition with tests for overyielding and superyielding in biodiversity experiments. *Proceedings of The Royal society of London,* 270:1713-1719.

Durand J-L. and Ghesquière M., 2002. Root biomass changes in tall fescue and Italian ryegrass swards under two irrigation regimes. In: Durand J. L., Emile J. C., Huyghe C. and Lemaire G. (eds) *Multi-function grasslands: quality forages, animal products and landscapes. Proceedings of the 19th General Meeting of the European Grassland Federation, La Rochelle, France, 2002*: 292-293.

Durand J-L. and Ghesquière M., 2002. Root biomass changes in tall fescue and Italian ryegrass swards under two irrigation regimes. In: Durand J. L., Emile J. C., Huyghe C. and Lemaire G. (eds) *Multi-function grasslands: quality forages, animal products and landscapes. Proceedings of the 19th General Meeting of the European Grassland Federation, La Rochelle, France, 2002*: 292-293.

Easton H.S., 1989. Variability of leaf shear strength in perennial ryegrass. *New Zealand Journal of Agricultural Research*, 32:1-6.

Easton H.S., Lee C.K. and Fitzgerald R.D., 1994. Tall Fescue in Australia and New Zealand. *New Zealand Journal of Agricultural Research,* 37:405-417.

Elgersma A. and Schelpers H., 1997. Performance of white clover/perennial ryegrass mixtures under cutting. *Grass and Forage Science,* 52 :134-146.

Emile J.C., Ghesquière M., Traineau R., Jadas-Hecart J. and Mousset C., 1992. Evaluation de la valeur alimentaire de génotypes de fétuque élevée obtenus par différents stratégies d'amélioration. *Fourrages,* 151 :373-386.

Emile J.C., Gillet M., Ghesquière M. and Charrier X., 1992. Pâturage continu de fétuques élevées par de vaches laitières: amélioration de la production par l'utilisation d'une variété sélectionnée pur l'appétibilité. *Fourrages,* 130 :159-169.

Emile J.C., Jadas-Hécart J., Traineau R., Mousset C. and Ghesquière M., 1997. Evaluation de la valeur alimentaire de fétuque élevée obtenus par différentes stratégies d'amélioration. *Fourrages,* 151:373-387.

Evans P.S., 1967. Leaf shear studies of pasture grasses. I Apparatus, techniques and some factors affecting leaf strength. *Journal of Agricultural Science,* 69:171-174.

Fearn T., 1996. Comparing standard deviations. *NIRnews,* 7:5-6.

Fernández Pierna J., Chauchard F., Preys S., Roger J., Galtier O., Baeten V. and Dardenne P., 2010. How to build a robust model against perturbation factors with only a few reference values: A chemometric challenge at ‘Chimiométrie 2007’. *Chemometrics and Intelligent Laboratory Systems,* 106:152-159.

Frame J., 1973. The yield response of a tall fescue/white clover sward to nitrogen rate and harvesting frequency. *Grass and Forage Science,* 28 :139-148.

Frame, 1992. Improved grassland management. Farming Press, Ipswich, UK.

Geves, 2013. Le Groupe d'Etude et de contrôle des Variétés Et des Semences, catalogue officiel. Available at: http://cat.geves.info/Page/ListeNationale. Last accessed 29 March 2013.

Ghesquière M., Humphreys M.W. and Zwierzykowski Z. 2010. Festulolium. In: Boller, B., Posselt, U.K. & Veronesi, F. (eds) *Handbook of Plant Breeding Vol. 5 Fodder crops and amenity grasses.* 293-316. Springer, Dordrecht, The Netherlands.

Gibson D.J. and Newman J.A., 2001. *Festuca arundinacea* (*F. elatior* L. ssp. *arundinacea* (Schreber) Hackel). *Journal of Ecology,* 89:304-324.

Gilbert W.B. and Chamblee D.S., 1965. Effect of submersion in water on tall fescue, orchardgrass and ladino clover. *Agronomy Journal,* 57 :502-504.

Gillet M., 1975. La sélection pour la facilité d’utilisation chez les fétuques élevées et les Dactyles. *Fourrages,* 64 :11-17.

Gillet M. and Jadas-Hecart J., 1965. La flexibilité des feuilles, critère de sélection de la fétuque élevée en tant que facteur d'appétibilité. *Fourrages,* 22 :6-11.

Gillet M., Noël C. and Jadas-Hecart J., 1983. La cafétéria d’auges, méthode d’étude de l’appétibilité des fourrages. *Agronomie,* 3 :817-822.

Gilliland T.J., Farrell A.D. and Grogan D., 2011. Differential responses to climatic conditions in Ireland by five grass species. *Grassland Science in Europe* In: Pötsch, E. M., Krautzer, B. and Hopkins A. (eds) *Grassland farming and land management systems in mountainous regions. Proceedings of the 16th Symposium of the European Grassland Federation, Gumpenstein, Austria, 2011*: 217-219.

Gilliland T.J., Farrell A.D., Mc Gilloway D. and Grogan D., 2010. The effect of nitrogen on yield and composition of grass-clover swards at three sites in Ireland: A comparison of six commonly grown species. In: Schnyder H., Isselstein J., Taube F., Auerswald K., Schellberg J., Wachendorf M., Herrmann A., Gierus M., Wrage N. and Hopkins, A. (eds) *Grassland in a* *changing world. Proceedings of the 23rd General Meeting of the European Grassland Federation, Kiel, Germany, 2010*: 946-948.

Gilliland T.J., Johnston J. and Connolly C., 2007. A review of forage grass and clover seed use in Northern Ireland, UK between 1980 and 2004. *Grass and Forage Science*, 62 :239-254.

GNIS, 2013.Groupement national interprofessionnel des semences et plants, chiffres. Available at: http://www.prairies-gnis.org/pages/chifftres.php. Last accessed 29 March 2013.

Gonzalez-Dugo V., Durand J.-L., Gastal F. and Picon-Cochard C. 2005. Short term response of the nitrogen status of tall fescue and Italian ryegrass swards under water deficit. Australian Journal of Agricultural Research, 56, 1269-1276.

Graiss W., Krautzer B. and Potsch E.M., 2011. Suitability of alternative grass species for grassland management in Austria under changing climatic conditions. In: Pötsch, E. M., Krautzer, B. and Hopkins A. (eds) *Grassland farming and land management systems in mountainous regions. Proceedings of the 16th Symposium of the European Grassland Federation, Gumpenstein, Austria, 2011:*440-442.

Haquin F., 2012. Semences fourragères, tableaux comparatifs. *Semences et Progrès*, 154:12-83.

Hayes, P., 1976. Seedling growth of four grasses. *Journal of the British Grassland Society*, 31:59-64.

Hejduk S. and Hrabe F., 2003. Influence of different systems of grazing , type of swards and fertilizing on underground phytomass of pastures. *Plant Soil and Environment*, 49:18-23.

Henry D.A., Simpson R.J. and Macmillan R.H., 1997. Intrinsic shear strength of leaves of pasture grasses and legumes. *Australian Journal of Agricultural Research*, 48:667-674.

Hodgson J., 1979. Nomenclature and definitions in grazing studies. *Grass and Forage Science*, 34:11–18.

Honek A., Martinkova Z. and Saska P., 2005. Post-dispersal seed predation of *Taraxacum officinale* (Dandelion) seed. *Journal of Ecology*, 93:345-352.

Humphreys M.W., Macleaod C.J.A., Whalley W.R., Turner L.B., Farrell M.S., Ghesquière M. and Haygarth P.M., 2012. designing grass cultivars for droughts and floods. In Barth S. and Milbourne D. (eds) *Breeding Strategies for sustainable forage and turf grass improvement. Proceedings of the 29th Eucarpia Meeting, Dublin, Ireland,2012.* 171-179. Springer, Dordrecht, The Netherlands.

Huyghe C., Litrico I. and Surault F., 2012. Agronomic value and provisioning services of multi species swards. In: Goliński P., Warda M. and Stypiński P. (eds) *Grassland – a European Resource? Proceedings of the 24th General Meeting of the European Grassland Federation Lublin, Poland, 2012:*35-46.

Image J, 2013. Image processing and analysis in Java. Available at: http://rsbweb.nih.gov/ij/. Last accessed 12 April 2013.

Ingwersen B., 2001. Einfluss von Bewirtschaftungsmaßnahmen auf die Leistungsfähigkeit von leguminosenbasiertem Dauergrünland unter besonderer Berücksichtigung der Nährstoffbilanzierung. Dissertation zur Erlangung des Doktorgrades der Agrar- und Ernährungswissenschaflichen Fakultät der Christian-Albrechts-Universität zu Kiel, Kiel, Deutschland.

Inoué T., Brookes I.M., John A., Hunt W.F. and Barry T.N.,1994a. Effects of leaf shear breaking load on the feeding value of perennial ryegrass (*Lolium perenne*) for sheep. I Effects on leaf anatomy and morphology. *Journal of Agricultural Science,* 123:129-136.

Inoué T., Brookes I.M., John A., Kolver E.S. and Barry T.N., 1994b. Effects of leaf shear breaking load on the feeding value of perennial ryegrass (*Lolium perenne*) for sheep. I Effects on feed intaken particle breakdown, rumen digesta outflow and animal performance. *Journal of Agricultural Science,* 123 :137-147.

INRA, 1978. L'alimentation des ruminants. INRA publications, Versailles, France.

IPCC (2007) Climate change 2007: Working Group II: Impacts, Adaptation and Vulnerability. Cambridge University Press, Cambridge, UK.

ISTA, 1993. International rules for seed testing-Rules 1993. International Seed Testing Association, Zürich, Switzerland.

Jadas-Hecart J., 1982. Etude de l'appétibilité de fétuques élevées (Festuca arundinacea Schreb.) à l'aide de moutons. *Agronomie,* 2:487-492.

Jones L. and Prickett, J., 1981.The rate of water loss from cut grass of different species dried at 20°C. *Grass and Forage Science,* 36:17-23.

Jones L. and Prickett, J., 1981. The rate of water loss from cut grass of different species dried at 20°C. *Grass and Forage Science,* 36:17-23.

Karcher D. E. and Richardson M. D., 2005. Batch analysis of digital images to evaluate turfgrass characteristics. *Crop Science,* 45:1536-1539.

Kendall W.A. and Sherwood R.T., 1975. Palatability of leaves of tall fescue and reed canarygrass and of some of their alkaloids to meadow voles. *Agronomy Journal,* 67:667-671.

Kneebone W.R., 1960. Tensile strength variations in weeping lovegrass (*Eragrostis curvula* (Schrad.) Nees.) and certain other grasses. *Agronomy Journal, 5*2: 539-542.

Landini G., 2013. Software. Available on: http://www.dentistry.bham.ac.uk/landinig/software /software.html. Last accessed 12 April 2013.

Lemaire G., Da Silva S. C., Agnusdei M., Wade M. and Hodgson J., 2009. Interactions between life span and defoliation frequency in temperate and tropical pastures: a review. *Grass and Forage Science,* 64:341-353.

Lewis, E. J. and Garcia, J. A.,1979. The effect of seed weight and coleoptile tiller development on seedling vigour in tall Fescue *Festuca aruninacea* Schreb. *Euphytica,* 28:393-402.

Locher F., Heuwinkel H., Gutser R. and Schmidhalter U., 2005a. Development of near infrared reflectance spectroscopy calibrations to estimate legume content of multispecies legume-grass mixtures. *Agronomy Journal*, 97:11-17.

Locher F., Heuwinkel H., Gutser R. and Schmidhalter U., 2005b.The legume content in multispecies mixtures as estimated with near infrared reflectance spectroscopy: Method validation. *Agronomy Journal*, 97:18-25.

Lock R., Rademacher I., Nonn H. and Künbauch W., 2004. Methods of digital image processing to quantify ground cover of turf grass. In: Lüscher A., Jeangros B., Kessler W., Huguenin O., Lobsiger M., Millar N., Suter D. (eds) *Land Use Systems in Grassland Dominated Regions: Proceedings of the 20th general meeting of the European Grassland Federation, Luzern, Switzerland, 2004:*790-792.

Luscher A., Nyfeler D., Suter M., Suter D. and Huguenin-Elie O., 2012. Grass-legume mixtures can fix more N_2 from the atmosphere than legume pure stands. *Grassland Science in Europe,* 15:818-820.

Luten W. and Remmelink G.J., 1984. Intake of perennial ryegrass, tall fescue and Italian ryegrass by dairy cattle. Proefstation voor Rudveehouderij, Lelystad, the Netherlands

MacAdam J.W. and Mayland H.F., 2003. The relation of leaf shear strength to cattle preference in tall fescue cultivars. *Agronomy Journal,* 95:414-419.

Mackinnon B.W., Easton H.S., Barry T.N. and Sedcole J.R., 1988. The effect of reduced leaf shear strength on the nutritive value of perennial ryegrass. *Journal of Agricultural Science,* 111:469-474.

Marshall A.H., Lowe M. and Vale J., 2012. Improved persistence of red clover (*Trifolium pratense* L.) varieties in mixed swards. In: Goliński P., Warda M. and Stypiński P. (eds) *Grassland – a European Resource? Proceedings of the 24th General Meeting of the European Grassland Federation Lublin, Poland 2012*: 73-75.

Martinkova Z., Honek A. and Pekar S., 2009. Seed availability and gap size influence seedling emergence of dandelion (*Taraxacum officinale*) in grasslands. *Grass and Forage Science,* 64:160-168.

Mayland H.F., Shewmaker G.E., Harrison P.A. and Chatterton N.J., 2000. Nonstructural carbohydrates in tall fescue cultivars: Relationship to animal preference. *Agronomy Journal,* 92:1203-1206.

Mc Bratney J.M., 1987. Productivity of red clover grown alone and with companion grasses; further studies. *Grass and Forage Science,* 39:167-175.

Mcevoy M. ,O' Donovan M. C. W. and Kennedy E., 2010. The effect of evaluation protocol on the dry matter yield performance of *Lolium perenne* varieties. In: H. Schnyder, J. Isselstein, F. Taube, K. Auerswald, J. Schellberg, M. Wachendorf, A. Herrmann, M. Gierus, N. Wrage & A. Hopkins (eds) *Grassland in a changing world. Proceedings of the 23rd General Meeting of the European Grassland Federation, Kiel, Germany, 29th August - 2nd September 2010:* 314-316.

Metoffice, 2013. Met Office Hadley Centre observations datasets: HadUKP. Available on: http://www.metoffice.gov.uk/hadobs/hadukp/, Last accesed on 1 May 2013.

Minson D.J., Harris C.E., Raymond W.F. and Millford R., 1964. The digestibility and voluntary intake of S22 and H.1 ryegrass, S170 tall fescue, S48 timothy, S215 meadow fescue and Germinal cocksfoot. *Grass and Forage science, 1*9: 298-305.

Mosimann E., Schmied R., Thuillard C.P. and Thompet P., 2010. Production de viande sur prairies temporaires: Intérêt de la fétuque élevée. *Recherche agronomique Suisse,* 1:194-201.

Naes T., Isaksson T., Fearn T. and Davies T., 2002. A user-friendly guide to multivariate calibration and classification. NIR Publications, Chichester, UK.

Nguyen Hung T., Sleper D.A. and Matches A.G., 1982. Inheritance of forage quality and its relationship to leaf tensile strength in tall fescue. *Crop Science,* 22:67-72.

NIAB, 2013. Recommended grass and clover lists for England and Wales. Available on: http://www.dairyco.org.uk/resources-library/technical-information/grass-management/grass-clover-list-2013/, Last accesed on 7 May 2013.

Norris I.B., 1982. Soil moisture and growth of contrasting varieties of *Lolium*, *Dactylis* and *Festuca* species. *Grass and Forage Science,* 37:273-283.

Nyfeler D., Huguenin-Elie O., Suter M., Frossard E., Connolly J. and Lüscher A., 2009. Strong mixture effects among four species in fertilized agricultural grassland led to persistent and consistent transgressive overyielding. *Journal of Applied Ecology,* 46:683-691.

Opitz V., Boberfeld W., Sterzenbach M. and Daniel P., 2004. Silage quality of tall fescue in comparison with other grass species. In: A. Lüscher, Jeangros B., Huguenin O., Lobsiger M., Millar N., Suter D. (eds) *Land use systems in grassland dominated regions.Proceedings of the 20th General Meeting of the European Grassland Federation, Luzern, Switserland, 2002:* 972-974.

Orr R.J., Cook J.E., Champion R.A., Penning P.D. and Rook A.J., 2003. Intake characteristics of perennial ryegrass varieties when grazed by sheep under continuous stocking management. *Euphytica,* 134:247-260.

Pannecoucque J., 2012. Grassen, resultaten CGW proeven 2011. Instituut voor Landbouw- en Visserijonderzoek, Merelbeke, Belgium.

Pannecoucque J., 2013. Grassen, resultaten CGW proeven 2012, Instituut voor Landbouw- en Visserijonderzoek, Merelbeke, Belgium.

Parsons A.J., Edwards G.R., Newton P.C.D., Chapman D.F., Caradus J.R., Rasmussen S. and Rowarth J.S., 2011. Past lessons and future prospects: plant breeding for yield and persistence in cool-temperate pastures. *Grass and Forage Science*, 66: 153-172.

Peratoner G., Florian C., Klotz C., Figl U. and Gottardi S., 2011. Effect of forage conservation on the leaf texture of tall fescue. In: Pötsch, E. M., Krautzer, B. and Hopkins A. (eds) *Grassland farming and land management systems in mountainous regions. Proceedings of the 16th Symposium of the European Grassland Federation, Gumpenstein, Austria, 2011:* 223-225.

Petersen J. C., Barton F. E., Windham W. R. and Hoveland C. S., 1987. Botanical composition definition of tall fescue white clover mixtures by near-infrared reflectance spectroscopy. *Crop Science*, 27:1077-1080.

Petersen R.G., Weswig P.H. and Cowan J.R., 1958. Measuring palatability differences in tall fescue by grazing sheep. *Agronomy Journal,* 50:117-119.

Peyraud J.L., Comeron E.A., Wade M.H. and Lemaire G., 1996. The effect of daily herbage allowance, herbage mass and animal factors upon herbage intake by grazing cows. *Annales de Zootechnie,* 45:201-217.

Peyraud J.L., Van den Pol-van Dasselaar A., Dillon P. and Delaby L., 2010. Producing milk from grazing to reconcile economic and environmental performances. In: Schnyder H., Isselstein J., Taube F., Auerswald K., Schellberg J., Wachendorf M., Herrmann A., Gierus M., Wrage N. and Hopkins, A. (eds) *Grassland in a changing world. Proceedings of the 23rd General Meeting of the European Grassland Federation, Kiel, Germany, 2010:* 864-879.

Phelan P., Keogh B., Fitzgerald E., Casy I.A. and Humphreys J., 2011. The effect of trampling on a rotationally grazed grass clover sward. In: *Proceedings of the tenth research conference of the British grassland society, Belfast, UK, 2011*: 7-8.

Pitman W. D., Piacitelli C. K., Aiken G. E. and Barton F. E., 1991. Botanical composition of tropical grass-legume pastures estimated with near-infrared reflectance spectroscopy. *Agronomy Journal,* 83:103-107.

Pontes L.S., Carrere P., Andueza D., Louault T F. and Soussana J.F., 2007. Seasonal productivity and nutritive value of temperate grasses found in semi-natural pastures in Europe: responses to cutting frequency and N supply. *Grass and Forage Science*, 62:485-496.

Posselt U.K., 2010.Breeding Methods in Cross-Pollinated Species. In: Boller, B., Posselt, U.K. & Veronesi, F. (eds) *Handbook of Plant Breeding Vol. 5 Fodder crops and amenity grasses.*39-87. Springer, Dordrecht, The Netherlands.

Purcell L. C., 2000. Soybean canopy coverage and light interception measurements using digital imagery. *Crop Science,* 40:834-837.

R development core team, 2011. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL http://www.R-project.org/.

Reheul D., De Cauwer B., Cougnon M. and Aper J., 2012. What global and/or European agriculture will need from grassland and grassland breeding over the next 10-15 years for a sustainable agriculture. In Barth S. and Milbourne D. (eds) *Breeding Strategies for sustainable forage and turf grass improvement. Proceedings of the 29th Eucarpia Meeting, Dublin, Ireland,2012.* 3-18. Springer, Dordrecht, The Netherlands.

Reheul D. and Ghesquière A., 1996. Breeding perennial ryegrass with better crown rust resistance. *Plant Breeding*, 115: 465-469.

Richardson M. D., Karcher D. E. and Purcell L. C., 2001. Quantifying Turfgrass Cover Using Digital Image Analysis. *Crop Science,* 41:1884-1888.

Rognli O.A., Saha M.C., Bhamidihari S. and van der Heijden S., 2010. Fescues. In: Boller, B., Posselt, U.K. & Veronesi, F. (eds) *Handbook of Plant Breeding Vol. 5 Fodder crops and amenity grasses.* 261-292. Springer, Dordrecht, The Netherlands.

Scehovic J., 1986. Appétibilité et caractéristiques organoleptiques de la fétuque élevée : Dernières connaissances. *Le sélectionneur Français,* 37:47-60.

Schübinger F. X. and 25 others, 2012. The EUCARPIA Multi-site rust Evaluation – Results 2010. In Barth S. and Milbourne D. (eds) *Breeding Strategies for sustainable forage and turf grass improvement. Proceedings of the 29th Eucarpia Meeting, Dublin, Ireland,2012.* 209-217. Springer, Dordrecht, The Netherlands.

Shaffer J. A., Jung G. A., Shenk J. S. and Abrams S. M., 1990. Estimation of botanical composition in alfalfa ryegrass mixtures by near-infrared spectroscopy. *Agronomy Journal,* 82:669-673.

Shenk J. and Westerhaus M. 1993. Analysis of agriculture and food products by near infrared reflectance spectroscopy. Infrasoft International, Port Matilda, USA.

Shenk J. S. and Westerhaus M. O., 1991. Population structuring of near-infrared spectra and modified partial least-squares regression. *Crop Science*, 31:1548-1555.

Shewmaker G.E., Mayland H.F. and Hansen S.B., 1997. Cattle grazing preference among eight endophyte-free tall fescue cultivars. *Agronomy Journal,* 89:695-701.

Sleper D.A. and Poehlman J.M., 2006. Breeding Field Crops, fifth edition. Blackwell, Ames, USA.

Snedecor G. and Cochran W., 1967. Statistical Methods, sixth edition. Iowa state university press, Ames, USA.

Staelens J., Wuyts K., Adriaenssens S., Van Avermaet P., Buysse H., Van den Bril B., Roekens E., Ottoy J.-P., Verheyen K., Thas O. and Deschepper E., 2012. Trends in atmospheric nitrogen and sulphur deposition in northern Belgium. *Atmospheric Environment,* 49:186-196.

Sugiyama S. and Nakashima H., 1991. Effect of tall fescue (*Festuca arundinacea* Schreb.) on the botanical composition of mixed swards. *Grass and Forage Science,* 46:365-373.

Surault F., Briand M., Veron R. and Huyghe C., 2006. NIRS calibration equations to determine species contribution in grassland swards. In: Lloveras J., González-Rodríguez A., Vázquez-Yañez O. Piñeiro J., Santamaría O., Olea L. and Poblaciones M. J. (eds) *Sustainable grassland productivity: Proceedings of the 21st General Meeting of the European Grassland Federation, Badajoz, Spain, 2006*:598-600.

Surault F., Veron F., Chataigner F. and Huyghe C., 2007. Comportement de prairies mono ou plurispécifiques en année à deficit hydrique marqué (2005). *Fourrages*, 192 :507-510.

Surault F., Veron R. and Huyghe C., 2006. Changes in species composition of grasslands induced by some agronomic practices In: Lloveras J., González-Rodríguez A., Vázquez-Yañez O. Piñeiro J., Santamaría O., Olea L. and Poblaciones M. J. (eds) *Sustainable grassland productivity: Proceedings of the 21st General Meeting of the European Grassland Federation, Badajoz, Spain, 2006*: 499-501.

Suter D., Frick R., Hirschi H. and Chapuis S., 2009. Rohrschwingel- und Timothesorten geprüft. *Agrarforschung,* 16:250-255.

Tabacco E., Borreani G., Valente M.E. and Peiretti P.G., 2004. Dry matter and water-soluble carbohydrate contents of Italian ryegrass at cutting as affected by environmental factors. *Italian Journal of Agronomy,* 8:63-74.

Tas B.M., Taweel H.Z., Smit H.J., Elgersma A., Dijkstra J. and Tamminga S., 2005. Effect of perennial ryegrass cultivar on intake, digestibility and milk yield in cows. *Journal of Dairy Science,* 88:3240-3248.

Tava A., Berardo N., Cunico C., Romani M. and Odoardi M., 1995. Cultivar differences and seasonal changes of primary metabolites and flavour constituents in tall fescue in relation to palatability. *Journal of Agricultural and Food Chemistry,* 43:98-101.

Thomas H.M., Morgan W.G. and Humphreys M.W., 2003. Designing grasses with a future – combining the attributes of *Lolium* and *Festuca*. *Euphytica,* 133:19-26.

Tilley J.M.A and Terry R.A., 1963. A two stage technique for the in-vitro digestion of forage crops. *Grass and Forage Science,* 18:104-111.

Turner L.R., Holloway-Phillips M.M., Rawnsley R.P., Donaghy D.J. and Pembleton K.G., 2012. The morphological and physiological responses of perennial ryegrass (*Lolium perenne* L.), cockcfoot (*Dactylis glommerata* L.) and tall fescue (*Festuca arundinacea* Schreb.; syn. *Schedonorus phoenix* Scop.) to variable water availability. *Grass and Forage Science,* 67:507-518.

Van den Pol-van Dasselaar A., Vellinga T.V., Johansen A. and Kennedy E., 2008. To graze or not to graze, that's the question. In: Hopkins A., Gustafsson T., Bertilsson J., Dalin G., Nilsdotter-Linde N., Spörndly E. (eds) *Biodiversity and Animal Feed Future Challenges for Grassland Production. Proceedings of the 22th General Meeting of the European Grassland Federation Uppsala, Sweden, 2012*: 706-716.

Van der Meijden R., 2005. Heukels' flora van Nederland. 23rd Edition, Wolters Noordhoff, Groningen, The Netherlands.

Van Dijk G.E., 1964. The inheritance of harsh leaves in tetraploid cocksfoot. *Euphytica,* 13:305-313.

Van Eekeren N., Van Bos M., De Wit J., Keidel H. and Bloem J., 2010. Effect of different grass species mixtures on soil quality in relation to root biomass and grass yield. *Applied Soil Ecology,* 45:275-283.

Van Landuyt W., Hoste I., Vanhecke L., Van den Brempt P., Vercruysse W., 2006. Atlas van de Flora van Vlaanderen en het Brussels gewest. Instituut voor natuur en bosbouwonderzoek, Nationale Plantentuin van Belgie en Flo.Wer, Meise, Belgium.

Verhelst J., 2011. Winterrye and Italian ryegrass as cover crops. Master Thesis, Ghent University, Ghent, Belgium.

Vignau-Loustau L., Huyghe C., 2008 Stratégies fourragères. Editions France agricole, Paris, France.

Vincent J.F.V., 1982. The mechanical design of grass. *Journal of Material Science,* 17:856-860.

Wachendorf M., Ingwersen B. and Taube F., 1999. Prediction of the clover content of red clover- and white clover-grass mixtures by near-infrared reflectance spectroscopy. *Grass and Forage Science*, 54:87-90.

Wichmann F., Widmer F., Vorhölter F.-J., Boller B. and Kölliker R., 2010. Breeding for Resistance to Bacterial Wilt in Ryegrass: Insights into the Genetic Control of Plant Resistance and Pathogen Virulence. In Barth S. and Milbourne D. (eds) *Breeding Strategies for sustainable forage and turf grass improvement. Proceedings of the 29th Eucarpia Meeting, Dublin, Ireland,2012.* 37-46. Springer, Dordrecht, The Netherlands.

Williams T., Evans D.R., Rhodes I. and Abberton M.T., 2003. Long term performance of white clover varieties grown with perennial ryegrass under rotational grazing by sheep with different nitrogen applications. *Journal of Agricultural Science,* 140:150-159.

Wilman D. and Gao Y., 1996. Herbage production and tiller density in five related grasses, their hybrids and mixtures. *Journal of Agricultural Science,* 127:57-65.

Wilman D., Mtengeti E.J. and Moseley G., 1996. Physical structure of twelve forage species in relation to rate of intake by sheep. *Journal of Agricultural Science,* 126:277-285.

Wilman D., Gao Y. and Leitch M., 1998. Some differences between eight grasses within the *Lolium-Festuca* complex when grown in conditions of severe water shortage. *Grass and Forage Science* 53:57-65.

Wims C., Mc Evoy M., Boland T. and O' Donovan M. A., 2010. A comparison between cut and intensively grazed grass swards on dry matter yield of perennial ryegrass. In: H. Schnyder, J. Isselstein, F. Taube, K. Auerswald, J. Schellberg, M. Wachendorf, A. Herrmann, M. Gierus, N. Wrage & A. Hopkins (eds) *Grassland in a changing world. Proceedings of the 23rd General Meeting of the European Grassland Federation, Kiel, Germany, 2010*: 314-316.

Wulfes R. and Taube F., 2010. Effect of maturity type of *Lolium perenne* cultivars on performance of grass-clover mixtures under frequent cutting. In: H. Schnyder, J. Isselstein, F. Taube, K. Auerswald, J. Schellberg, M. Wachendorf, A. Herrmann, M. Gierus, N. Wrage & A. Hopkins (eds) *Grassland in a changing world. Proceedings of the 23rd General Meeting of the European Grassland Federation, Kiel, Germany, 2010*: 374-376.

Xu W.W., Sleper D.A. and Chao S., 1992. Detection of RFLPs in perennial ryegrass, using heterologous probes from tall fescue. *Crop Science,* 32:1366–1370.

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Scientific Publications

International Peer Reviewed Journals (A1 Publications)

Cougnon M., Van Waes C., Baert J., Poinsard L. and Reheul D., 2013. Rendement de mélanges de fétuque élevée et de ray-grass d’Italie avec ou sans trèfle violet en Belgique. Submitted in April 2013 to *Fourrages.*

Cougnon M., Van Waes C., Baert J. and Reheul D., 2013. Performance and quality of tall fescue and perennial ryegrass and mixtures of both species grown with or without white clover under cutting management. Submitted in January 2013 to *Grass and Forage Science.*

Cougnon M., Van Waes C., Dardenne P., Baert J. and Reheul D., 2013. Comparison of near infrared reflectance spectroscopy calibration strategies for the botanical composition of grass-clover mixtures. Published on line in *Grass and Forage Science,* doi: 10.111/gls12031.

D'Hose T., Cougnon M., De Vliegher A., Willekens K., Van Bockstaele E. and Reheul D., 2012. Farm compost application : effects on crop performance. *Compost Science & Utilization*, 20, 49-56.

D'Hose T., Cougnon M., De Vliegher A., Van Bockstaele E. and Reheul D., 2012. Influence of farm compost on soil quality and crop yields. *Archives of Agronomy and Soil Science*, 58, 71-75.

Cougnon M., De Frenne P., Bommelé L., De Cauwer B., Verheyen K. and Reheul D., 2011. The short term agronomic impact of raising canal water levels in grassland areas : a case study in the Belgian polders. *Agriculture Ecosystems & Environment*, 144, 159-166.

De Cauwer B., D'Hose T., Cougnon M., Leroy B., Bulcke R., and Reheul D., 2011. Impact of the quality of organic amendments on size and composition of the weed seed bank. *Weed Research*, 51, 250-260.

De Cauwer B., Van den Berge K., Cougnon M., Bulcke R. and Reheul D., 2010. Weed seedbank responses to 12 years of applications of composts, animal slurries or mineral fertilisers. *Weed Research*, 50, 425-435.

Devos Y., Cougnon M., Vergucht S., Bulcke R., Haesaert G., Steurbaut W. and Reheul D., 2008. Environmental impact of herbicide regimes used with genetically modified herbicide-resistant maize. *Transgenic Research*, 17, 1059-1077.

Devos Y., Thas O., Cougnon M., De Clercq E.M., Cordemans K. and Reheul, D., 2008.. Feasibility of isolation perimeters for genetically modified maize. *Agronomy for Sustainable Development*, 28, 195-206.

Devos Y., Reheul D., Thas O., De Clercq E.M., Cougnon M. and Cordemans K., 2007. Implementing isolation perimeters around genetically modified maize fields. *Agronomy for Sustainable Development*, 27, 155-165.

A2 publications

Devos Y., Cougnon M., Thas O. and Reheul D., 2008. A method to search for optimal field allocations of transgenic maize in the context of co-existence. *Environmental Biosafety Research*, 7, 97-104.

Cougnon M., Van Waes C., Baert J. and Reheul D., 2011. Prediction of the botanical composition of grass-clover mixtures by near infrared reflectance spectroscopy. *Communications in Agricultural and Applied Biological Sciences*, 76, 133-136.

Chapters in books

Reheul D., De Cauwer B., Cougnon M. and Aper J., 2012. What global and/or European agriculture will need from grassland and grassland breeding over the next 10-15 years for a sustainable agriculture. In: Barth S. and Milbourne D. (eds) *Breeding Strategies for sustainable forage and turf grass improvement.* p. 3-18. Springer, Dordrecht, The Netherlands, 391pp.

Cougnon M., Van Waes C., Baert J. and Reheul D., 2012. NIRS calibration strategies for the species composition of grass-clover mixtures. In: Barth S. and Milbourne D. (eds) *Breeding Strategies for sustainable forage and turf grass improvement.* p. 371-375. Springer, Dordrecht, The Netherlands, 391pp.

Cougnon M. Verhelst J., De Dauw K. and Reheul D., 2012. Quantifying early vigour and ground cover using digital image analysis In: Barth S. and Milbourne D. (eds) *Breeding Strategies for sustainable forage and turf grass improvement.* p.147-153. Springer, Dordrecht, The Netherlands, 391pp.

Reheul D., De Cauwer B. and Cougnon M., 2010. The role of forage crops in multifunctional agriculture. In: Boller, B., Posselt, U.K. & Veronesi, F. (eds) Handbook of Plant Breeding Vol. 5 Fodder crops and amenity grasses. P 1-12. Springer, Berlin, Germany, 523 pp.

Devos Y., Cougnon M., Thas O., De Clercq E.M., Cordemans K. and Reheul, D., 2009. Forecasting the feasibility of implementing isolation perimeters between GM and non-GM maize fields under agricultural conditions. In Computing anticipatory systems, 338-350. D. Dubois (univ of Liège), Springer, Berlin, Germany, 428 pp.

Devos, Y., Cougnon, M., Reheul, D., Laenens, L., 2008. Co-existentie tussen transgene en niet-transgene maïs. 82-92. In: MIRA-T 20071 : Milieu- en Natuurrapport Vlaanderen : Focusrapport, p 82-92. Vlaamse Milieumaatschappij, Leuven, Belgium, 295 pp.

Proceedings

Cougnon M., Baert J., Van Waes C. and Reheul D., 2012. Effect of grass species and ploidy on clover content in grass-clover mixtures. In: Goliński P., Warda M. and Stypiński P. (eds) *Grassland – a European Resource? Proceedings of the 24th General Meeting of the European Grassland Federation Lublin, Poland, 2012*, 35-46.

Cougnon M., Baert J., Van Waes C. and Reheul, D., 2012. Tall fescue and Italian ryegrass : an ideal mixture for intensive cutting management. In: Goliński P., Warda M. and Stypiński P. (eds) *Grassland – a European Resource? Proceedings of the 24th General Meeting of the European Grassland Federation Lublin, Poland, 2012*, 35-46.

Cougnon M., Baert J., Van Waes C. and Reheul, D., 2012. Yield and botanical composition of mixtures and monocultures of tall fescue and perennial or Italian ryegrass with or without clover. *Proceedings of the 10 th British Grassland Society Conference*, 99-100.

Cougnon M., Van Waes C., Baert J. and Reheul D., 2012. Prediction of the botanical composition of complex mixtures of grasses and clovers by NIRS. *Proceedings of the 10 th British Grassland Society Conference*, 47-48.

De Cauwer B., Van Den Berge K., Cougnon M., Bulcke R. and Reheul D., 2010. Weed seed bank response to 12 years of different fertilisation systems. *Proceedings of 17th Australasian Weeds Conference, Christchurch, New Zealand*, 76.

Others

Devos, Y., Cougnon, M. and Reheul, D., 2007. Implementeerbaarheid van isolatieperimeters tussen percelen met transgene en niet-transgene maïs in de context van co-existentie in Vlaanderen. MIRA-onderzoeksrapporten.

Verhelst J., Cougnon M. and Reheul D., 2011. Winterrogge als groenbedekker na maïs. Landbouw en Techniek 15, 23-25.

Cougnon M., Reheul D., D'Hose T. and Vandaele E., 2010. Het gebruik van GFT-compost in de maïsteelt. Landbouw en Techniek 18-21.

Supervision of MSc thesis students

De Dauw K., 2009. Veredeling van rietzwenkgras. Masterproef voorgedragen voor tot behalen van de graad van Master in de Bio-ingenieurswetenschappen: Landbouwkunde, Universiteit Gent, Gent, Belgium.

Vanden Nest T., 2009. Het gebruik van CMC compost in de Landbouw. Masterproef voorgedragen voor tot behalen van de graad van Master in de Bio-ingenieurswetenschappen: Landbouwkunde, Universiteit Gent, Gent, Belgium.

Druant D., 2011. Wisselbouw. Masterproef voorgedragen voor tot behalen van de graad van Master in de Bio-ingenieurswetenschappen: Landbouwkunde, Universiteit Gent, Gent, Belgium.

Verhelst J., 2011. Winterrogge en Italiaans raaigras als groenbemenster na maïs. Masterproef voorgedragen voor tot behalen van de graad van Master in de Bio-ingenieurswetenschappen: Landbouwkunde, Universiteit Gent, Gent, Belgium.

Vandermoere S., 2013. Het zaaien van winterrogge en Italiaans raaigras als groenbedekkers na maïs. Masterproef voorgedragen voor tot behalen van de graad van Master in de Bio-ingenieurswetenschappen: Landbouwkunde, Universiteit Gent, Gent, Belgium.

Vermeersch P., 2013. Opbrengst en botanische smenstelling van gras-klaver mengsels onder begrazing. Masterproef voorgedragen voor tot behalen van de graad van Master in de Bio-ingenieurswetenschappen: Landbouwkunde, Universiteit Gent, Gent, Belgium.