Forage potential of *Thinopyrum intermedium*.

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Forage potential of *Thinopyrum intermedium* through near-infrared spectrometry and grown in mixture with various legumes.

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Abstract

Intermediate wheatgrass [IWG; *Thinopyrum intermedium* (Host) Barkworth & D.R. Dewey] is a perennial grass, dual-purpose crop that can provide environmental services. In addition to the grain production, assessing its forage potential is crucial. We developed models for near-infrared (NIR) spectrometry prediction of the chemical composition and digestibility of IWG across various experimental sites. Among these, a Belgian field was used to compare its dual production in pure stands or in mixture with legumes. Good quality NIR predictions were observed, offering an efficient tool to characterize the forage composition of IWG. Its forage parameters were mainly influenced by the phenological stage with an increase of fibers and a decrease of protein, digestibility, and energy content (NE_L) during the growing season. IWG forage at vegetative stages could be used to feed lactating dairy cattle with a NE_L of 1625 kcal kg^{-1} of DM but, its biomass was low averaging 1.8 t of DM ha^{-1}. At grain maturity, biomass was higher (i.e., 5.3 t of DM ha^{-1}), representing 73 to 92 % of the total biomass production, and could replace straw in high-starch dairy diets with a NE_L averaging 849 kcal kg^{-1} of DM. Although the mixture of IWG with legumes enhanced some forage parameters, its value as animal feed was not improved. In mixture, we observed a tradeoff between the increase of the forage yield and the reproductive potential of IWG. These insights can inform the on-going process of breeding and help farmers to design relevant systems to experiment this new crop.

Keywords

Perennial grain – *Thinopyrum intermedium* – Intermediate wheatgrass – Kernza® – Forage evaluation – Legume intercropping

1. Introduction

To reconcile the objective of dedicated land to arable cropping for human food consumption and grasslands area for ecological intensification, intermediate wheatgrass [IWG; *Thinopyrum intermedium* subsp. *intermedium* (Host) Barkworth & D.R. Dewey] is currently proposed as a dual-use perennial grass. The crop can provide both forage and grain as well as other ecosystem services thanks to its year-round soil cover and its persistent root system (Crews et al., 2016; Rasche et al., 2017; Ryan et al., 2018). Due to its actual low grain yield (Fagnant et al., 2024), forage valorization could be a way to generate additional agricultural production to increase crop profitability (Bell et al., 2008; Larkin et al., 2014; Newell & Hayes, 2017). Forage harvesting can be achieved at different time during the year depending on the farmer's objectives. While grains and straws are harvested in summer, additional cuts or grazing operations could be performed in fall, early-spring, or both for forage production (Culman et al., 2023; Hunter et al., 2020). However, knowledge of the forage quality is essential to foster an adequate use of unconventional forage resources such as *Th. intermedium* in the feeding system of domestic herbivores.

Pasture species with high feeding value can increase farm profitability and reduce externalities through the improvement of feed autonomy (Thomas et al., 2021). Chemical composition, intake, digestibility, and efficiency of utilization are the main features determining the nutritive value of a given forage. Notably, the fiber, lignin, and protein contents of forage tissues are associated with voluntary feed intake and digestibility properties (Cherney, 2000). Deriving forage quality from multiple chemical analyses is time-consuming. The near-infrared (NIR) spectrometry is successfully used since multiple decades as a non-destructive alternative to lab reference methods. Nevertheless, a crucial calibration step is required to relate the NIR optical measurements to the desired constituent or property used to define the nutritional quality of forages (Deaville & Flinn, 2000). As for other grasses, *Th. intermedium* forage quality depends on its phenological stage at harvest. Early in spring, before or at the beginning of the reproductive growth, the proportion of leaves is significantly higher than that of stems and represents more than 80 % of the aboveground biomass (Barriball, 2020; Fagnant et al., 2023). After this stage, the proportion of stems increases until spikes are fully emerged. At grain maturity, leaves only represent 13 % of the aboveground biomass, while stems and spikes are respectively 71 % and 16 % of it (Fagnant et al., 2023). This progressive change during the growing season is linked with an increase in total aboveground biomass but is known to lead to a significant decrease in forage quality (Thomas et al., 2021; Wróbel et al., 2023).

Agronomic management of *Th. intermedium* can help to increase the global forage yield potential in terms of quantity and quality. In this way, additional forage harvesting in spring, fall or both increases the total production of biomass of *Th. intermedium* compared to a unique harvest at grain maturity (Hunter et al., 2020). In the study of Culman et al. (2023), harvesting forage in the spring resulted in the lowest forage biomass but with the highest forage quality, fall harvest was intermediate in terms biomass and quality and summer harvest maximized biomass but resulted in the lowest quality. Although the forage quality at grain maturity (i.e., straw) is relatively poor, Hunter et al. (2020) highlighted that the higher quantity of biomass harvested is of great interest as it provides a tangible second outcome besides grains. The impact of forage harvesting, particularly the timing and frequency, on grain yields also needs to be considered. As observed by Culman et al. (2023), harvesting forage in the summer and fall increased grain yield as harvesting forage in the spring reduced grain yield. Besides forage harvesting, the mixture of legumes with forage grasses is known to secure forage yield potential (Louarn et al., 2016) and can improve forage quality. Compared to pure grass forage, protein content can be reinforced, the fiber content lowered (Baumont et al., 2016) and the digestibility and therefore the energy value enhanced, according to the legume species and its chemical and morphological traits. For example, the digestibility of legumes such as lucerne or red clover (i.e., *Medicago sativa* L. and *Trifolium pratense* L.) is generally lower than or equivalent to that of grasses. By contrast, white clover (i.e., *Trifolium repens* L.) stands out for its very high digestibility, superior to that of grasses, due to its notable absence of stems and lower fiber content. In addition, legume digestibility decreases less rapidly over the growing cycle than that of grasses, so their nutritional value is more stable over time (Baumont et al., 2016). In the study of Favre et al. (2019), the forage provided by the mixture of *Th. intermedium* with red clover tended to have lower fiber and higher protein contents compared to a *Th. intermedium* monoculture and increased forage yield. As a dual-use crop, a range of effects of the legume mixtures on the grain yield of *Th. intermedium* is documented. Some experiments showed lower grain yields in lucerne or red clover mixtures than monoculture (Favre et al., 2019; Mårtensson et al., 2022; Pinto et al., 2022; Tautges et al., 2018). Other experiments demonstrated similar grain yields in lucerne, berseem clover (*Trifolium alexandrinum* L.), kura clover (*Trifolium ambiguum* M. Bieb), sweet clover (*Melilotus officinalis* L.), and white clover mixtures than monoculture (Dick et al., 2018; Pinto et al., 2022; Reilly et al., 2022; Tautges et al., 2018). Thus, the interaction between the species within the mixture needs to be studied to favor complementary and reduce competitive relationships.

In this study, we aimed to develop models for near-infrared (NIR) spectrometry prediction of the chemical composition and enzymatic in vitro digestibility of *Th. intermedium* forage. Through the speed of analysis, such model should facilitate the characterization of forage nutritive value of this novel multifunctional species where efforts are still ongoing to describe and improve its dual-use productions. Secondly, using the developed prediction models, we evaluated the grain and especially the forage production of the crop, either in monoculture or in mixture with different legume species.

2. Materials and methods

2.1 *Experimental sites*

To characterize the chemical composition and enzymatic in vitro digestibility of the forage of *Thinopyrum intermedium* (intermediate wheatgrass, IWG) through near-infrared spectra models, samples from different experimental sites were used to cover a wide range of pedoclimatic conditions, agronomic managements, crop ages and phenological stages (Table 1).

Data collected on the BE3 experimental site in Belgium (Table 1) was used to characterize the forage production of *Th. intermedium* under a dual-use management (i.e., spring and autumn forage harvest coupled to grain and forage harvest at grain maturity). This field experiment was conducted during two successive cropping years using a complete randomized split-plot design (4 x 8 m subplots) with a forage harvest factor as the main-plot treatment and a species mixture factor as the split-plot treatment, with four replicates. The forage harvest factor compared different treatments, not studied in this study (Table S1). An autumn forage harvest was performed both years while spring harvest was only performed in the second year due to insufficient plant establishment in the first year. During these mechanical forage harvests (i.e., spring and autumn forage harvest), the aboveground biomass was cut to a height of 7 cm (i.e., above the apex height) and exported from the field. In this experiment, four treatments compared the effects of the mixture with different legumes species: I) *Th. intermedium* monoculture (IWG), II) *Th. intermedium* in mixture with white clover, III) *Th. intermedium* in mixture with red clover and IV) *Th. intermedium* in mixture with lucerne. Legumes were seeded in the interrow of *Th. intermedium*, but only on the half of all interrow to reduce light competition as described in Figure 1. Each subplot (i.e., 4 x 8 m) was split in two: one plot was dedicated to destructive sampling during the growing season and the other one to grain yield measurement in summer.

Table 1 : Detailed information about experimental sites, their design and their management.

	Experimental sites					
Site code:	***BE1***	***BE2***	***BE3***	***FR1***	***FR2***	***FR3***
Location:						
Country	Belgium			France		
GPS Long. (DD)	4.7063	4.7052	4.7091	5.1251	5.0920	5.143
GPS Lat. (DD)	50.5664	50.5659	50.5652	45.4250	45.2746	45.3323
Soil type:	Clay loam			Loam	Sandy-loam (stony)	Sandy-loam
Climate:						
Average annual rainfall (mm)	852			881	984	983
Average annual min temperature (°c)	7			7.8	6.3	6.3

Average annual max temperature (°c)	14.2			16.5	16.1	16.1
Type of experiment:	Research station (microplots)			On-farm experiment		
	Randomized split-plot design (4 replicates)			Strips design (3 replicates)		
Implementation:						
Sowing date	22-09-2017	15-05-2019 23-08-2019	09-09-2021	20-09-2017	15-09-2018	05-09-2017
Seed population	The Land Institute (TLI-C5)			The Land Institute (TLI-C3)		
Seeding rate (kg/ha)	20		12	18		
Interrow spacing (cm)	25	12,5 or 25	28	25	12	20
Field management:						
N fertilization (kg N/ha) BBCH30	0 or 100	50	50	50	50	50
BBCH39	0 or 50	50	0	0	0	0
Fall	0 or 0	50	50	0	0	0
Weeding	Chemical + mechanical		Mechanical	/	/	/
Crop protection	/		/	/	/	/
Post-harvest residue management	Chipping or mowing at 5cm from the ground					
Cropping year for data collection:	2020*, 2021	2019*, 2020, 2021	2022, 2023	2021	2021	2021
Growing stages for data collection (BBCH scale):	BBCH2., BBCH30, BBCH39, BBCH65, BBCH89			BBCH29, BBCH30, BBCH65	BBCH29, BBCH30	BBCH29, BBCH30, BBCH65

N.B.: * is indicating that only the autumn vegetative stage was collected this year. BBCH2. is corresponding to the autumn vegetative stage; BBCH29 to the spring vegetative stage; BBCH30 to the stem elongation stage; BBCH39 to the flag leaf stage; BBCH65 to the flowering stage and BBCH89 to the grain maturity stage.

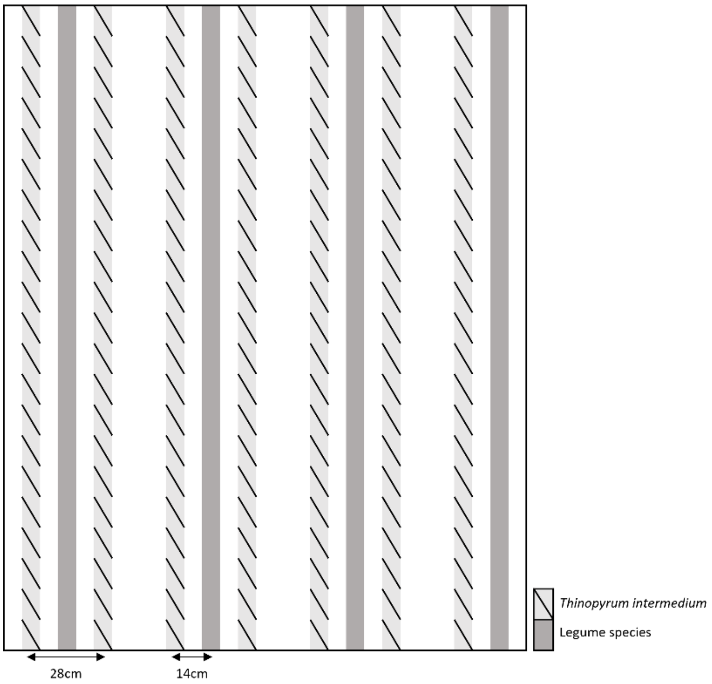

Figure 1 : Interrow disposition in subplots between Th. intermedium and the different legume species in the BE3 experimental site.

2.2 *Data collection*

For all experimental sites, aboveground biomass was sampled during the growing season with a 50 x 50 cm quadrat and cut at 5 cm above soil surface. Fresh samples were weighted to obtain the fresh matter and then oven-dried (72 h at 60 °C) and weighted again to obtain dry matter (DM60). Samples were collected at different phenological stages, rated with the BBCH scale (Meier, 2018), from the vegetative stage (BBCH2.) to the grain maturity stage (BBCH89) as mentioned in Table 1. Samples were then ground to a particle size of 1 mm in a FOSS Tecator Cyclotec 1093 mill; Foss company; Hillerød; Denmark.

The dried and ground samples were subjected to near-infrared radiation with wavelengths ranging from 400 to 2498 nm by using a XDS Monochromator Type XM-1000 FOSS spectrometer; Foss company; Hillerød; Denmark. The spectral data were recorded with a step of 2 nm. The spectral absorbance values were recorded as log (1 R^{-1}), where R is the sample reflectance.

2.3 *Development of near-infrared spectra models*

2.3.1 Chemical analyses

To develop NIRS prediction models for the chemical composition and enzymatic in vitro digestibility of the forage of *Th. intermedium,* 223 samples covering the variation range within the database (Table 1) were selected to undergo the chemical reference analyses.

The parameters described below were expressed on a dry matter basis (method 967.03; AOAC, 1990). The total ash content was determined by weighing the sample fraction remaining after complete calcination at 550 °C (method 942.05; AOAC, 1990). Crude protein (CP) was calculated as total Kjeldahl N multiplied by 6.25 (method 981.10; AOAC, 1990). Fiber contents were analyzed with the Fibercap system (Foss Electric, Bagsvaerd, Denmark). Neutral detergent fiber (NDF) and acid detergent fiber (ADF, method 973.18; AOAC, 1990) contents were determined as described by Van Soest et al. (1991). NDF was analyzed using Termamyl (Novo Nordisk, Bagsværd, Denmark). The acid detergent lignin (ADL) was analyzed according to Van Soest (1963) and the crude cellulose (CEL) according to Weende (method 978.10; AOAC, 1990). The enzymatic in vitro digestibility of organic matter (OMD_{cel}) was analyzed according to De Boever et al. (1986). All analyses were performed with two replicates. Some sample masses were insufficient to perform all chemical analyses, inducing a lower number of observations for the OMD_{cel} parameter (i.e., 126 observations compared to more than 200 observations for the other chemical parameters; Table 2).

2.3.2 Characterization of the database

To identify the factors underlying the variability within the database composed of the different chemical parameters and digestibility of *Th. intermedium* forage (section 2.3.1), a principal component analysis (PCA) was conducted. The *PCA* function of R program version 4.1.2 (R Core Team, 2024) was used on centered and scaled data.

2.3.3 Predictive models and assessment of their quality

All the following analyses were performed on the MatLab R2018a software. First, the NIR spectra of the 223 samples were subjected to pre-processing to remove noisy regions. The *SNV* (Standard Normal Variate) function of Matlab was firstly performed to reduce the effects of interference related to the dispersion and particle size of the sample (Eylenbosch, 2018). Then, the *detrend* function was used to reduce the curvature and offset of the spectra. Finally, the Savitzky-Golay algorithm was applied allowing for curve smoothing and background reduction (Eylenbosch, 2018).

For each forage parameter (Ash, CP, NDF, ADF, ADL, CEL, OMD_{cel}), a PLS regression was performed on the pre-processed spectra (shown in Figure 3) with an explanation of the variance set at 50 %. The standard coefficients (i.e., ß-coefficients) from the PLS regression were used to identify the most significant wavelengths to explain the variability within the parameter values (Eylenbosch et al., 2018). If significant wavelengths of similar sign were too close (i.e., distance set at 25 nm), only the wavelength with the highest ß-coefficient was kept. Finally, these wavelengths were fed into a multiple linear regression to predict the different forage parameters. Of these 223 analyzed samples, 70 % were randomly selected to calibrate the models. The remaining 30 % samples were used for model validation as an internal validation (Table 2).

To evaluate the model quality, the following criteria were used: the modelling efficiency (EF; or the Nash-Sutcliffe model efficiency coefficient), the standard error of calibration (SEC), the standard error of prediction (SEP; i.e., when the validation is performed on a set of independent samples) and the ratio of the standard deviation (RPD; i.e., standard deviation between the database reference values (SD) and the SEP). To define a model as acceptable, EF value should reach at least 0.5, the SEC value should be as low as possible and close to the SEP and finally, the RPD should be greater than 3 (Beaudoin et al., 2008; Minet et al., n.d.; Murphy et al., 2022).

2.4 *Productivity assessment on the BE3 site*

2.4.1 Forage productivity assessment

To assess the forage production of *Th. intermedium* in a dual-use management and compare the effect of the mixture with different legume species, the data was collected from the BE3 experimental site (Table 1). As described in section 2.2, samples of aboveground biomass were collected during the growing season to quantify forage biomass (in DM) of each species separately (i.e., *Th. intermedium*, white clover, red clover and lucerne). Spectral data were collected alongside to predict their forage chemical composition and digestibility. Forage parameters of *Th. intermedium* were derived from the predictive model described in section 2.3 as forage parameters of legumes were derived from a referenced spectral database as described by Minet et al. (2018). To go further in the characterization of the forage composition, the organic matter (OM) content of the samples was determined as the percentage of dry matter excluding the ash content. Specifically for *Th. intermedium*, the crude fat content was analyzed on 26 samples selected to capture the different phenological stages (i.e., diethyl ether extraction with a Soxhlet device, method 920.39; AOAC, 1990) to assign the crude fat content at each phenological stage (i.e., BBCH2. and BBCH30: 30 g kg^{-1} of DM, BBCH39: 25 g kg^{-1} of DM and BBCH65 and BBCH89: 20 g kg^{-1} of DM). The nonfibrous carbohydrates (NFC) content was calculated by removing the crude fat, the CP and the NDF content from the OM content. Finally, from the predicted forage parameters, the net energy for lactation (NE_L) of the different legume species and *Th. intermedium* was calculated according to the Dutch feed evaluation system for ruminants (CVB, 1991). Depending on the forage composition of samples (i.e., ash, fiber content, OMD_{cel} or CP), various feed equations can be used to estimate NE_L values. Following the PCA analysis (see section 2.3.2 and 3.1.1), the composition of *Th. intermedium* varied with the phenological stage of the crop. Different equations were used to estimate the NE_L value of *Th. intermedium*: 'fresh grass' equations for the vegetative stages (i.e., BBCH2. and BBCH30); 'hay' equations for the BBCH39 stage and 'straw' equations for the BBCH65 and BBCH89 stages. The NE_L values of legumes were all calculated with the 'fresh grass' equations.

The different forage parameters (i.e., OM, CP, NDF, ADF, ADL, CEL, OMD_{cel}, NE_L) of the grass-legume mixture were then calculated as the weighted average of *Th. intermedium* and the legume species based on their respective DM proportion of the total mixture DM.

2.4.2 Grain yield productivity assessment

From BBCH30 to BBCH89 stages, tillers and spikes, when present, were counted from the aboveground biomass samples of *Th. intermedium* (as described in section 2.2.) to estimate tiller and spike density. At grain maturity, plots were harvested with a trial combine harvester to obtain grain yield on a cleaned, but unsorted seeds basis (i.e., a mix of hulled and dehulled seeds).

2.4.3 Standard statistical analysis

All data analyses were conducted in the R program version 4.1.2 (R Core Team, 2024). As spring forage harvest was only performed in the second year, an ANOVA was performed for each cropping year separately. Within the different ANOVA, mixed models were applied with *lmer* function from the *lme4* package. Two-way ANOVA was performed with the species mixture and the phenological stage considered as fixed effect, while replicates as random effect. Models were evaluated to ensure they met the assumptions of independence and normality of residuals through the *plotresid* function. Transformation of variables was not necessary as the assumptions were met. Following the ANOVA, pairwise comparisons among treatment means were evaluated with the *emmeans* function from the *emmeans* package with a Tukey adjustment for multiple comparisons. If a significant interaction between fixed factors within the model was observed, this was considered in the post hoc test. Statistical significance was set at 0.05. Grain yield, tiller density and spike density of *Th. intermedium*, aboveground biomass of *Th. intermedium*, legumes and the grass-legume mixture, as well as their forage parameters (i.e., OM, CP, CEL, NDF, ADF, ADL, OMD_{cel}, NE_L) were the analyzed variables.

3. Results

3.1 *Prediction of Th. intermedium forage parameters*

3.1.1 Database characterization

The performed principal component analysis is illustrated in Figure 2, OMD_{cel}, CP and fiber parameters (i.e., ADF, NDF and CEL) were relatively well represented by the first principal components (PC1), explaining 67.5 % of the variance. As expected, principal component analysis indicated that there was a clustering effect on the database through phenological stages. Globally, vegetative stages (i.e., BBCH2., BBCH29 and BBCH30) were on the negative axis of the PC1 indicating higher OMD_{cel}, CP and lower fiber content, while late reproductive stages (i.e., BBCH65 and BBCH89) had the opposite behavior. The flag leaf stage (i.e., BBCH39) represented an intermediate situation (Figure 2).

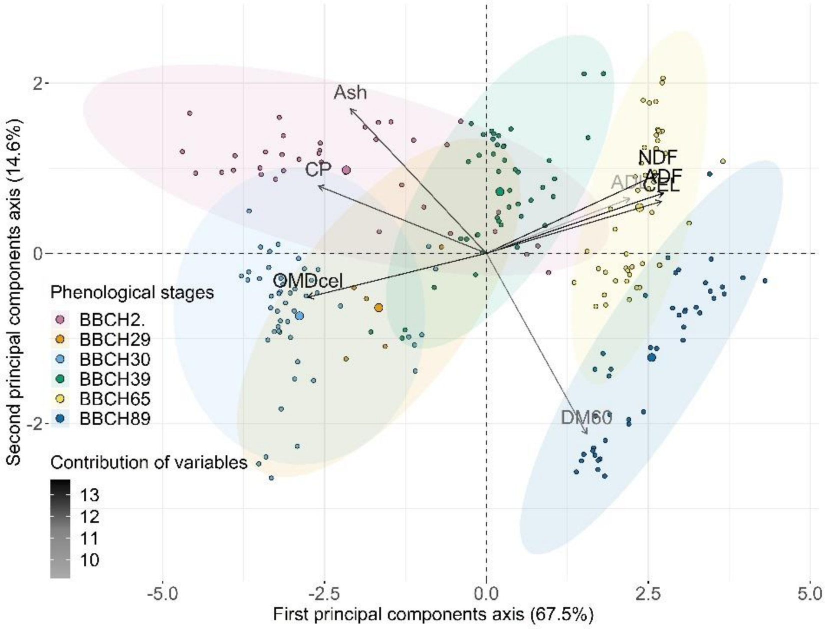

Figure 2 : Principal component analysis for the consolidated dataset used to develop the NIRS predictive models: first and second principal component axis.

Through the variability mainly induced by phenological stages, the contents expressed in g kg^{-1} of DM obtained by standard chemical analyses ranged from 31 to 133 for Ash, from 28 to 313 for CP, from 345 to 799 for NDF, from 164 to 514 for ADF, from 11 to 112 for ADL and from 144 to 528 for CEL. OMD_{cel} ranged from 28 to 93 g per 100g of DM.

3.1.2 *NIRS models performances*

The 223 generated spectra exhibited spectral features expected for dried forage plant samples (Figure 3).

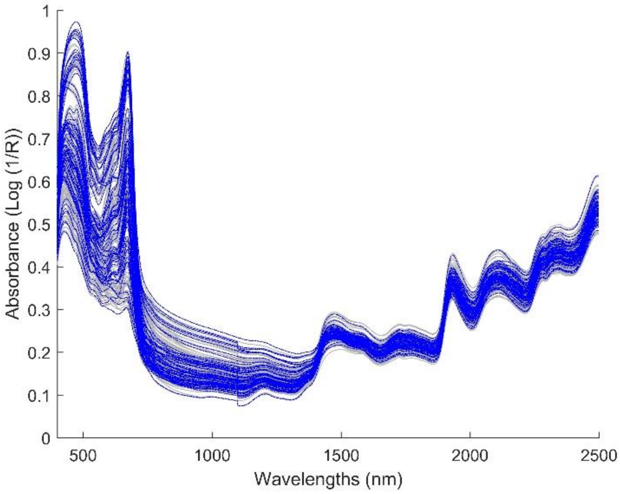

Figure 3 : The 223 generated pre-processed spectra of the consolidated dataset used to develop the NIRS predictive models, spectra in grey were used for calibration and spectra in blue were used for validation. As highlighted by Deaville & Flinn (2000), this type of spectra has prominent absorption bands including water at 1450 and 1940 nm; aliphatic carbon - hydrogen bands (lipids) at 1210, 1400, 1725 and 2310 nm; oxygen - hydrogen bands (carbohydrates) at 1600 and 2100 nm and nitrogen - hydrogen bands (amide structures in protein) at 2055 and 2180 nm.

Performances of the PLS models developed for the NIRS prediction (Table 2) and the relationship between observed and predicted values for the calibration and validation (supplementary materials – Figure S1) indicated that the developed models performed quite well. Except for the ADL content, modelling efficiency (EF) was always above 0.95 for calibration and 0.90 for validation, the standard error of calibration (SEC) values were relatively low and close to standard error of prediction (SEP) values, and the ratio of standard deviation (RPD) values were above 3. Reduced quality of prediction for the ADL content was observed with lower values of EF and RPD (Table 2).

Table 2 : summary statistics for the calibration and validation of PLS models for the various forage parameters.

		Calibration						Validation					
	FPLS	N	Mean	SD	EF	SEC	RPD	N	Mean	SD	EF	SEP	RPD
Ash	20	156	67.2	21.4	0.95	0.50	4.30	67	66.3	22.6	0.90	0.68	3.27
CP	18	155	112.1	61.4	0.99	0.66	9.51	67	117.5	68.8	0.99	0.75	8.90
NDF	17	155	607.1	119.4	0.99	1.26	9.23	66	591.3	118.0	0.96	2.45	5.12
ADF	17	155	344.8	93.8	0.98	1.27	7.38	66	332.7	96.7	0.98	1.46	6.63
ADL	24	154	47.6	20.5	0.77	1.01	2.07	66	43.8	19.8	0.64	1.11	1.67
CEL	17	152	332.7	89.5	0.98	1.29	6.80	65	318.2	88.9	0.96	1.84	5.10
OMD_{cel}	17	88	57.47	18.61	0.98	2.82	6.57	38	60.55	19.80	0.97	3.20	6.31

N.B.: FPLS: number of PLS factors to explain 50 % of variability; N: number of observations; Mean: mean of forage parameters, SD: standard deviation of forage parameters; EF: modelling efficiency; SEC: standard error of calibration; SEP: standard error of prediction; RPD: ratio of standard deviation. Ash, CP, NDF, ADF, ADL and CEL were expressed in g kg^{-1} of DM and OMD_{cel} in g 100g^{-1} of DM.

3.2 *Forage production of Th. intermedium in mixture with legumes*

3.2.1 *Forage quantity*

As illustrated in Figure 4, an establishment year was observed for both *Th. intermedium* and legumes through an increase of biomass from the first to the second cropping year. Particularly for legumes, this establishment was marked by the increase in their relative importance (in terms of DM) in the mixture over the first growing season (i.e., less than 10 % at BBCH30 compared to 23 % for white clover and more than 40 % for red clover and lucerne at BBCH89).

Globally, biomass production of *Th. intermedium* was low (Figure 4). Grown alone, it ranged from 4.8 to 5.8 t of DM ha^{-1} at grain maturity (i.e., BBCH89). In 2023, the spring forage harvest (only performed this year) allowed an additional exportation of biomass of 1.7 t ha^{-1} at the beginning of the reproductive phase (i.e., BBCH30). Low autumn regrowth (i.e., 0.4 t of DM ha^{-1} at BBCH2.) was also observed for *Th. intermedium*, representing lower levels compared to the biomass production at the BBCH30 stage ($p < 0.001$).

The biomass production at grain maturity and at autumn regrowth was increased ($p < 0.01$) when a mixture was performed, especially with lucerne and red clover, as they were the two most productive legumes (e.g., red clover reached 4 to 5.2 t of DM ha^{-1} and lucerne reached 3 to 7.1 t of DM ha^{-1}, compared to 0.2 to 1.2 t of DM ha^{-1} for white clover at BBCH89; Figure 4). At the autumn vegetative stage (i.e., BBCH2.), the legume mixtures increased the biomass production from 225 to 600 % compared to the production of *Th. intermedium* in monoculture. At this stage, *Th. intermedium* was completely dominated by lucerne and red clover (i.e., more than 70 % of legumes within the mixture). Indeed, a strong competition from red clover and lucerne on *Th. intermedium* was observed, especially in 2023, reducing its production ($p < 0.001$). The relative loss of biomass of *Th. intermedium* in mixture compared to its monoculture at grain maturity was from 0.5 to 4.8 t ha^{-1} when associated to red clover or lucerne and from 0.4 to 1.1 t of DM ha^{-1} when associated to white clover (Figure 4). In contrast, white clover was dominated by *Th. intermedium* and represented less than 10 % of the mixture at BBCH89 in 2023.

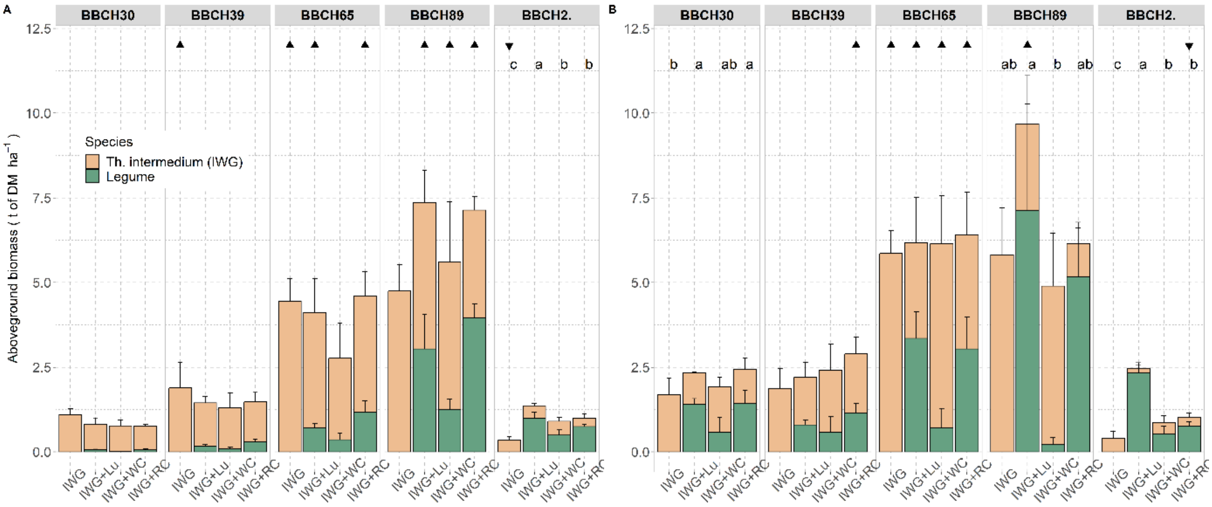

Figure 4 : Total aboveground biomass of the various species mixture treatments during the growing season of (A) 2022 and (B) 2023. Standard errors are indicated by error bars. Letters represent the results of the post hoc analysis of the effect of the species mixture for each phenological stage (i.e., each letter is assigned to a boxplot representing the species mixture treatment). Bar plot with a symbol indicates the results of the post hoc analysis of the effect of the phenological stage for each species mixture treatment; ∇ specifying a decreased value; and Δ specifying an increased value compared to the previous phenological stage, except at BBCH2. where the comparison is with the BBCH30 stage. IWG for intermediate wheatgrass - Th. intermedium, Lu for lucerne, WC for white clover and RC for red clover.

3.2.2 Forage composition and nutritive value

The forage composition of legumes is presented in supplementary materials – Table S2. Globally, lucerne had the highest content in CEL, NDF and ADF and the lowest in OMD_{cel} and NE_L. The opposite was observed with white clover, and red clover showed intermediate values ($p < 0.05$). The CP content of legumes didn't vary across species (Table S2).

Concerning organic matter (OM), it ranged from 888 to 966 g kg^{-1} of DM (Figure 5A) and was composed from 21 to 31 % of hemicellulose (i.e., NDF minus ADF), from 16 to 40 % of cellulose (i.e., ADF minus

ADL), from 2 to 7 % of lignin (i.e., ADL), from 2 to 31 % of crude protein (CP) and from 2 to 3 % of crude fat for *Th. intermedium* monoculture. The remaining part was represented by the nonfibrous carbohydrates (NFC), ranging from 15 to 42 % of the OM. Globally, the OM increased during the growing season ($p < 0.001$). When an effect of the species mixture treatments was observed, OM was generally lower in mixtures compared to the monoculture ($p < 0.01$).

As shown by the PCA (Figure 2), the forage quality of the different mixture treatments was influenced by the phenological stages. Fiber content (i.e., CEL, NDF, ADF and ADL) increased during the growing season, while CP, OMD_{cell} and NE_L decreased ($p < 0.001$; Figure 5). For the monoculture of *Th. intermedium*, the highest CEL, NDF and ADF contents were observed at the flowering stage (i.e., BBCH65; mean, in g kg^{-1} of DM, of 396 for CEL, 690 for NDF and 415 for ADF). The highest content of ADL was observed at the grain maturity stage (i.e., BBCH89; mean of 65 g kg^{-1} of DM). Concerning CP, OMD_{cel} and NE_L the highest values were observed at vegetative stages (i.e., BBCH30 and BBCH2.) with a mean of 201 g kg^{-1} of DM, 84 g per 100 g of DM and 1625 kcal kg^{-1} of DM, respectively.

The forage quality was modified by legumes once they were well established (i.e., representing roughly 20 % of the mixture, Figure 4 and 5) at a leafy vegetative stage, as early phenological stage induced the best forage quality (Table S2). Thus, red clover had globally the major impact on the forage quality compared to white clover and lucerne (Figure 5). In mixture, the NDF content was reduced by about 87 g kg^{-1} of DM regardless of the legume species ($p < 0.01$). At late phenological stages (i.e., BBCH65 and BBCH89), CEL and ADF decreased in the red clover mixture by about 43 g kg^{-1} of DM ($p < 0.05$). Focusing on the CP content, the mixture with legumes buffered the decrease over the growing season, especially with red clover and lucerne ($p < 0.001$) as no effect was observed at the autumn vegetative stage (i.e., BBCH2.). Concerning the OMD_{cel}, the highest increase was observed at late phenological stages with the red clover mixture (e.g., maximal increase of 13 g per 100 g of DM). Finally, legumes increased the energy value (NE_L) and particularly the red clover mixture (e.g., maximal increase of 313 kcal kg^{-1} of DM). Focusing on *Th. intermedium* forage parameters in the mixture, the CP content was the only parameters influenced by legumes at the beginning of the second year (i.e., BBCH30 and BBCH39), with a maximal increase of 30 g kg^{-1} of DM with red clover at BBCH30 (supplementary materials – Figure S2).

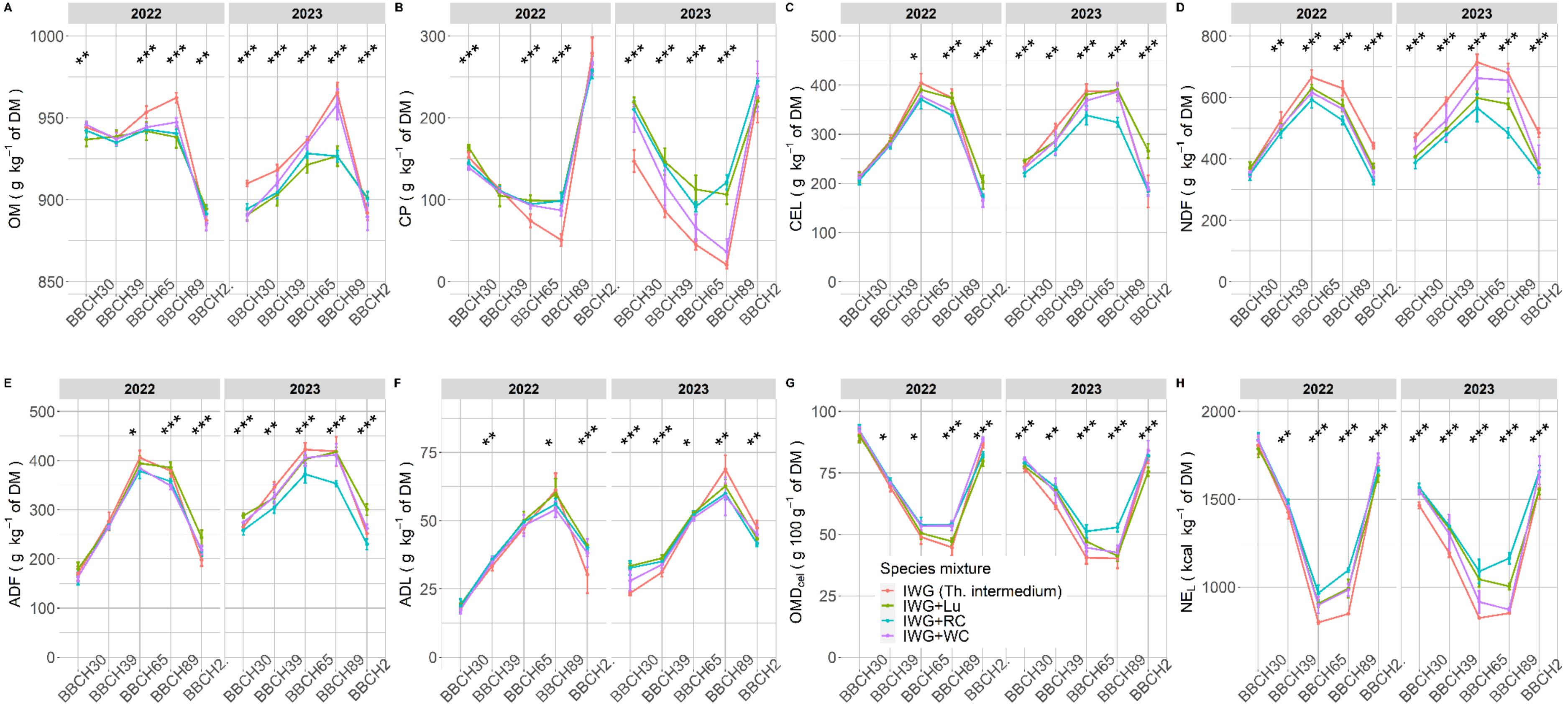

Figure 5 : Evolution of the forage parameters (A) organic matter (OM), (B) crude protein (CP), (C) crude cellulose (CEL), (D) neutral detergent fiber (NDF), (E) acid detergent fiber (ADF), (F) acid detergent lignin (ADL), (G) enzymatic in vitro digestibility of OM (OMD_{cel}), (H) net energy for lactation (NE_L) of the different species mixture treatments during the growing season of 2022 and 2023. Statistical differences (post hoc analysis) between species mixture treatment for each phenological stage are indicated by "" with a statistical significance at $p < 0.05$, "**" with a statistical significance at $p < 0.01$, "***" with a statistical significance at $p < 0.001$. IWG for intermediate wheatgrass - Th. intermedium, Lu for lucerne, WC for white clover and RC for red clover.*

3.3 *Grain production of Th. intermedium in mixture with legumes*

Overall, *Th. intermedium* grain yields were low (Figure 6) and ranged from 345 to 616 kg ha^{-1}. This was partly explained by a weak establishment of the crop during the two first years with an average tiller density of 260 tillers m^{-2} during the entire first reproductive phase and 690 tillers m^{-2} at the beginning of the second reproductive phase, regardless of the species mixture treatment. This led to low spike densities in the first year (i.e., ranging from 165 to 257 spikes m^{-2}). In addition, the spring forage harvest performed in the second year reduced the grain yield potential by about half compared to no spring forage harvest (data not shown).

As observed in Figure 6, the mixture with a legume impacted the grain yield ($p < 0.05$) with the highest grain yield obtained with *Th. intermedium* monoculture and the lowest with the mixture of *Th. intermedium* with lucerne and red clover. Furthermore, in the second year, the mixtures with lucerne and red clover led to the impossibility of harvesting grains due to lodging. Spike density was also impacted by the mixture with legume ($p < 0.01$), with the highest densities obtained with the monoculture (i.e., 260 and 440 spike m^{-2} in the first and second year, respectively) and the lowest with *Th. intermedium* associated with red clover (i.e., 165 and 56 spikes m^{-2} in the first and second year, respectively).

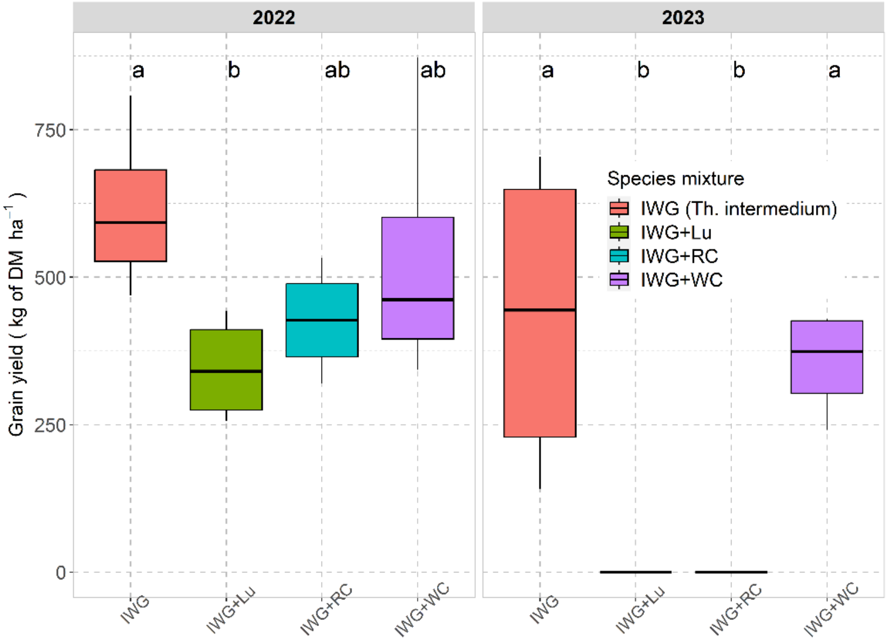

Figure 6 : Grain yield of Th. intermedium under various species mixture treatments in 2022 and 2023. Statistical differences (post hoc analysis) between species mixture treatment are indicated by letters (i.e., each letter is assigned to a boxplot representing the species mixture treatment), with a p < 0.05 in 2022 and a p < 0.01 in 2023. IWG for intermediate wheatgrass - Th. intermedium, Lu for lucerne, WC for white clover and RC for red clover.

4. Discussion

4.1 NIR spectrometry prediction

Th. intermedium samples used to develop the NIR models showed a wide range of values for the different forage parameters. Although the database contained various cropping sites, this variability mainly reflected the ageing tissues from different phenological stages. This was also observed in Culman et al. (2023) where the forage nutritive value was primarily driven by seasonal trends and to a much lesser extent by stand ages. Good quality predictions were obtained for most forage parameters through the different quality criteria. An exception can be mentioned with ADL with a lower quality of prediction due to the weak repeatability of the chemical analysis (Table 2). As our NIR calibration was based on a single species, the predictions were fairly accurate, but could involve less robustness (Deaville & Flinn, 2000). Although satisfactory, this initial model should be further completed with additional data coming from new climatic years to broaden the database. Nevertheless, this first calibration offered a fast and efficient tool to characterize the forage composition of *Th. intermedium* across various pedoclimatic conditions and management practices. In addition, as present-day breeding efforts on *Th. intermedium* for grain yield improvements could constrain the production of vegetative tillers to the benefit of reproductive ones (Altendorf et al., 2021), this method could be helpful to assess the effect of breeding advances on forage quality.

4.2 Forage production potential of *Th. intermedium*

The different forage parameters of *Th. intermedium* were in agreement with other studies on the species (Table 3). Forage quality of *Th. intermedium* at vegetative stages (i.e., BBCH30 and BBCH2.) was satisfactory with an energy content (NE_L) of 1625 kcal kg^{-1} of DM (Figure 5), which was close to a fresh grass (Table 3). Indeed, the various forage parameters of *Th. intermedium* were close to those of common pasture grasses such as *Phleum pratense* L., *Lolium perenne* L., *Festuca pratensis* Huds., *Festuca arundinacea* Schreb. and *Dactylis glomerata* L. at the same stage (Table 3). The protein (CP) content was more variable ranging from 147 to 279 g kg^{-1} of DM and tended to be higher in autumn. Consequently, this fodder could be used to feed lactating dairy cattle with an eventual proper supplementation for balancing the amount of protein and energy in the diet (Cuvelier et al., 2021). However, the biomass production of *Th. intermedium* at vegetative stages was weak, averaging 1.4 t ha^{-1} at the beginning of the spring (i.e., BBCH30) and 0.4 t ha^{-1} at the autumn regrowth. Although our spring production was close to other studies, the autumn production was lower, generally between 1 and 2 t ha^{-1} (Culman et al., 2023; Hunter et al., 2020). The mean total production of vegetative biomass averaged 1.8 t ha^{-1} over a growing season (Figure 4). Pugliese (2017) reported that the production in spring and autumn can, both, reach 4 t ha^{-1}, but only one of the two forage harvests was performed over the growing season.

At grain maturity, the quality of *Th. intermedium* forage was relatively low with NE_L averaging 849 kcal kg^{-1} of DM (Figure 5). The fiber content of *Th. intermedium* was quite close to the reference values of the different common pasture grasses previously mentioned at a flowering stage. However, the OMD_{cel}, CP and NE_L values of *Th. intermedium* were close to common cereals straw (i.e., wheat, barley and oat; Table 3). As already mentioned in the study of Favre et al. (2019), this crop residues could replace straw in high-starch dairy diets to maintain proper rumen function and prevent acidosis (Hurdebise et al., 2023). The biomass production at grain harvest averaged 5.3 t ha^{-1} (Figure 4). This was relatively low compared to the yield potential of *Th. intermedium* in our pedoclimatic conditions that ranged from 7 to 16 t ha^{-1} (Fagnant et al., 2023). It can be explained by the poor establishment of the crop in the first year (i.e., only 260 tillers m^{-2} during the first year) and the spring forage harvest performed in the second year. As observed by Culman et al. (2023), the summer yield potential was highly variable ranging from 2 to 11 t of DM ha^{-1}.

Over a growing season, the yield potential of *Th. intermedium* ranged from 5.2 to 7.9 t ha^{-1} (Figure 4). As observed by Pugliese (2017), when a spring, an autumn or both forage harvests are performed, this yield potential varied widely, but was generally exceeding 9 t ha^{-1} (Favre et al., 2019; Hunter et al., 2020). While the biomass production *Th. intermedium* could reach that of sowed grasslands (i.e., sown European grassland range from 5 to 12 t of DM ha^{-1} y^{-1} and up to 20 t ha^{-1} y^{-1}; Wilkins (2000)), its forage potential was limited. Indeed, most of the biomass was obtained at grain maturity (i.e., more than 70 % of the biomass of the year) with a forage quality comparable to cereal straw, which was of little value in animal feed. In contrast, only 2 to 4 t of DM ha^{-1}, in best cases, could be valorized as good quality *Th. intermedium* fodder. Spring forage harvest represented a way to increase the proportion of good quality fodder, as it decreased the proportion of biomass harvested at grain maturity from 95 to 73 % and converted the remaining percentage into valuable fodder. However, a trade-off between grain and forage harvest was observed. The spring forage harvest decreased grain yield of *Th. intermedium* in our second year (data not shown), as also observed in other studies (Culman et al., 2023; Hopkins et al., 2003; Zimbric et al., 2021). This could be explained by the removal of the leaf area essential for grain production and the limited accumulation of reserves after the spring harvest (Culman et al., 2023). In addition, *Th. intermedium* regrowth was not sufficient to justify an autumn forage harvest. All these insights highlighted the complexity to produce sufficient high-quality fodder in a growing season dedicated to grain production, suggesting a potential forage valorization within extensive livestock production with moderate production goals. As mentioned by Duchene et al. (2021), *Th. intermedium*, as a slow-growing species, could be more suited to harsh pedoclimatic conditions (e.g., fields at high altitudes or with low resource-availability) through its capacity to produce high levels of biomass with low resources requirements such as water or nitrogen (Clément et al., 2022; Fagnant et al., 2023) .

Table 3 : Forage parameters of common pastures grasses and other feedstuffs compared to Th. intermedium at different phenological stages found in the literature.

	Vegetative stages						Maturity stages						References
	OMD_{cel}	CP	CEL	NDF	ADF	NE_L	OMD_{cel}	CP	CEL	NDF	ADF	NE_L	
Dactylis glomerata L.	78	245	177	490	206		57	95	353	680	393		(INRA, 2018)
Festuca pratensis Huds.	82	235	186	499	215		65	113	342	663	367		(INRA, 2018)
Lolium perenne L.	82	223	197	482	221		60	97	328	629	356		(INRA, 2018)
Phleum pratense L.	79	202	224	500	257		51	72	361	664	375		(INRA, 2018)
Festuca arundinacea Schreb.	74	204	235	546	261		57	10	33	65	36		(INRA, 2018)
Fresh grass	84	219				1665							(CVB, 2022)
Hay of "poor quality"							63	106				1152	(CVB, 2022)
Grass seed straw							55	62				990	(CVB, 2022)
Cereal straw							45	35	420	785	493	812	(CVB, 2022; INRA, 2018)
Th. intermedium		[125-225]		[456-590]	[249-337]			[41-73]		[672-828]	[382-501]		(Barriball, 2020; Culman et al., 2023; Favre et al., 2019; Pinto et al., 2022)

N.B.: enzymatic in vitro digestibility (OMD_{cel}) is expressed in g per 100 g of DM, crude protein (CP), crude cellulose (CEL), neutral detergent fiber (NDF) and acid detergent fiber (ADF) in g per kg of DM and net energy for lactation (NE_L) in kcal per kg of DM.

4.3 Production potential of *Th. intermedium* grown in mixture

The mixture of *Th. intermedium* with legumes could improve the forage potential through quantity and, to a lesser extent, quality. We observed this positive impact when legumes represented at least 20 % of the mixture. As observed in Figure 4, this proportion was not encountered before the flowering stage of the establishment year. In addition, we observed differences between legumes, with lower levels of biomass of white clover which was dominated within the mixture contrarily to red clover and lucerne. We also noticed better forage qualities of red clover and white clover compared to lucerne. Through its high biomass production and its good forage quality, red clover had the major impact on the forage quality of the mixture (Figure 4; Table S2). The positive effect of the legume mixture was mainly observed at late phenological stages, with a reduction of the fiber content (i.e., CEL, NDF, ADF) and the increase of the protein (CP) content, digestibility (OMD_{cel}) and net energy (NE_L) (Figure 5). However, at grain maturity, the production of *Th. intermedium* in mixture with legume resulted in a forage with still little value in animal feed. Depending on the forage parameter compared and the legume used within the mixture, the forage was comparable to common pasture grasses at a flowering stage or grass seed and cereal straws that had lower forage nutritive value than a hay characterized by 'poor quality' (Table 3). Concerning the forage quantity, at grain harvest, it was increased with the lucerne and red clover mixtures (i.e., mean increase of 2.3 t of DM ha^{-1}; Figure 4). In autumn, due to the lack of regrowth of *Th. intermedium*, a significant increase of the forage quantity was observed for all the legume mixtures (i.e., mean increase of 1 t ha^{-1}; Figure 4). In the study of Favre et al. (2019) red clover mixture increased forage yield around 3 t ha^{-1} over the year and its CP content as it decreased fiber content in autumn.

The increase of the total biomass production when *Th. intermedium* was associated to red clover or lucerne came at the expense of *Th. intermedium* growth. The crop showed little competitiveness over these two species with a loss of biomass compared to its monoculture at BBCH89 from 1 to 5 t ha^{-1} (Figure 4). This was also reflected in grain yield component with a loss from 22 to 82 % of spike density and therefore a reduction of the grain yield from 37 to 100 % (Figure 6). Indeed, the strong competition of these productive forage legumes (i.e., production level always above 4 t of DM ha^{-1}; Figure 4) induced an impossibility of grain harvesting due to lodging at grain maturity. It was also highlighted by Tautges et al. (2018), where a reduction of the grain yield was observed when *Th. intermedium* was grown in mixture with lucerne that produced from 2 to 4 t of DM ha^{-1}. As Pinto et al. (2022) observed that the high level of red clover and lucerne biomass compromised the establishment of *Th. intermedium* and its grain and forage production. On the contrary, through its low production (i.e., maximum of 1.2 t of ha^{-1} observed at BBCH89 in 2022; Figure 4), white clover had little effect on grain yield with similar spike density and limited reduction of the grain yield compared to *Th. intermedium* monoculture (Figure 6). In the study of Dick et al. (2018), the mixtures with white clover and lucerne didn't impact the production of *Th. intermedium* since their production of biomass didn't exceed 1 t ha^{-1}. Pinto et al. (2022) suggested that the early *Th. intermedium* biomass accumulation in the establishment year was essential with aggressive legume's establishment such as red clover and lucerne. Thus, new agroecosystems should be designed to optimize the complementarity and stability of the mixture of *Th. intermedium* with legume under a dual-use management. Some research was performed to understand how to regulate the competition between *Th. intermedium* and perennial legumes with agronomic management. This included forage cuttings in the interrow (Crews et al., 2022), legume frost seeded in the spring on *Th. intermedium* crop planted in the previous fall (Olugbenle et al., 2021; Pinto et al., 2022) or the implantation of annual legume such as berseem clover (Pinto et al., 2022) to reduce competition. As suggested by Culman et al. (2023), management of the crop could also be shifted from grain production to a single-purpose forage production over the cropping years, enabled by the stability of the forage quantity and quality of *Th. intermedium* over time. Finally, the implantation of legumes may take place after the first years of *Th. intermedium* grain production to allow proper establishment of the crop and maximize the benefits of the legume mixtures for forage production.

5. Conclusion

Through proper model calibrations, near-infrared spectrometry offered an efficient and easy-to-use tool to predict the forage chemical composition and enzymatic in vitro digestibility of *Th. intermedium*, with the need to continuously supply the database to catch the maximal variability of forage constituents. *Th. intermedium* forage potential was reduced as most of the biomass harvested in a dual-use perspective had poor nutritional value. The intensification of forage production through the spring forage harvest or the mixture with competitive legumes came at the expense of the grain production of *Th. intermedium*. Therefore, in the perspective of a dual-use management, the implantation of companion legume such as white clover, in case of good stand establishment, could slightly enhance the forage yield potential (i.e., increase of nutritive value and of forage quantity at autumn regrowth) without hampering the grain production. More competitive legumes, like red clover and lucerne, require more work to find the best varieties or innovative management options in fields. All these insights can inform the on-going process of *Th. intermedium*'s breeding and help farmers to design relevant systems to experiment this new crop.

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The authors have no conflict of interests related to this publication.

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8. Author contribution

L.F.: Conceptualization and planning of the experiments. Writing – original draft Preparation. Carrying out the samplings, data curation, formal analyses (statistical and mathematical).
L.F., V.D., Y.B., B.D.: Formulation of research goals and aims.
L.F., V.D., B.D.: Development and design of methodology. Formal analyses (statistical).
L.F., V.D., Y.B.: Contribution to the interpretation of result.
O.D.; V.D., Y.B., J.B.: Help provided for data presentation and visualization, critical review, commentary and revision, validation.

9. Statement for Data availability

Data will be available on request.

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