EVALUATING THE ESTABLISHMENT OF PERENNIAL FORAGES WITH SUDANGRASS OR SORGHUM-SUDANGRASS AS ANNUAL COMPANION CROPS ON FORAGE YIELD, FORAGE AND SILAGE QUALITY, AND PREDICTED MILK YIELDS

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ABSTRACT

Two studies were conducted to evaluate the use of sudangrass and sorghum-sudangrass as new annual companion crops for the establishment of perennial forages. In Experiment 1, we evaluated the effects of underseeding a perennial forage mix (alfalfa, clover and tall fescue) seeded alone (**control**) or with different annual companion crops [sudangrass (**SG**), sudangrass brown midrib [BMR] (**BSG**), sorghum-sudangrass BMR (**BSSG**), oat or wheat] on forage yields and quality, in vitro total-tract fiber digestibility (**TTNDFD**) using rumen fluids and predicted milk yields. Experiment 2 was conducted to confirm findings of Experiment 1 (i.e. different climatic conditions and soil types). Furthermore, in Experiment 2, all forages (control, SG, BSG and BSSG) harvested at 90 d were ensiled in laboratory silos over 42 d for silage quality evaluations. In both studies, experimental plots (7 or 8 replicates/treatment) were harvested at d 60 (1st cut) and d 90 (2nd cut) at bud stage of alfalfa. Forage indigestible NDF (**iNDF**) was calculated by in vitro incubation at 240 h and potentially degradable NDF (**pdNDF**) was calculated by subtracting iNDF from total NDF. Digestion rate (kd) of pdNDF was estimated by in vitro incubation at 24, 30 and 48 h. The TTNDFD was estimated from pdNDF, kd and passage rate (kp) (Lopes et al., 2015). Data were analyzed using the MIXED procedure of SAS with fixed effects of treatment and cut.

In Experiment 1, results showed that total forage yields (cuts 1 and 2) were higher ($P <$ 0.0001) with SG, BSG and BSSG than control. Oat treatment produced 72% more forage yield in the first than second cut. Yields of individual perennial forages and weeds were lower ($P < 0.0001$) with companion forages than control. When considering companion crops only, yield was lowest with oat, intermediate with BSG and highest with SG and BSSG ($P < 0.0001$). Companion crops reduced ($P < 0.0001$) crude protein (**CP**) and ADL but increased ($P < 0.0001$) the concentrations of NDF, ADF and water-soluble carbohydrates (**WSC**). In vitro TTNDFD followed the order (P < 0.0001): BSG and BSSG (average 59.2%) > SG (55.7%) > control (49.9%) > oat (46.0%).

Evidently, iNDF was lower ($P < 0.0001$) with SG, BSG and BSSG than control and oat treatment. Finally, estimated milk yield was higher ($P < 0.0001$) with SG, BSG and BSSG than control and oat treatment.

In Experiment 2, total forage yield (2 cuts) was higher ($P < 0.0001$) for SG, BSG and BSSG than control. Oat treatment produced 87% less forage yield in the second than first cut. Companion forages reduced ($P < 0.0001$) yield of perennial legumes and weeds, reduced ($P < 0.0001$) ADL and CP concentrations but increased ($P < 0.0001$) NDF and ADF contents. The WSC concentration was highest with oat, intermediate with SG, BSG and BSSG and lowest with control ($P < 0.0001$). The iNDF was lower ($P < 0.0001$) with BSSG than control and oat treatment. In vitro TTNDFD was higher ($P < 0.0001$) for SG, BSG and BSSG compared with control and oat treatment in the second cut. For experimental silages, companion crops reduced ADL ($P < 0.0001$) and CP ($P = 0.0006$) concentrations but increased ($P < 0.0001$) NDF and ADF contents compared with control. Neutral detergent insoluble protein, WSC, total digestible nutrients and net energy of lactation were similar across treatments whereas acid detergent insoluble protein was higher ($P < 0.0001$) for control than remaining treatments. Indigestible NDF fractions were lower ($P = 0.002$) with SG, BSG and BSSG than control. Digestion rate of pdNDF was higher ($P < 0.0001$) in SG, BSG and BSSG than control. In vitro TTNDFD was greater ($P < 0.0001$) with SG, BSG and BSSG than control. Estimated milk yield was higher ($P < 0.0001$) for SG, BSG and BSSG than control and oat treatment.

It was concluded that establishing perennial forages with SG, BSG or BSSG may improve forage yields, nutritive value and fiber digestibility of both and silages, and increase milk yields when fed to lactating cows.

RÉSUMÉ

Deux projets de recherche ont été menés pour évaluer l'utilisation de l'herbe de Soudan et de sorgho-soudan comme nouvelles plantes de compagnonnages annuel pour l'implantation des fourrages vivaces. Dans l'expérience 1, nous avons évalué les effets de semer un mélange de fourrages vivaces (luzerne, trèfle et fétuque élevée) seules (**contrôle**) ou avec différentes plantes de compagnes annuelles [l'herbe de Soudan (**SG**), l'herbe de Soudan avec nervure brune [BMR] (**BSG**), sorgho-soudan BMR (**BSSG**), avoine ou blé] sur les rendements et la qualité des fourrages, digestibilité totale in vitro des fibres (TTNDFD) à l'aide de jus du rumen et l'estimation pour la production de lait. L'Expérience 2 a été réalisée afin de confirmer les résultats de l'Expérience 1 (c.-à-d. dans différentes conditions climatiques et types de sol). En outre, dans l'expérience 2, tous les fourrages (contrôle, SG, BSG et BSSG) récoltés à 90 jours ont été ensilés dans des silos de laboratoire pour une durée de 42 jours afin d'évaluer la qualité des ensilages. Dans les deux études, des parcelles expérimentales (7 ou 8 répétitions/traitement) ont été récoltées à 60 jours (1re coupe) et 90 jours (2^{e} coupe) au stade du bouton de la luzerne. L'indigestibilité de la fibre au détergent neutre (iNDF) a été calculée par l'incubation in vitro à 240 h et le NDF potentiellement dégradable (pdNDF) a été calculé en soustrayant iNDF du NDF total. La vitesse de digestion (kd) pour pdNDF a été estimée par l'incubation in vitro à 24, 30 et 48 h. Le TTNDFD a été estimé à partir de pdNDF, kd, et vitesse de passage dans le rumen (kp) (Lopes et al., 2015).

Dans l'expérience 1, les résultats ont démontré que le total des rendements en fourrage (coupes 1 et 2) était plus élevé ($P < 0.0001$) avec SG, BSG et BSSG comparativement au contrôle. Le traitement avec l'avoine a produit 72% plus de rendement en fourrages dans la première que la seconde coupe. Les rendements des fourrages vivaces et mauvaises herbes étaient plus faibles ($P < 0.0001$) avec l'utilisation des plantes de compagnes que le contrôle. Pour les plantes de compagnes seulement, le rendement était plus faible avec l'avoine, l'intermédiaire avec BSG et

plus élevé avec SG et BSSG ($P < 0.0001$). Les plantes de compagnes avaient réduit ($P < 0.0001$) les niveaux de protéines brutes (CP) et de lignine (ADL), mais ont augmenté ($P < 0.0001$) les concentrations de NDF, ADF et glucides solubles dans l'eau (WSC). Le TTNDFD a suivi l'ordre ($P < 0.0001$): BSG et BSSG (moyenne de 59.2 %) > SG (55.7 %) > contrôle (49.9 %) > avoine (46.0 %). Évidemment, iNDF était plus faible ($P < 0.0001$) avec SG, BSG et BSSG que le contrôle et l'avoine. Finalement, la production de lait estimée était plus élevé ($P < 0.0001$) avec SG, BSG et BSSG comparativement au contrôle et l'avoine.

Dans l'expérience 2, le rendement total des fourrages (2 coupes) été plus élevé ($P < 0.0001$) pour SG, BSG et BSSG qu'au contrôle. Le traitement avec l'avoine a produit 87% moins de rendement en fourrages dans la deuxième que la première coupe. Les plantes de compagnonnages ont réduit ($P < 0.0001$) les rendements des légumineuses vivaces et les mauvaises herbes, réduit ($P < 0.0001$) les niveaux de CP et lignine, mais ont augmenté ($P < 0.0001$) les teneurs en NDF et ADF. La concentration de WSC était plus élevée avec l'avoine, l'intermédiaire avec SG, BSG et BSSG et plus faible avec le contrôle ($P < 0.0001$). L'iNDF était plus faible ($P < 0.0001$) avec BSSG comparativement au contrôle et à l'avoine. Pans la deuxième coupe, le TTNDFD était plus élevé ($P < 0.0001$) pour SG, BSG et BSSG comparativement au contrôle et à l'avoine. Pour les ensilages, les plantes de compagnonnages ont réduit les niveaux de lignine ($P < 0.0001$) et CP ($P = 0.0006$) mais ont augmenté ($P < 0.0001$) les concentrations de NDF et ADF en comparaison au contrôle. La protéine insoluble au détergent neutre, le WSC, nutriments digestibles totaux, et l'énergie nette de lactation ont été similaires pour tous les traitements tandis que la protéine insoluble au détergent acide était plus élevée ($P < 0.0001$) pour le groupe contrôle comparativement aux autres traitements. Les fractions indigestibles de NDF été plus faible ($P = 0.002$) avec SG, BSG et BSSG comparativement au contrôle. Les taux de digestion pour pdNDF étaient plus élevés ($P < 0.0001$) pour SG, BSG et BSSG que le contrôle. Le TTNDFD était plus élevé ($P < 0.0001$) avec SG, BSG et BSSG que le contrôle. La production laitière estimée était plus élevée ($P < 0.0001$) pour SG, BSG et BSSG comparativement au contrôle et à l'avoine.

Il a été conclu que l'établissement de fourrages vivaces avec SG, BSG ou BSSG peut améliorer les rendements en fourrage, la valeur nutritive et la digestibilité des fibres pour les fourrages et ensilages, et améliorer la production de lait lorsque nourrie aux vaches en lactation.

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LIST OF ABBREVIATIONS

ADF	acid detergent fiber
ADICP	acid detergent insoluble crude protein
ADL	acid detergent lignin
BSG	bmr sudangrass
BSSG	bmr sorghum-sudangrass
CP	crude protein
EE	ether extract
iNDF	indigestible neutral detergent fiber
kd	digestion rate
kp	passage rate
NDF	neutral detergent fiber
NDFD	neutral detergent fiber digestibility
NDICP	neutral detergent insoluble crude protein
NE_L	net energy of lactation
pdNDF	potentially digestible neutral detergent fiber
SAS	statistical analysis method
SG	sudangrass
TDN	total digestible nutrients
TTNDFD	total-tract neutral detergent fiber digestibility
WSC	water soluble carbohydrate

CONTRIBUTION OF AUTHORS

This thesis has been written according to the "Guidelines for Manuscript-based Thesis preparation" which has been approved by the Faculty of Graduate Studies, McGill University. I conducted all the experiments, analyzed data and wrote the thesis and manuscripts under the supervision of Dr. Arif Mustafa and Dr. Bushansingh Baurhoo. The studies were designed by Dr. Bushansingh Baurhoo. Both first and second manuscripts are co-authored by Dr. Arif Mustafa and Dr. Bushansingh Baurhoo who supervised and reviewed the written thesis and manuscripts, provided valuable suggestions and contributed to the accomplishment of this thesis.

CHAPTER I. GENERAL INTRODUCTION

In Canada, perennial forages are established as mixtures of legumes (i.e. alfalfa and clover) and gramineaes (i.e. tall fescue and timothy). Legumes are rich in protein whereas grasses are rich in fiber, and a mixture of both provide an excellent source of nutrients for ruminant animals. However, because perennial forages establish slowly in the first year of cultivation (Canevari, 2000), forage yields are very low, forage quality is poor due to insufficient gramineaes proportions and weed infestation is problematic. Traditionally, small grain cereals (i.e. oat or wheat) have frequently been used to establish perennial forages in order to increase harvestable biomass, to suppress weed growth and to reduce risks of soil erosion during the establishment year (Klebesadel and Smith, 1959; Johnston et al., 1998). However, small grain cereals are known to reduce alfalfa yields and density both in the establishment and subsequent years (Lanini et al., 1991). Moreover, it is important to maintain a balance between legumes and grasses because feeding cows high levels of legume silage increases the risks for bloating (Sheaffer et al., 1992; Mourino et al., 2003; Seguin, 2007) and reduces the efficiency of nitrogen utilization (Dewhurst et al., 2003). Inappropriate proportion of grasses and legumes affects the quality of both forage and silage (Xue et al., 2020). Indeed, the high buffering capacity and low concentrations of water-soluble carbohydrates (WSC) of legumes greatly affect the fermentation process during ensiling by resisting pH reduction, therefore causing proteolysis (Xue et al., 2020).

Therefore, there is interest to identify companion crops that could grow harmoniously with perennial forages, increase forage yields and quality during the seeding year without any detrimental competitive effects on perennial forages, and to efficiently control weeds.

Sudangrass and sorghum-sudangrass, warm-season annual forages, are attractive to dairy producers because of their high-yielding capacity over short periods of time (Wright et al., 2012). These annual gramineaes are drought tolerant and contain high levels of protein and digestible fiber (Dangi, 2012; Wright et al., 2012). Moreover, sudangrass and sorghum-sudangrass contain high WSC concentrations (Kondo et al., 2004; Han et al., 2015) thereby improving its ensiling characteristics (Adesogan et al., 2004). In Canada, the cultivation of sudangrass and sorghum-sudangrass is very minimal. Moreover, scientific evaluations about the use of sudangrass and sorghum-sudangrass as companion crops for the establishment of perennial forages are very limited.

To the best of our knowledge, there are no data regarding the use of sudangrass and genetically engineered brown midrib (BMR) hybrids of sudangrass and sorghum-sudangrass as companion crops for the establishment of mixtures of perennial forages (i.e. alfalfa, clover and tall fescue). Data pertaining to forage yield and quality, silage quality and milk yields are still not available.

Therefore, the objectives of this study were to evaluate the effects of underseeding a mixture of perennial forages with regular and BMR hybrids of sudangrass and sorghum-sudangrass on:

1. forage yields, nutritive value, in vitro total-tract NDF digestibility and estimated milk yields when cultivated in a loam soil (Experiment 1) and clay-loam or clay soil (Experiment 2).
2. nutritive value and in vitro total-tract NDF digestibility of experimental silages (Experiment 2).

CHAPTER II. LITERATURE REVIEW

2.1 Significance of forages

The livestock industry including the dairy sector is a key driver to the Canadian economic and agricultural sectors. In Canada, about 81% of commercial dairy farms are located in the provinces of Ontario and Quebec (CDIC, 2018). In Quebec, the dairy sector is the number one agricultural activity with annual cash receipts of $ 2.4 billion (MAPAQ, 2018). Agricultural lands are mostly dedicated to the production of fodder crops and to support the increasing number of livestock animals (CQPF, 2016). Most of the forages can be classified into grasses (Graminae) and legumes (Fabaceae) family. In Quebec, the most commonly cultivated forages include alfalfa (*Medicago sativa* L.), red clover (*Trifolium pratense* L.), white clover (*Trifolium repens* L.), timothy (*Phleum pratense* L.), tall fescue (*Festuca arundinacea* Schreb.), orchardgrass (*Dactylis glomerate* L.), smooth bromegrass (*Bromus inermis* L.) and meadow bromegrass (*Bromus riparius* Rehm.) (CQPF, 2016). These forage species are preferred due to better field management, adaptability to climatic conditions, high yield, and forage quality (CQPF, 2016). Legumes (i.e. alfalfa and clovers) are excellent protein sources whereas grasses (i.e. tall fescue) provide fiber for healthy rumen functions and high levels of water-soluble carbohydrates for proper silage quality and conservation. A mixture of both legumes and grasses provides good quality forage (Wiersma et al., 1999; Sleugh et al., 2000).

2.2 Advantages of grass-legume mixtures

Over the years, several studies have emphasized the importance of cultivating forages as grass-legume mixtures. Seeding a mixture of grasses and legumes can increase forage yield in both the seeding year and post-seeding years (Berdahl et al., 2001; Bélanger et al., 2014). Forage quality of a grass-legume mixture is greater due to high protein content and improved digestibility (Sleugh et al., 2000; Bélanger et al., 2014). This underseeding strategy can also suppress weed infestation in forage cultivations compared with monocultures (Bélanger et al., 2014).

From an animal production perspective, feeding a mixture of grass-legume can alleviate the risks for bloating, reproduction abnormalities and milk fever in dairy cows (Baylor, 1974) and may increase milk yields (Austenson et al., 1959). However, varietal choice of compatible species is important in order to obtain maximum forage productivity (Bélanger et al., 2017) and to lengthen the survival of a grass-legume stand (Berdahl et al., 2001).

2.3 Seeding of perennial forages with companion crops

The major advantages of seeding perennial forages with companion crops are increased forage yield and forage quality (Wiersma et al., 1999), better weed control (Matteau et al., 2020) and reduced soil erosion (Simmons et al., 1992). However, choice of a companion crop depends on several factors such as rate of physiological growth and ability to produce forage, grain or straw (Simmons et al., 1992). Simmons et al. (1992) conducted a survey in Minnesota to determine producer practices and perceptions about alfalfa establishment. From all respondents (351), 85% used companion crops out of which 87% preferred to use oat as a companion crop for alfalfa while the remaining respondents preferred to use spring barley and spring wheat. Oat has been the most preferred and utilized companion crop. Over the years, several studies have evaluated the potential use of oat as a companion crop for alfalfa establishment (Peters, 1961; Genest, 1969; Lanini et al., 1991; Hurley, 1994; Hoy et al., 2002; Sheaffer et al., 2014). However, recently, there has been scientific interests in using warm-season annual forages such as sorghum-sudangrass (La Vallie, 2019) and sudangrass (Matteau et al., 2020) as companion crops for alfalfa establishment.

2.3.1 Effects of small grain cereals as companion crop for perennial forage establishment

2.3.1.1 Forage yield

Several studies have evaluated the effects of underseeding alfalfa with oat on forage yield. Lanini et al. (1991) showed that intercropping alfalfa with oat at various seeding rates improved total forage yield from 1.54 to 5.05 Mg/ha in the seeding year. However, in the establishment year, oat produced higher forage yield in the first harvest only rather than in subsequent harvests. In contrast, another study reported greater total forage yield in both seeding and post-seeding years as a result of oat intercropping (Wiersma et al., 1999). Similar improvement in total forage yield due to oat companion crop have been reported by Hoy et al. (2002) and Matteau et al. (2020). However, oat reduced alfalfa yield (Wiersma et al., 1999; Matteau et al., 2020) and alfalfa density in the post-seeding year (Lanini et al., 1991) because of oat's aggressive growth. These negative effects of oat on alfalfa is even more dramatic at higher seeding rates of oat. In contrast, Sheaffer et al. (2014) reported no effect of oat intercropping on alfalfa yield probably due to variations in the growing environment.

2.3.1.2 Weed control

Small grain companion crops is as an effective and equivalent technique as herbicides to control weeds (Lanini et al., 1991; Hurley, 1994; Hoy et al., 2002). Several studies have evaluated the potential of oat in controlling weeds as a companion crop for alfalfa. Oat seeded with alfalfa at a rate of 36 kg/ha reduced weeds by 75% in the post-establishment year (Lanini et al., 1991). Other studies also reported that oat suppressed weed population in the seeding year (Wiersma et al., 1999; Matteau et al., 2020) and the following year (Wiersma et al., 1999). Barley and wheat intercropping decreased weed population in the first season up to 89% and 84%, respectively (Janson and Knight, 1973). Similar reduction was reported with wheat and barley in alfalfa fields

(Sheaffer et al., 2014). Establishment of oat as a companion crop decreased weed population in the post-seeding year due to the mulching effect of oat straw (Hurley, 1994). In contrast, Hoy et al. (2002) found that the oat residue mulch increased weed biomass compared with oat companion crops.

2.3.1.3 Forage quality

Seeding of alfalfa with oat companion has been reported to reduce forage quality (Table 2.2) by reducing the protein content compared with solo-seeded alfalfa (Wiersma et al., 1999; Matteau et al., 2020). Sheaffer et al. (1988) found that alfalfa seeded together with oat harvested at boot stage had higher in vitro dry matter digestibility and NDF but lower crude protein content than solo-seeded alfalfa. Hoy et al. (2002) evaluated the different establishment methods for alfalfa using oat and reported that oat harvested green for silage had greater crude protein content (i.e. more than 20%) and in vitro dry matter digestibility (i.e. more than 77%) at first harvest in the seeding year. However, forage quality decreased as oat matured at second harvest due to lower crude protein contents and lower in vitro dry matter digestibility and increment in the cell wall contents. Therefore, harvesting stage of oat is important to obtain high-quality forages (Table 2.1; Johnston et al., 1998). Differences in seeding rate of oat also influences forage quality. For instance, oat seeded at higher rate may reduce alfalfa yields (Lanini et al., 1991) and crude protein content (Wiersma et al., 1999) while lower seeding rate can increase weed infestation (Lanini et al., 1991), thereby reducing the forage quality. Therefore, seeding rate of selected companion crops is a critical factor with regards to forage quality and should be determined according to the soil and environmental conditions.

Table 2.1 Forage quality of oat at different maturity

% of DM	Boot	Headed	Milk	Dough
CP	16.4	13.5	10.1	8.4
ADF	35.2	40.9	43.5	43.3
NDF	53.7	60.1	61.2	62.4

Source: Johnston et al. (1998)

Table 2.2 Nutrient concentration of legumes established using oat companion in the seeding year

Establishment method	Legume	CP %	ADF %	NDF %
Solo seeded	Alfalfa	23.4	29.6	35.6
	Red clover	19.6	23.7	34.1
Oat	Alfalfa	21.5	23.1	35.9
	Red clover	23.0	20.6	35.0

Source: Wiersma et al. (1999)

2.3.2 Annual warm-season grasses

Warm-season grasses, with origin from tropical regions, thrive well and are highly-productive under hot climatic conditions (Valenzuela and Smith, 2002). Amongst warm-season grasses, sudangrass and sorghum-sudangrass hybrids have recently been chosen by producers as it offers an adequate amount of forages over shorter periods of time (AERC, 2007; Wright et al., 2012). In addition, warm-season grasses can be advantageous in improving soil structure and suppressing weeds (Wright et al., 2012).

Forage quality of sudangrass hybrid is greater than corn with more protein (15 *vs* 8.5%). However, the quality of sudangrass decreases as the forage matures. In addition, it can regrow rapidly after each harvest and withstand multiple cuts (3 cuts; AERC, 2007). Moreover, hydrocyanic acid (prussic acid) levels, a toxic compound to cows, is very low with sudangrass hybrid (Dangi, 2012; Wright et al., 2012). Sorghum-sudangrass hybrids are developed from forage sorghum and sudangrass (Undersander et al., 1990). Sorghum-sudangrass is adapted to warm climatic conditions and require less water for growth. These hybrids contain high amount of fiber, however, harvesting at the vegetative stage provides an excellent quality forage with more protein and digestible nutrients and less fiber (Wright et al., 2012). However, sorghum-sudangrass usually contain higher prussic acid concentrations than sudangrass, but this risk can be minimized by proper management and harvesting techniques (Wright et al., 2012).

The insertion of brown mid rib (BMR) gene can provide another superior qualities into the sorghum-sudangrass forage by lowering the lignin deposition and cell wall composition (Beck et al., 2013). This genetic-engineered advancement improves forage quality and animal performance. However, harvesting sorghum-sudangrass at late maturity reduces the quality (Beck et al., 2013). Therefore, suitable management practices of warm-season grasses from the establishment to harvest is necessary to obtain high quality forages (Wright et al., 2012).

2.3.3 Effects of seeding perennial forages with or without warm-season forages

2.3.3.1 Forage yield

Data about the effects of underseeding perennial forages with warm-season annual grasses as a companion crop on forage yields are limited. A study in Nebraska (La Vallie, 2019) concluded that seeding perennial legumes (alfalfa, birdsfoot trefoil, Illinois bundle flower, red clover, roundhead lespedeza, and purple prairie clover) with sorghum-sudangrass increased total forage dry matter production but reduced legume yields. The author also reported that three-time cutting frequency could be adopted to obtain more yield from sorghum-sudangrass rather than four times cutting frequency by allowing the plants to produce adequate tillers until late maturity.

Matteau et al. (2020) evaluated the establishment of alfalfa intercropped with annual forage species (i.e. sudangrass, annual ryegrass and oat) in Southwestern Quebec. The authors reported that sudangrass as a companion forage produced greater total forage yield in two of the four environments compared with oat and annual ryegrass. Sudangrass was less competitive than oat and annual ryegrass for growth of alfalfa in the establishment and following years, which resulted in higher alfalfa yields. Similar findings were also reported for alfalfa seeded together with sorghum (Buxton et al., 1998) and soy and cowpea (Basaran et al., 2017). Sudangrass grew well and yielded more when the environmental conditions are favorable (Noland et al., 2017).

Solo-seeded BMR sorghum harvested at different maturity stage (i.e. boot, flower, milk, and soft dough) increased dry matter yield from boot stage (10.7 Mg/ha) to soft dough stage (15.8 Mg/ha; Lyons et al., 2019). Therefore, when alfalfa stands are damaged or weakened due to severe weather conditions, intercropping forage species with sudangrass or sorghum-sudangrass can be adopted by farmers in order to increase forage yields (Buxton et al., 1998).

2.3.3.2 Weed control

The efficacy of companion crops in controlling weeds can be influenced by seeding rate, forage establishment and growth rate (Holmes et al., 2017). In monocropping cultivation system, sorghum-sudangrass was found to be highly competitive against weeds and weed seed production (Lenssen and Cash, 2011). Sorghum-sudangrass contains allelopathic compounds which affect the growth of weed species (Valenzuela and Smith, 2002). Valenzuela and Smith (2002) established a solo stand of sorghum-sudangrass hybrid and reported that 98% weeds were suppressed after six weeks of planting. Therefore, cover crop strategies may be used to control weeds. In order to evaluate the potential of various forage crops in controlling weeds, cover crop mixtures comprising of 12 different species (i.e. spring and summer cover crops) were seeded (Holmes et al., 2017). Among the six summer cover crops, sudangrass effectively suppressed weeds in solo and mixed cultures of all summer cover crops compared to remaining summer crops (corn, radish, cowpea and soybean). Moreover, sudangrass was found to be effective in controlling certain weed species (Bicksler and Masiunas, 2009).

Other studies have investigated the potential of warm-season forages (i.e. sorghum-sudangrass and sudangrass) to control weeds when seeded in mixtures. Sorghum-sudangrass performed well against weeds in binary mixtures of grasses and legumes (Creamer and Baldwin, 2000; Brainard et al., 2011). Similarly, sorghum-sudangrass as a companion crop for perennial legumes (alfalfa, birdsfoot trefoil, Illinois bundle flower, red clover, roundhead lespedeza, and purple prairie clover) also reduced weed growth (La Vallie, 2019). Sudangrass can also be used as a companion crop when establishing alfalfa fields to reduce weed invasion (Matteau et al., 2020).

2.3.3.3 Forage quality

Sorghum-sudangrass hybrids contain more protein (17 *vs* 8.5%), energy (NE_L: 1.6 *vs* 1.4%) and digestible nutrients (70 *vs* 62%) than corn (Table 2.3; Wright et al., 2012). The Canadian forage sorghum-sudangrass hybrid (CFSH 30) has an excellent forage quality with high protein contents (15.71%) and in vitro dry matter disappearance (84.7%; Dangi, 2012). Moreover, forage quality is high when seeded together with alfalfa (i.e. 17.1, 31.4, 49.3% of CP, ADF and NDF, respectively; Dangi, 2012).

Brown midrib sudangrass has 9% less lignin and 7.2% higher in vitro digestibility (Table 2.4; Casler et al., 2003). Backcrossing of BMR genes with regular varieties can also reduce the lignin content (Fritz et al., 1981). However, fiber contents and in vitro digestibility can be influenced by maturity stage (Lyons et al., 2019). Among all physiological stages, harvesting BMR sudangrass before the soft dough stage of grain is desirable for higher forage quality in terms of crude protein, fiber and NDF digestibility (Lyons et al., 2019).

Intercropping of legumes with sudangrass and sorghum-sudangrass can influence the chemical composition of established forages (Buxton et al., 1998; Matteau et al., 2020). The primary objective of such cropping is to increase protein content of harvested forage, thereby reducing supplemental dietary protein to cows (Contreras-Govea et al., 2009). However, chemical composition profiles vary according to botanical composition of forage mixture. For instance, higher percentage of legumes in a forage mixture is strongly correlated with protein concentration (Wiersma et al., 1999). In a companion cropping study for alfalfa using sudangrass, the mixed forages contained lower protein and high fiber concentrations compared with solo-seeded alfalfa (Matteau et al., 2020). Similarly, seeding forage sorghum together with alfalfa produced higher NDF (53.1%), crude protein (15.6%) and less lignin (6.8%; Buxton et al., 1998). In another study, intercropping sorghum-sudangrass with soybean and cowpea (Basaran et al., 2017) produced higher hay quality with higher protein contents and relative feed value. However, in mixed cropping systems, forage quality depends on species, variety, seeding rate and year (Basaran et al., 2017).

Table 2.3 Chemical composition of sudangrass and sorghum-sudangrass hybrids seeded alone or with alfalfa

Chemical composition (% DM)	Sudangrass[1]	Sorghum-sudangrass[2]	Alfalfa + Sudangrass[3]
CP	14-16	17	13.6
NDF	56-58	55	46.6
ADF	29-31	29	28.1
TDN	66-67	70	-

Adapted from [1]AERC (2007); [2]NRC (1989) and Wright et al. (2012); [3]Matteau et al. (2020)

Table 2.4 Neutral detergent fiber (NDF), acid detergent fiber (ADF), acid detergent lignin (ADL) and in vitro NDF digestibility for sudangrass varieties at first harvest

Sudangrass line	%			
	NDF	ADF	ADL	NDFD
Piper-BMR	64.2	38.5	6.4	49.2
Piper-normal	66.6	39.7	7.3	44.9

Source: Casler et al. (2003)

2.4 Determination of nutritive value of forages

Chemical analyses of forages are important for ration formulation. Nutritional value of forages is dependent on several factors such as forage species, maturity stage and environmental conditions (Ball et al., 2001). Nutritive analyses include dry matter, crude fiber, crude protein, ether extract and ash (Ball et al., 2001).

Based on laboratory analytical measurements, fiber can be separated as neutral and acid detergent fiber (Van Soest et al., 1991). The fiber fractions which are insoluble in neutral detergent solution include cellulose, hemicellulose and lignin while the acid detergent insoluble fiber fractions include cellulose, lignin and ash (Ball et al., 2001). Lignin is the final fraction that remains following the digestion of cellulose (Van Soest et al., 1991). Estimation of NDF and ADF fractions is important as it determines the dry matter intake and fiber digestibility, respectively. For instance, higher levels of NDF and ADF reduce feed intake and digestibility, respectively (Schroeder, 2004). The standard level of NDF is 40% for alfalfa and 50% for grasses (Wood et al., 2018) while the ADF level is less than 30% for all forages (Belisle, 2016). Higher or lower levels of fiber than the optimum level have a huge impact on productivity (Wood et al., 2018).

Protein is a major nutrient component in feed quality evaluations. High producing dairy cows require higher levels of protein to be absorbed in the hindgut (Ball et al., 2001). Therefore, protein of a forage can be measured as crude protein (CP = 6.25 × N content of forage). On the other hand, undegradable fraction of protein (i.e. neutral and acid detergent insoluble protein) is measured as nitrogen content that remains in neutral or acid detergent fiber (Ball et al., 2001).

Digestible nutrient contents of forages are crucial in determining milk production. For instance, higher total digestible nutrients (TDN) will improve milk production (Hoffman et al., 2001). Therefore, TDN is estimated using chemical composition of forage such as NDF, CP, ether extract, lignin, NDF nitrogen and ADF nitrogen (Ball et al., 2001). However, digestible NDF is a better estimate to calculate the energy value of a forage (NRC, 2001).

2.5 Forage fiber and fiber digestibility

2.5.1 Importance of fiber

Forage cell wall consists of cellulose, hemicellulose, pectin and lignin (Table 2.5). Analytically, it can be measured as residues following digestion in a neutral detergent solution (Van Soest et al., 1991). Based on digestibility, fiber can be fractionated into potentially digestible (pdNDF) and indigestible fiber (iNDF; Allen and Oba, 1996).

Table 2.5 Fiber fractions of legumes and grasses[1]

Fiber fractions	Legumes	Grasses
Hemicellulose	8 – 12% (alfalfa)	20 - 30%
Cellulose	15 – 30%	15 – 30%
Lignin (iNDF)	<6 -10%<	<3 – 7%<

[1]Adapted from Allen and Oba (1996)

Lignin is the indigestible fiber fraction of the cell wall. The iNDF concentration varies according to forage species (Table 2.5) where grasses have a lower level of iNDF than legumes (Oba and Allen, 1999b). Other factors that affect iNDF content in harvested forages include stage of maturity and environmental conditions (Oba and Allen, 1999b). Forage indigestible fiber and lignin content are strongly correlated and can be estimated as $6.17 \times (\text{lignin/NDF})^{0.77}$ (Traxler et al., 1998). An increase in lignin deposition increases the level of iNDF and therefore reduces the pdNDF fraction (Traxler et al., 1998). Other studies have reported the impact of higher level of iNDF that reduced digestion rate of potentially digestible fiber (Smith et al., 1972) and total-tract NDF digestibility (Lopes et al., 2015a). Moreover, it accounts for lower rumen retention time and higher passage rate than digestible fiber due to indigestible nature (Lund et al., 2007). Therefore, indigestible fiber is an important factor to determine fiber digestibility (Lopes et al., 2015a) and forage nutritional value (Krämer et al., 2012; Van Amburgh et al., 2015; Gallo et al., 2019).

2.5.2 Importance of fiber digestibility and animal performance

Fiber digestibility is a major factor in forage quality evaluations that has a direct impact on dairy cow’s performance (Oba and Allen, 1999b). Ruminal forage NDF digestibility ranges from less than 25% to over 75% (Table 2.6; NRC, 2001). Increase in fiber digestibility influences the dietary energy content and dry matter intake (Allen and Oba, 1996). Digestion of fiber depends on type of forage, retention time and microbial activity (Allen and Oba, 1996). For instance, grasses have lower digestion rate than legumes, and therefore have higher retention time than legumes (Allen and Oba, 1996).

Table 2.6 Ruminal NDF digestibility of different forages[1]

Forages	Ruminal NDF digestibility %
Alfalfa hay	33-63
Alfalfa silage	31-41
Corn silage	32-68
Orchardgrass hay	53-63
Orchardgrass silage	41-48
Red clover hay	31-59
Timothy hay	66-77
Timothy silage	49-52

[1]Adapted from Nocek and Russell (1988)

Several studies have compared the relationship between fiber digestibility and dairy cow performance. Oba and Allen (1999b) reported an increase in dry matter intake and 4% fat-corrected milk yield by 0.17 kg and 0.25 kg, respectively for a unit increase in vitro or in situ NDF digestibility. A similar increase in milk yeld (3.15 kg/d) has been reported by Miller et al. (1990) as NDF digestibility increases. However, the same study showed no effect on dry matter intake. Kendall et al. (1999) studied the effects of different dietary fiber concentrations on in vitro NDF digestibility in early lactating cows. The authors reported greater intake of digestible NDF and higher milk yield when cows consumed high digestible diet compared with low digestible diets. However, Kendall et al. (1990) found no effect of in vitro NDF digestibility on dry matter intake. Similar to Kendall et al. (1990), Lopes et al. (2015b) reported that feeding corn silages with high and low fiber digestibility to dairy cows had no effects on dry matter intake and milk yield.

2.5.3 Effect of BMR gene on fiber digestibility and animal performance

Forage varieties containing BMR gene are less lignified than regular varieties (Cherney et al., 1991; Grant et al., 1995; Casler et al., 2003; Beck et al., 2013). For instance, BMR sudangrass has 9% lower lignin than regular sudangrass (Casler et al., 2003). Forage crops with BMR genes have several advantages. For example, Grant et al. (1995) evaluated the feeding value of BMR sorghum silage for mid-lactating dairy cows and reported that the BMR sorghum-based diet (46.7%) had higher in situ NDF digestibility than regular sorghum-based diet (44.8%). The authors also observed an increase in dry matter intake and 4% fat-corrected milk yield by 24 and 46% per day, respectively as a result of BMR sorghum-based diet. Similarly, Aydin et al. (1999) reported that feeding BMR sorghum silage to cows resulted in 10% greater in situ total-tract NDF digestibility compared with regular sorghum. The authors also observed that milk production (i.e. 24.3 *vs* 21.5 kg/day) and 4% fat-corrected milk yield (i.e. 23.7 *vs* 20.7 kg/day) were higher for dairy cows fed with BMR sorghum silage compared with those fed regular sorghum silage. The improvement in milk yield and 4% fat-corrected milk yield as a result of feeding BMR sorghum silage was estimated as 13%. Muller et al. (1972) studied the effects of BMR corn silage (34% less lignin than regular corn) on NDF digestibility in lambs. The authors found that BMR corn silage had 20% greater in vitro NDF digestibility and 29% higher dry matter intake than lambs fed regular corn silage. Similarly, Oba and Allen (1999a) observed greater dry matter intake (2.1 kg/d) and milk yield (2.6 kg/d) for cows fed BMR corn silage diet (56% forage and 44% concentrate) than cows fed normal corn silage diet.

2.6 Using in vitro method to estimate total-tract fiber digestibility

In vitro digestibility of forages is estimated by anaerobic incubation of dried ground samples in buffered rumen fluid over a specific time period with the provision of optimum environmental conditions (i.e. pH and temperature; Oba and Allen, 2005). A two-stage conventional in vitro procedure was developed by Tilley and Terry (1963) to estimate fiber digestibility. The first stage involved anaerobic incubation of forage samples in rumen fluid for 48 h followed by pepsin-HCl digestion of residues for 48 h (second stage). This method was modified by Goering and Van Soest (1970) by influxing in neutral detergent solution after 48 h of in vitro incubation. The reasons for estimating NDF digestibility are that forages with high NDF digestibility are important in milk production and to determine the energy value (Hoffman et al., 2001).

2.6.1 Modification of in vitro fiber digestibility

In vivo methods used to measure fiber digestibility have been replaced by in situ and in vitro methods to reduce cost and complexity (Lopes et al., 2015b). The conventional in vitro procedures performed by Tilley and Terry (1963) and Van Soest et al. (1991) have not been justified at ad libitum feed intake in dairy cows. Consequently, different values from in vitro NDF digestibility of forages may not be accurate for in vivo digestibility results (Goeser, 2008). Moreover, Lopes et al. (2015) reported that the use of in vitro single time point to estimate total-tract NDF digestibility (TTNDFD) is problematic due to the insignificance relationship (P = 0.54 and R^2 = 0.02) between in vivo TTNDFD at a particular in vitro time point (i.e. 30 h incubation). Single time point determines digestibility by estimating residual fiber after the period of incubation (e.g. 30 or 48 h). The residue consists of both potentially digestible and indigestible fiber fractions. Furthermore, single time point failed to estimate the retention time of forages and thus cannot be utilized to predict fiber digestibility (Lopes et al., 2015b).

According to Allen (2011) inaccuracy in the prediction of digestion rate and passage rate of forages using in vitro methods may either overestimate or underestimate in vivo fiber digestibility. Therefore, certain aspects should be considered when utilizing a model to estimate fiber digestibility. These include differentiation of fiber into potentially digestible fiber and indigestible fiber, determination of accurate digestion rate (kd) and passage rate (kp) together with "selective retention of feed particle" (Huhtanen et al., 2008).

2.6.2 Total-Tract Neutral Detergent Fiber Digestibility (TTNDFD)

Researchers from the University of Wisconsin-Madison have recently proposed a direct in vitro model with added aspects for estimating fiber degradation (Combs, 2013):

(***TTNDFD = 100 × {pdNDF × [kd / (kd + kp)]}/0.9***

The TTNDFD model was based on the fact that fiber digestion starts primarily in the rumen and ends in the hindgut. The model composed of pdNDF, kd and kp (Lopes et al., 2015a) where the pdNDF can be estimated by the difference between total NDF and iNDF (pdNDF = NDF-iNDF; NRC, 2001) while forage iNDF can be determined from 240 h in vitro incubation (Goeser and Combs, 2009). Digestion rate can be estimated from NDF residues calculated at multiple ruminal incubation time points (i.e. 24, 30 and 48 h; Goeser and Combs, 2009), using first order kinetics model (Mertens, 1993) while passage rate can be estimated using a regression model (Krizsan et al., 2010). Estimation of NDF digestibility using a direct model would be essential to formulate dairy cattle rations and to improve effective use of forages (Lopes et al., 2015b).

To validate the TTNDFD model, an in vivo study with two types of corn silage with different fiber digestibility (i.e. high fiber digestibility corn silage; HFDCS and low fiber digestibility corn silage; LFDCS) was conducted by Lopes et al. (2015b) to estimate digestion rate of pdNDF and total-tract NDF digestibility. The researchers observed similar pdNDF, digestion and passage rates between in vivo and in vitro methods (Table 2.7). The estimated value of TTNDFD for in vivo and in vitro was 50.3 and 50.2% respectively for cows fed with HFDCS.

However, the in vitro method (42.9%) underestimated the in vivo (48.6%) with TTNDFD of LFDCS. Nevertheless, the researchers reported that the TTNDFD model can be used to predict fiber digestibility and rate of fiber digestion in high producing lactating cows (Lopes et al., 2015a).

Table 2.7 Comparison of NDF digestion of diets predicted from TTNDFD model and observed in vivo[1]

Item	Method		SEM	*P*-value
	Predicted	In vivo		Method[2]
Input				
pdNDF,[3] kg/d	4.74	4.44	0.20	0.20
pdNDF kd,[4] %/h	4.11	4.27	0.46	0.72
pdNDF kp,[5] %/h	2.67	2.79	0.35	0.56
Output				
NDF digested in rumen, kg	2.73	2.63	0.22	0.64
NDF digested in hindgut, kg	0.36	0.64	0.19	0.05
NDF digestibility in total-tract, kg	3.09	3.27	0.22	0.42
Total-tract NDF digestibility, % of total NDF	46.4	49.5	2.07	0.13

[1]Adapted from Lopes et al. (2015b)
[2]Method = comparison between in vitro and in vivo values.
[3]pdNDF = potential digestible NDF fraction.
[4]kd = digestion rate.
[5]kp = passage rate.

CHAPTER III.

ESTABLISHMENT OF PERENNIAL FORAGES WITH ANNUAL SUDANGRASS OR SORGHUM-SUDANGRASS HYBRIDS IMPROVED FORAGE YIELDS, IN VITRO TOTAL-TRACT FIBER DIGESTIBILITY AND PREDICTED MILK YIELDS

3.1 ABSTRACT

This study investigated the effects of underseeding a mixture of perennial forages (alfalfa, clover and tall fescue) with different annual companion crops on forage yields, chemical composition and total-tract NDF digestibility (TTNDFD). Treatments included the perennial forage mix seeded alone (control) or with a companion crop [sudangrass (SG), sudangrass brown midrib (BMR) gene 12 (BSG), sorghum-sudangrass BMR gene 6 (BSSG), oat or wheat]. Experimental plots (7 replicates / treatment) were harvested on d 60 (1^{st} cut) and d 90 (2^{nd} cut) at the bud stage of alfalfa. Forage indigestible NDF (iNDF) was calculated by in vitro incubation with rumen fluid at 240 h whereas potentially degradable NDF (pdNDF) was calculated by subtracting iNDF from total NDF. Digestion rate (kd) of pdNDF was estimated by in vitro incubations at 24, 30 and 48 h. The TTNDFD was estimated from pdNDF, kd and passage rate (kp) (Lopes et al., 2015). Data were analyzed as repeated measures using the MIXED procedure of SAS with fixed effects of treatment and cut. Results showed that total forage yields (cuts 1 and 2) were higher ($P < 0.0001$) with warm-season forages (i.e. SG, BSG and BSSG) than control and oat treatment. The use of companion crops reduced ($P < 0.0001$) yields of individual perennial forages and weeds. Yields of individual companion crops followed the order ($P < 0.0001$): SG = BSSG > BSG > oat. Chemical analysis of harvested forages showed that companion forages reduced ($P < 0.0001$) CP and ADL concentrations but increased ($P < 0.0001$) NDF and ADF levels. In vitro iNDF fraction was lower ($P < 0.0001$) with warm-season forages than control and oat treatment. In vitro TTNDFD followed the order ($P < 0.0001$): BSG = BSSG (average 59.2%) > SG (55.7%) > control (49.9%) > oat (46.0%). Finally, estimated milk yield was higher ($P < 0.0001$) with SG, BSG and BSSG than control and oat treatment. In conclusion, seeding perennial forages with SG, BSG or BSSG may improve forage yields, fiber digestibility and milk yields.

Key words: dairy cows, forages, alfalfa, sudangrass, fiber digestibility

3.2 INTRODUCTION

High quality forage is not only the major source of nutrients (i.e. protein, energy and fiber) to dairy cows, but it can also lower feed costs by reducing the use of concentrated feeds (i.e. grains and soybean meal) for higher farm's profitability. In Canada, forages are mostly cultivated as mixtures of perennial legumes (i.e. alfalfa) and grasses (i.e. tall fescue and timothy) for higher yields and better nutritive values. Alfalfa, the most commonly fed legume, is an excellent source of protein and energy whereas grasses provide adequate fiber for cud chewing and rumination. Therefore, grass is an important component in cow's diet to reduce bloat caused by feeding high quantity of legumes (Sheaffer et al., 1992; Mourino et al., 2003; Seguin, 2007).

However, in the establishment year, forage yields are usually low because physiological growth of perennial gramineaes is extremely slow. Moreover, harvested forages are more concentrated in legumes, thereby increasing the risks for bloating when fed to cows. Traditionally, small grain cereals (i.e. oat) have been used as companion crop when establishing perennial forages. However, oat has been reported to reduce alfalfa yield both in the establishment and post-establishment years (Lanini et al., 1991). Therefore, we believe that warm-season grasses may represent new promising options as companion crops. We have special interest in sudangrass, an annual Gramineae, because of its high yield and interesting nutrient profile for dairy cows. For example, sudangrass has higher crude protein and digestible fiber contents than corn (Wright et al., 2012). Most importantly, we recently showed that establishing alfalfa with sudangrass produced higher forage yields compared to alfalfa seeded alone or with annual ryegrass or oat, and that sudangrass did not affect the physiological growth of alfalfa (Matteau et al., 2020). However, to the best of our knowledge, there is still no information regarding the use of sudangrass as a companion crop for the establishment of mixtures of perennial legumes and grasses. Moreover, detailed nutrient evaluations about this mixed cropping strategy with sudangrass pertaining to dairy cow's nutrition is still unknown. Finally, the use of brown midrib (BMR) hybrids of sudangrass as a companion crop has not been evaluated yet.

Therefore, the objectives of the present study were to evaluate the effects of underseeding a perennial forage mix with different annual companion crops (sudangrass, brown midrib (BMR) sudangrass, BMR sorghum-sudangrass, wheat and oat) on forage yields, forage quality, in vitro total-tract neutral detergent fiber digestibility (TTNDFD) and estimated milk yields.

3.3 MATERIALS AND METHODS

3.3.1 Experimental site and treatments

This study was conducted over two successive years (i.e. the seeding year and first post-seeding year) at McGill University Agronomy Research Centre located in Sainte-Anne-de-Bellevue, QC (45° 26' N, 73° 55' W). Experimental plots were established in late May 2018 in a St-Bernard loam soil. Forage treatments included a mixture of alfalfa (11 kg/ha), red clover (2 kg/ha), white clover (1 kg/ha) and tall fescue (5 kg/ha) seeded alone (control) or with 1 of 5 companion crops [sudangrass (20 kg/ha; **SG**), sudangrass brown midrib (BMR) gene 12 (20 kg/ha; **BSG**), sorghum-sudangrass BMR gene 6 (46 kg/ha; **BSSG**), oat (90 kg/ha) or wheat (160 kg/ha)]. Seeding rate for BSSG was adjusted to contain equal number of seeds as SG or BSG. For the purpose of this study, all seeds were provided by Belisle Solution Nutrition Inc. (Saint-Mathias, Quebec, Canada). Each plot (5 x 1.5 m^2) consisted of seven rows separated by 18 cm at a target depth of 2 cm and seeded using a no-till experimental seeder (Fabro ltd, Swift Current, SK, Canada). Treatments were assigned to experimental plot in a randomized complete block design with seven blocks.

Soil was fertilized according to soil test analysis and CRAAQ guidelines for alfalfa establishment (CRAAQ, 2010) without the use of herbicides and pesticides. During the seeding year, all plots (except for the wheat treatment) were harvested at the budding stage of alfalfa for a total of two harvests at 60 (first harvest) and 90 (second harvest) days post-seeding. Wheat was manually harvested at the dough grain stage and with no forage yield in the seeding year. During the post-seeding year, all plots were harvested four times at the budding stage of alfalfa.

3.3.2 Field data collection

Experimental plots (area of 4 x 0.6 m^2) were harvested using an experimental flail mower (Swift Machine and Welding ltd, Swift Current, SK, Canada) and fresh forage yield was recorded per plot. For each plot, a 500 g subsample of harvested forages was dried at 55^oC for 48 h in a forced-air oven to determine forage yield on a dry matter (**DM**) basis and subsequently ground through a 1 mm screen using a Wiley mill (Standard model 4, Arthur H. Thomas Co., Swedesboro, NJ) for laboratory analyses.

From the same experimental plots, another area of 0.35 m^2 was manually harvested and hand-separated by plant species (i.e. alfalfa, clover, tall fescue, companion crop and weeds). Plant components were then dried at 55^oC for 48 h in a forced-air oven to determine DM yield contribution of each species to total forage yields. Alfalfa tillers (representative area of 0.35 m^2) were counted in fall of the seeding year and in spring of the following year to assess alfalfa establishment and winter survival (Palmer and Wynn-Williams, 1972).

3.3.3 Chemical analyses

Dried and ground forage samples were analyzed for DM, ash and acid detergent lignin (**ADL**) according to the Association of Official Analytical Chemists (AOAC, 1990). Crude protein (**CP** = N x 6.25) was analyzed using Leco Nitrogen Analyzer (TruSpec Nitrogen Determinator System; Leco Corp., St Joseph, MI) while ether extract (**EE**) was analyzed using an Ankom^{XT15} Extractor (Ankom Technology Corp., Macedon, NY) and following standard procedures (AOAC, 1990). Neutral (**NDF**) and acid (**ADF**) detergent fiber were determined using an Ankom Fiber Analyzer (Ankom Technology, Macedon, NY, USA) as previously described by AOAC (1990). Heat stable α-amylase was used in NDF analysis without the use of sodium sulfite (Van Soest et al., 1991). Acid (**ADICP**) and neutral (**NDICP**) detergent insoluble protein were determined by analyzing ADF and NDF residues for CP, respectively.

Water-soluble carbohydrates (**WSC**) were determined using extractions of dried forage samples (0.2 g) into 35 mL deionized water for 1 h at 40°C (Hall, 2014). Following extraction, samples were centrifuged at 12,000xg for 10 min at ambient temperature and supernatant was used for WSC determination using the phenol-sulfuric acid method (Dubois et al., 1956).

Total digestible nutrients (**TDN;** Weiss et al., 1992) and net energy of lactation (**NE_L**; NRC, 2001) were calculated from chemical composition using standard equations. Finally, milk yield was estimated for each treatment using the alfalfa-grass spreadsheet of Milk2016 (Undersander et al., 2016).

3.3.4 In vitro fiber digestibility of experimental forages

All animal procedures were approved by the Animal Care Committee of the Faculty of Agriculture and Environmental Sciences of McGill University. Total-tract NDF digestibility (**TTNDFD**) of dried forages was determined using a Daisy^{II} incubator (Ankom Technology, Fairport, NY) as described by Lopes et al. (2015). The method was based on in vitro incubations at multiple time intervals using rumen fluid followed by NDF analysis. All in vitro procedures were conducted using rumen fluid obtained from a cow in late lactation. Forage indigestible NDF (**iNDF**) was calculated by in vitro incubation for 240 h (Goeser and Combs, 2009) whereas potentially degradable NDF (**pdNDF**) was calculated by subtracting iNDF from total NDF (NRC, 2001). Degradation rate (**kd**) of pdNDF was calculated from NDF residues of forage samples incubated for 24, 30 and 48 h (Goeser and Combs, 2009) using a first order kinetics model (Mertens, 1993). The passage rate of potentially digestible NDF (**kp** = 2.38% /h) was calculated from standard equation (Sniffen et al., 1992). Total-tract NDF digestibility was estimated using the following equation (Lopes et al., 2015):

$$TTNDFD = 100 \times \{pdNDF \times [kd / (kd + kp)]\}/0.9$$

3.3.5 Statistical analysis

Data were analyzed as a randomized complete block design using PROC MIXED procedure of SAS (SAS Institute, 2014). Differences between treatments means were tested using Scheffe's multiple comparison *t*-test and statistical significance was declared at $P < 0.05$ level.

3.4 RESULTS AND DISCUSSION

3.4.1 Forage yield in the establishment year

3.4.1.1 *Total forage yield*

The establishment of perennial forages with annual warm-season companion forages (SG, BSG or BSSG) significantly increased ($P < 0.0001$) total forage yields in the seeding year when compared to both control and oat treatments (Table 3.1). Higher forage yields were due to higher proportions of companion forages used for establishment. In agreement with our findings, Matteau et al. (2020) reported 133% increase in forage yield when alfalfa was established together with sudangrass compared with solo-seeded alfalfa. The authors also reported higher forage yield with sudangrass than oat (Matteau et al., 2020). Similar increase in forage yield was observed when sorghum-sudangrass was seeded with alfalfa and clover (La Vallie, 2019).

Forage yields were different between harvests in the establishment year. In the first cut, all companion forages produced higher (P < 0.0001) total forage yields than control while in the second cut SG, BSG and BSSG produced higher (P < 0.0001) yields than the oat treatment. In contrast to the first cut, total forage yields in the second cut were increased in control (135.4%), SG (9.5%) and BSG (34.3%) but reduced for BSSG (-34.1%) and oat (-71.85%). For the oat treatment, lower forage yield in the second compared to the first cut was likely due to the poor regrowth of oat after the first harvest. Our later findings are in agreement with previous reports indicating that establishment of alfalfa with oat also produced higher forage yield in the first harvest compared to subsequent harvests when compared with alfalfa seeded alone (Lanini et al., 1991; Matteau et al., 2020). In our study, forage yield was consistently increased with SG and BSG treatments throughout the harvests, which agreed with the findings of Matteau et al. (2020). Overall, all of the warm-season forages produced higher total forage yields compared to control and oat treatments.

3.4.1.2 *Yield of perennial and companion forages*

Establishment of perennial forages with companion crops negatively impacted alfalfa, clover and tall fescue yields (Table 3.1). In the first harvest, when compared with control, alfalfa and clover yields were reduced (P < 0.0001) by all companion forages whereas tall fescue yield was severely affected (P = 0.0059) by the oat treatment only. In the second harvest, alfalfa and clover yields were reduced (P < 0.0001) by all companion forages while tall fescue yield was reduced ($P < 0.0001$) by BSG, BSSG and oat treatments compared with control. When compared to SG and BSG, BSSG also reduced alfalfa and clover yields, but these reductions were not statistically different. Perennial forages grew poorly due to competitive growth exerted by companion crops and/or adverse drought climatic conditions that prevailed during the growing periods. The negative effect of sudangrass on alfalfa growth and yield has previously been reported (Matteau et al., 2020). Nevertheless, the reduction in alfalfa yield was less significant with sudangrass than oat, and agree with previous findings (Matteau et al., 2020). Oat can drastically reduce alfalfa yield in the seeding year due to its vigorous growth, especially at high seeding rates (Lanini et al., 1991). This oat strategy leads to stand thinning for alfalfa favoring weeds to invade alfalfa fields in the following year (Lanini et al., 1991).

Amongst all companion forages evaluated in this study, yields of warm-season forages were higher ($P < 0.0001$) than those of oat. Sudangrass and sorghum-sudangrass had more tillers after harvest (i.e. in the second than first cut). Indeed, warm-season forages have the capacity to accumulate adequate reserves between harvest to produce more tillers and higher yields (La Vallie, 2019). Our findings that higher total forage yields were due to higher proportion of companion forages (sudangrass and sorghum-sudangrass) are in agreement with previous studies (La Vallie, 2019; Matteau et al., 2020).

3.4.1.3 *Weed control*

Establishing perennial forages with companion crops significantly reduced ($P < 0.0001$) weed biomass compared with control. In particular, more than 65% of weeds were suppressed by SG, BSG and BSSG compared with control, suggesting that establishing perennial forages with warm-season crops is an effective strategy to control weeds. Sudangrass may selectively control certain weed species (Bicksler and Masiunas, 2009), and this may explain less weed suppression in certain cases. The capability of sorghum-sudangrass to effectively control weeds when seeded in binary mixture of grasses and legumes or with different legumes (i.e. alfalfa and clover) has previously been demonstrated (Creamer and Baldwin, 2000; Brainard et al., 2011; La Vallie, 2019). In the latter study, more than 50% of weeds were suppressed in the field with sorghum-sudangrass. Oat also suppressed weed growth in contrast to control. Similar findings were observed when oat was established with alfalfa (Lanini et al., 1991; Sheaffer et al., 2014). Our findings that both sudangrass and oat are effective in controlling weeds are in agreement with the study by Matteau et al. (2020).

3.4.2 Forage yield in the post-establishment year

In the post-establishment year, none of the companion crop had any detrimental effect on total yield (4 cuts) of the perennial forage mix, except that the wheat treatment produced higher (P = 0.002) forage yield compared to BSSG (Table 3.2). Moreover, weed infestation was not different between treatments. Despite the absence of companion crops (annuals) in the second year, total forage yield was 24.2, 26.9, 68.4 and 146.4% higher in the post-seeding year compared to the seeding year for SG, BSSG, oat and control, respectively. However, BSG produced similar yields in both years. In agreement with our findings, Matteau et al. (2020) also reported similar alfalfa yields in the post-seeding year when alfalfa was established with sudangrass, oat or wheat as companion crop. However, when alfalfa was seeded together with oat or wheat, alfalfa yield was found to be inconsistent (similar, higher or lower) between locations in the post-establishment year (Sheaffer et al., 2014). There are indications that, in post-establishment years, any detrimental effect of companion crops on the yield of alfalfa or perennial forage may be due to high seeding rate of companion crops (Lanini et al., 1991) and environmental conditions (Matteau et al., 2020).

3.4.3 Alfalfa stem density in the establishment and post-seeding years

In the establishment year (fall of 2018), alfalfa tiller counts were higher (*P* < 0.0001) in control and wheat treatments compared to remaining treatments. However, in spring of the following year (June 2019), alfalfa stem density was similar across treatments. According to Banks (2000), alfalfa tiller counts in our experimental forage stands were at medium yield potential (i.e. 430 to 592 stems/m^2) in fall of 2018 and less than the minimum level (i.e. 430 stems/m^2) in spring of 2019. Therefore, our alfalfa stands were at a critical stage of production after one winter. In contrast, Matteau et al. (2020) reported no detrimental effects of sudangrass on the tillering capacity of alfalfa such that alfalfa stem density was similar in both the seeding and post-seeding years whether alfalfa was established solely or with sudangrass.

3.4.4 Chemical composition of forages

Our findings show significant variations in CP concentration between treatments in the establishment year (Table 3.5). For instance, in both harvests, control had higher (*P* < 0.0001) CP concentration than any of the companion treatment because of its higher legume (i.e. alfalfa and clover) proportions. Indeed, in forage mixtures, there is a strong relationship between legume proportions and protein concentrations (Wiersma et al., 1999). In the second harvest, however, CP concentration in harvested forages decreased by 13.6, 23.5 and 14.5% for SG, BSG and BSSG, respectively but increased by 15.9% in the oat treatment (higher legumes:oat due to poor regrowth of oat). Lower CP concentrations with the use of warm-season forages is attributed to their rapid growth or regrowth resulting in lower legume:Gramineae ratios.

In contrast to control, forage concentrations of NDF and ADF were increased (*P* < 0.0001) in all companion forage treatments (first harvest) or in SG, BSG and BSSG (second harvest) only (Table 3.5). Forage ADL concentrations were higher (*P* < 0.0001) in control and oat treatment than BSSG in the first harvest. In the second harvest, ADL concentrations were higher ($P < 0.0001$) in control than remaining treatments. Oat was at milk stage of grain maturity at time of first harvest and this could explain its high ADL level. Interestingly, ADL level was lowest ($P < 0.0001$) in BSSG but not different to BSG. Our later findings demonstrate the capacity of BMR genes to reduce ADL deposition in sorghum-sudangrass and sudangrass, but apparently to a greater extent with BMR6 gene.

When comparing second to first harvests, control and BSG produced 7.9 and 4.5% higher NDF respectively whereas BSSG and oat treatment produced 2.0 and 9.3% lower NDF, respectively (Table 3.5). Differences in fiber fraction is likely to be due to relative proportions of forage species at time of harvest. For example, oat proportion was lower in the second harvest such that legumes contributed more to forage yield. All treatments (except oat) had higher ADF content in the second harvest as a result of greater lignin depositions in forage cell walls. However, Matteau et al. (2020) reported that when alfalfa was established with sudangrass and oat, NDF and ADF levels were lower in the second than first harvest. Moreover, according to the study by Wiersma et al. (1999), when oat was used as a companion crop for alfalfa, NDF and ADF concentrations were higher in the second than first harvest.

Neutral detergent insoluble CP (NDICP) was lower ($P < 0.0001$) in the oat treatment compared to remaining treatments in both harvests whereas ADICP was higher ($P < 0.0001$) in control than BSG, BSSG and oat treatments in the second harvest (Table 3.5). In general, NDICP and ADICP values describe CP contents that are associated with NDF and ADF, respectively and indicating slow ruminal CP degradation (NDICP) or indigestible CP (NRC, 2001). Both NDICP and ADICP values are used to estimate energy requirements for dairy cows (NRC, 2001).

Concentrations of WSC were higher ($P < 0.0001$) in SG and oat treatment compared to remaining treatments in the first harvest (Table 3.5). The high WSC level in oat treatment was due to presence of oat seeds that contained starch. However, in the second harvest, WSC levels were higher (P < 0.0001) in BSSG and BSG than both control and oat treatment. Forage WSC concentrations are usually affected by several factors including forage type, forage maturity, time of harvest and environmental conditions (Buxton and O'Kiely, 2003). Our WSC values for sorghum-sudangrass are within the range (i.e. 9 to13.7%) observed in previous studies (Han et al., 2015; Dillard et al., 2017).

3.4.5 In vitro fiber digestibility

The effects of companion crops on in vitro NDF digestibility and TTNDFD are depicted in Table 3.6. For both harvests, SG, BSG and BSSG had higher (P < 0.0001) pdNDF and lower (P < 0.0001) iNDF than control and oat treatments. Degradation rate of NDF was not influenced by treatments in the second harvest but was higher (P < 0.0001) in control than oat treatment in the first harvest. Interestingly, SG, BSG and BSSG significantly increased (P < 0.0001) TTNDFD when compared with control and oat treatments in the second harvest. Moreover, BSG and BSSG had higher (P < 0.0001) TTNDFD than SG due to lower cellular depositions of ADL as a result of expression of BMR genes. The association between BMR varieties and lower lignin depositions and greater fiber digestibility is well documented (Cherney et al., 1991; Oba and Allen, 1999a; Beck et al., 2007; Beck et al., 2013). Lower TTNDFD in control and oat may be due to their higher ADL (i.e. iNDF) which might limit fiber digestion (Buxton and Redfearn, 1997). It is well documented that fiber in grasses is more digestible than fiber in legumes (Weiss and Shockey, 1991; Messman et al., 1992; Holden et al., 1994; Hoffman et al., 1998; Oba and Allen, 1999b). Although control and oat treatments had equal iNDF fraction in the first harvest, control had higher (P < 0.0001) TTNDFD than the oat treatment due to faster digestion rate (5.4% *vs* 3.6%) of its fiber contents. According to Lopes et al. (2015), forages with faster digestion rate and less indigestible fiber have higher TTNDFD compared with forages having a slow rate of digestion and more iNDF.

3.4.6 Estimated total digestible nutrients, net energy of lactation and milk yields

In this study, TDN and NE_L were not affected by any treatments for both harvests (Table 3.5). In diet formulations, TDN is a critical parameter to estimate energy requirements of cows at different stages of lactation (NRC, 2001). Our NE_L result for BSSG is very close to solo-seeded sorghum-sudangrass (1.6 $Mcal kg^{-1}$; Wright et al., 2012). Indeed, BSSG significantly suppressed growth of all perennial forages in our study.

Estimated milk yield was significantly improved ($P < 0.0001$) with SG, BSG, and BSSG than control and oat treatment (Table 3.7). In the first harvest only, the oat treatment had similar milk yield to SG, BSG and BSSG due to its higher forage yield in the first than second harvest (72% lower). Our findings agree with previous reports showing that estimated milk yield is positively correlated to forage yield (Kilcer et al., 2003; Ketterings et al., 2004, 2005). Moreover, there is a positive effect of BMR gene on fiber digestibility and therefore milk yield (Aydin et al., 1999). However, comparisons between SG, BSG and BSSG show that this BMR effect in that to increase milk yield was not evidenced in our companion strategy most likely as a result of their similar forage yields. Previous findings reported that fiber digestibility and BMR forages are strongly correlated with milk yield and DM intake (Grant et al., 1995; Dado and Allen, 1996; Oba and Allen, 1999a). Therefore, when fed to lactating cows, it is feasible to believe that the higher fiber digestibility of SG, BSG and BSSG may increase feed intake and milk production. Finally, these increases in feed intake and milk yield may be even more significant in BSG and BSSG than SG because of their higher fiber digestibility.

3.5 CONCLUSIONS

Establishment of perennial forages with SG, BSG and BSSG significantly increased total forage yields, total-tract fiber digestibility and predicted milk yields when compared to control and oat treatment. However, fiber digestibility was even higher with BSG and BSSG than SG, most likely due to expression of BMR genes which reduced ADL depositions. In all companion treatments, harvested forages contained lower CP level but higher NDF and ADF concentrations. Likewise, all companion crops effectively controlled weeds. Finally, SG, BSG and BSSG are better companion crops than oat due to higher forage yields, fiber digestibility and greater estimated milk yields.

Table 3.1 Effects of seeding perennial forages with different annual companion crops on yield contribution of perennial forages, companion crops and weeds in the establishment year (T/ha; DM basis)

		Forage treatments[1]						
		Control	Control + SG	Control + BSG	Control + BSSG	Control + Oat	SEM[2]	*P*-value
Cut 1	Alfalfa	0.39^{a}	0.10^{b}	0.10^{b}	0.05^{b}	0.03^{b}	0.022	<0.0001
	Clover	0.02^{a}	0.01^{b}	0.01^{b}	0.006^{bc}	0.0009^{c}	0.002	<0.0001
	Tall fescue	0.009^{a}	0.004^{ab}	0.006^{ab}	0.004^{ab}	0.002^{b}	0.001	0.0059
	Companion	-	2.91^{ab}	2.06^{b}	3.34^{a}	2.92^{ab}	0.208	<0.0001
	Weeds	0.39^{a}	0.13^{b}	0.19^{b}	0.12^{b}	0.07^{b}	0.041	<0.0001
	Total	0.82^{c}	3.15^{ab}	2.36^{b}	3.52^{a}	3.02^{ab}	0.201	<0.0001
Cut 2	Alfalfa	1.04^{a}	0.27^{b}	0.25^{b}	0.06^{b}	0.16^{b}	0.046	<0.0001
	Clover	0.12^{a}	0.05^{b}	0.03^{b}	0.01^{b}	0.02^{b}	0.011	<0.0001
	Tall fescue	0.031^{a}	0.020^{ab}	0.015^{bc}	0.004^{c}	0.005^{c}	0.003	<0.0001
	Companion	-	2.95^{a}	2.65^{a}	2.23^{a}	0.57^{b}	0.201	<0.0001
	Weeds	0.74^{a}	0.16^{b}	0.22^{b}	0.06^{b}	0.09^{b}	0.098	0.0002
	Total	1.93^{c}	3.45^{a}	3.17^{ab}	2.32^{bc}	0.85^{d}	0.214	<0.0001
Total	Alfalfa	1.43^{a}	0.34^{b}	0.35^{b}	0.10^{b}	0.18^{b}	0.061	<0.0001
	Clover	0.140^{a}	0.064^{b}	0.042^{b}	0.014^{b}	0.022^{b}	0.011	<0.0001
	Tall fescue	0.04^{a}	0.024^{ab}	0.021^{bc}	0.0068^{c}	0.0072^{c}	0.004	<0.0001
	Companion	-	5.9^{a}	4.7^{ab}	5.1^{a}	3.5^{b}	0.305	<0.0001
	Weeds	1.13^{a}	0.29^{b}	0.40^{b}	0.16^{b}	0.16^{b}	0.112	<0.0001
	Total	2.7^{b}	6.6^{a}	5.5^{a}	5.4^{a}	3.9^{b}	0.296	<0.0001

$^{a\text{-}d}$Means in the same row with different superscripts are different ($P < 0.05$).

[1]Forage treatments: sudangrass (SG), BMR sudangrass (BSSG) or BMR sorghum-sudangrass (BSSG).

[2]SEM: Pooled standard error of the mean.

Table 3.2 Effects of seeding perennial forages with different annual companion crops on forage yield in the post-establishment year (T/ha; DM basis)

	Forage Treatments[1]						SEM[2]	*P*-value
	Control	Control + SG	Control + BSG	Control + BSSG	Control + Oat	Control + Wheat		
Cut 1	3.7[ab]	2.3[bc]	2.5[bc]	1.8[c]	3.6[abc]	4.4[a]	0.358	<0.0001
Cut 2	1.4	1.1	1.2	1.1	1.3	1.4	0.129	0.3378
Cut 3	0.99	0.88	0.94	0.82	0.90	1.1	0.057	0.0274
Cut 4	0.81	0.70	0.79	0.79	0.68	0.83	0.081	0.714
Total yield	6.9[ab]	5.0[ab]	5.5[ab]	4.6[b]	6.4[ab]	7.7[a]	0.552	0.002

[a-c]Means in the same row with different superscripts are different ($P < 0.05$).

[1]Forage treatments: sudangrass (SG), BMR sudangrass (BSSG) or BMR sorghum-sudangrass (BSSG).

[2]SEM: Pooled standard error of the mean.

Table 3.3 Effects of seeding perennial forages with different annual companion crops on yield contribution of perennial forages, companion crops and weeds in the post-establishment year (T/ha; DM basis)

Species	Cut	Forage Treatments[1]						SEM[2]	*P*-value
		Control	Control + SG	Control + BSG	Control + BSSG	Control + Oat	Control + Wheat		
Alfalfa	cut 1	2.3	1.6	1.2	1.5	2.1	2.2	0.442	0.7166
	cut 2	1.3	0.8	0.9	0.8	1.0	1.2	0.172	0.3337
	cut 3	0.72	0.37	0.59	0.34	0.55	0.54	0.105	0.1276
	cut 4	0.57	0.32	0.52	0.35	0.48	0.46	0.111	0.584
	Total	4.9	3.1	4.0	3.0	4.2	4.4	0.693	0.5101
Clover	cut 1	0.99^{ab}	0.65^{ab}	0.43^{b}	0.26^{b}	1.14^{ab}	1.91^{a}	0.262	0.0012
	cut 2	0.11	0.22	0.20	0.24	0.23	0.18	0.055	0.5624
	cut 3	0.21	0.41	0.28	0.42	0.30	0.49	0.088	0.2263
	cut 4	0.14	0.27	0.15	0.34	0.13	0.26	0.056	0.049
	Total	1.4	1.5	1.1	1.3	1.8	2.8	0.402	0.05
Tall fescue	cut 1	0.35^{a}	0.088^{b}	0.12^{ab}	0.086^{b}	0.22^{ab}	0.28^{ab}	0.051	0.0026
	cut 2	0.06	0.05	0.08	0.07	0.07	0.05	0.019	0.8205
	cut 3	0.06	0.08	0.06	0.05	0.05	0.06	0.018	0.8432
	cut 4	0.08	0.08	0.08	0.08	0.08	0.08	0.015	0.997
	Total	0.56	0.29	0.33	0.28	0.42	0.47	0.080	0.136
Weeds	cut 1	0.023	0.009	0.002	0.002	0.053	0.003	0.013	0.0437
	cut 2	0.0047	0.015	0.017	0.019	0.014	0.0036	0.007	0.571
	cut 3	0.0039	0.019	0.01	0.012	0.002	0.008	0.006	0.3807
	cut 4	0.017	0.026	0.037	0.026	0.01	0.03	0.012	0.737
	Total	0.05	0.07	0.07	0.06	0.08	0.04	0.025	0.896

[a-b]Means in the same row with different superscripts are different ($P < 0.05$).

[1]Forage treatments: sudangrass (SG), BMR sudangrass (BSSG) or BMR sorghum-sudangrass (BSSG).

[2]SEM: Pooled standard error of the mean.

Table 3.4 Effects of seeding perennial forages with different annual companion forages on alfalfa stem density in the post-establishment year (number of tillers/m^2)

	Forage Treatments[1]							
	Control	Control + SG	Control + BSG	Control + BSSG	Control + Oat	Control + Wheat	SEM[2]	*P*-value
Fall 2018	564^{a}	303^{bc}	252^{bc}	187^{c}	420^{ab}	525^{a}	37.8	<0.0001
Spring 2019	383	286	379	299	419	320	74.4	0.764

[a-c]Means in the same row with different superscripts are different ($P < 0.05$).
[1]Forage treatments: sudangrass (SG), BMR sudangrass (BSSG) or BMR sorghum-sudangrass (BSSG).
[2]SEM: Pooled standard error of the mean.

Table 3.5 Effects of seeding perennial forages with different annual companion crops on chemical composition of harvested forages (% DM)

	Forage Treatments[1]	Ash	CP[2]	NDF[2]	ADF[2]	ADL[2]	NDICP[2]	ADICP[2]	EE[2]	WSC[2]	TDN[2]	NE_L[2] (Mcalkg^{-1})
Cut 1	Control	12.7[a]	19.4[a]	36.9[b]	22.2[b]	2.6[a]	4.3[a]	0.8	2.5[b]	8.3[b]	66.7	1.51
	Control + SG	7.3[b]	11.0[b]	56.6[a]	29.9[a]	2.3[ab]	4.3[a]	0.9	1.8[c]	13.7[a]	67.0	1.52
	Control + BSG	8.4[b]	12.9[b]	53.1[a]	27.6[a]	2.2[ab]	4.8[a]	0.9	2.2[bc]	9.9[b]	67.6	1.53
	Control+ BSSG	8.0[b]	11.0[b]	56.3[a]	28.6[a]	1.8[b]	4.7[a]	0.9	2.1[bc]	8.4[b]	67.9	1.54
	Control + Oat	7.7[b]	12.9[b]	51.4[a]	29.6[a]	2.7[a]	2.6[b]	0.8	3.4[a]	15.0[a]	68.7	1.56
	SEM[3]	0.407	0.435	1.503	1.206	0.129	0.195	0.063	0.131	0.665	0.545	0.013
	P-value	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.211	<0.0001	<0.0001	0.116	0.110
Cut 2	Control	9.9[a]	19.4[a]	39.9[b]	26.6[c]	4.9[a]	3.8[ab]	1.2[a]	2.3[b]	7.1[c]	66.2	1.50
	Control + SG	8.0[c]	9.5[c]	56.7[a]	34.0[a]	3.3[b]	3.7[b]	1.0[ab]	2.0[b]	9.5[bc]	64.3	1.46
	Control + BSG	8.2[bc]	9.9[c]	55.5[a]	32.4[ab]	2.9[bc]	3.9[ab]	0.9[b]	2.0[b]	12.0[ab]	65.3	1.48
	Control+ BSSG	9.7[a]	9.4[c]	55.1[a]	31.7[ab]	2.2[c]	4.2[a]	0.8[b]	2.0[b]	13.7[a]	65.1	1.48
	Control + Oat	9.1[ab]	14.9[b]	46.6[b]	29.6[bc]	3.5[b]	2.8[c]	0.9[b]	2.9[a]	7.2[c]	66.6	1.51
	SEM[3]	0.232	0.476	1.564	0.881	0.22	0.107	0.051	0.114	0.721	0.799	0.019
	P-value	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.327	0.336
Average	Control	11.0[a]	19.3[a]	39.7[c]	25.5[b]	4.1[a]	4.0[a]	1.1[a]	2.4[b]	7.4[c]	66.2[ab]	1.50[ab]
	Control + SG	7.6[c]	10.1[c]	56.8[a]	32.2[a]	2.9[b]	3.9[a]	1.0[ab]	1.9[b]	11.5[ab]	65.4[b]	1.48[b]
	Control + BSG	8.2[bc]	11.0[c]	54.7[ab]	30.7[a]	2.6[bc]	3.9[a]	0.9[ab]	2.1[b]	11.1[ab]	66.2[ab]	1.50[ab]

Control+ BSSG	9.0^{b}	10.5^{c}	55.9^{ab}	30.0^{a}	2.1^{c}	4.5^{a}	0.9^{ab}	2.1^{b}	10.7^{b}	66.3^{ab}	1.51^{ab}
Control + Oat	8.0^{c}	13.3^{b}	50.4^{b}	29.7^{a}	2.9^{b}	2.6^{b}	0.8^{b}	3.3^{a}	13.3^{a}	68.2^{a}	1.55^{a}
SEM[3]	0.184	0.319	1.249	0.754	0.142	0.264	0.048	0.111	0.519	0.548	0.013
P-value	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.0003	0.003	<0.0001	<0.0001	0.018	0.016

[a-c]Means in the same row with different superscripts are different ($P < 0.05$).

[1]Forage treatments: sudangrass (SG), BMR sudangrass (BSSG) or BMR sorghum-sudangrass (BSSG).

[2]CP: crude protein; NDF: neutral detergent fiber; ADF: acid detergent fiber; ADL: acid detergent lignin; NDICP: neutral detergent insoluble protein; ADICP: acid detergent insoluble protein; EE: ether extract; WSC: water-soluble carbohydrate; TDN: total digestible nutrient; NE_L: net energy of lactation.

[3]SEM: Pooled standard error of the mean.

Table 3.6 Effects of seeding perennial forages with different annual companion crops on in vitro fiber digestibility

		Forage treatments[1]					SEM[2]	*P*-value
		Control	Control + SG	Control + BSG	Control + BSSG	Control + Oat		
Cut 1	NDFD24h[3], %	47.8^{a}	51.5^{a}	53.2^{a}	56.4^{a}	39.3^{b}	1.429	<0.0001
	NDFD30h[3], %	53.4^{b}	59.4^{ab}	63.3^{a}	63.9^{a}	46.2^{c}	1.273	<0.0001
	NDFD48h[3], %	62.5^{c}	66.3^{bc}	69.4^{ab}	70.3^{a}	55.9^{d}	0.818	<0.0001
	pdNDF[3], % of NDF	69.1^{b}	78.7^{a}	81.6^{a}	79.9^{a}	69.6^{b}	1.495	<0.0001
	iNDF[3], % of NDF	30.9^{a}	21.3^{b}	18.4^{b}	20.1^{b}	30.4^{a}	1.495	<0.0001
	kd[3], %/h	5.4^{a}	4.2^{ab}	4.3^{ab}	4.8^{ab}	3.6^{b}	0.278	0.0016
	TTNDFD[3], % of NDF	54.2^{c}	55.7^{bc}	58.3^{ab}	59.2^{a}	46.3^{d}	0.610	<0.0001
Cut 2	NDFD24h[3], %	39.4^{b}	53.2^{a}	54.8^{a}	57.4^{a}	36.8^{b}	1.077	<0.0001
	NDFD30h[3], %	46.6^{c}	58.3^{b}	62.5^{a}	61.8^{ab}	44.8^{c}	0.837	<0.0001
	NDFD48h[3], %	53.8^{c}	66.7^{b}	70.6^{a}	71.7^{a}	53.8^{c}	0.739	<0.0001
	pdNDF[3], % of NDF	59.6^{b}	76.1^{a}	79.1^{a}	78.9^{a}	62.3^{b}	1.048	<0.0001
	iNDF[3], % of NDF	40.4^{a}	23.9^{b}	20.9^{b}	21.1^{b}	37.7^{a}	1.048	<0.0001
	kd[3], %/h	5.3	4.6	5.1	5.12	4.8	0.299	0.4289
	TTNDFD[3], % of NDF	45.6^{c}	55.7^{b}	59.6^{a}	59.7^{a}	45.7^{c}	0.683	<0.0001

$^{a\text{-}d}$Means in the same row with different superscripts are different ($P < 0.05$).

[1]Forage treatments: sudangrass (SG), BMR sudangrass (BSSG) or BMR sorghum-sudangrass (BSSG).

[2]SEM: Pooled standard error of the mean.

[3]NDFD: neutral detergent fiber digestibility; pdNDF: potentially digestible neutral detergent fiber; iNDF: indigestible fiber; kd: digestion rate; TTNDFD: total-tract neutral detergent fiber digestibility.

Table 3.7 Effects of seeding perennial forages with different annual companion forages on estimated milk yield per hectare of cultivated land (T/ha)

	Forage Treatments[1]					SEM[2]	*P*-value
	Control	Control + SG	Control + BSG	Control+ BSSG	Control + Oat		
Cut 1	1.13^{c}	4.28^{ab}	3.43^{b}	5.10^{a}	3.76^{ab}	0.267	<0.0001
Cut 2	2.38^{bc}	4.58^{a}	4.56^{a}	3.29^{ab}	1.04^{c}	0.280	<0.0001
Total	3.51^{c}	8.86^{a}	7.99^{a}	8.41^{a}	4.81^{b}	0.244	<0.0001

$^{a-c}$Means in the same row with different superscripts are different ($P < 0.05$).

[1]Forage treatments: sudangrass (SG), BMR sudangrass (BSSG) or BMR sorghum-sudangrass (BSSG).

[2]SEM: Pooled standard error of the mean.

CHAPTER IV. CONNECTING STATEMENT

In Experiment 1, we evaluated the effects of underseeding a mixture of perennial forages (alfalfa, clover and tall fescue) with different annual companion crops [sudangrass (SG), BMR sudangrass (BSG), BMR sorghum-sudangrass (BSSG), oat or wheat] on forage yields and quality, in vitro total-tract NDF digestibility and predicted milk yields. Our findings showed that SG, BSG and BSSG increased the yields, nutritive value and total-tract NDF digestibility of harvested forages, thereby increasing estimated milk yields in the establishment year. Unlike oat and wheat, SG, BSG and BSSG are new companion strategies with little scientific evaluations. Therefore, Experiment 2 was conducted to confirm findings of Experiment 1, especially in different climatic conditions and soil types. Moreover, in Experiment 2, we conducted silage quality evaluations due to treatments for more direct relevance to dairy cow's nutrition.

CHAPTER V.

EVALUATING THE EFFECT OF ESTABLISHING PERENNIAL FORAGES WITH SUDANGRASS OR SORGHUM-SUDANGRASS AS ANNUAL COMPANION CROPS ON FORAGE YIELD, NUTRITIONAL VALUE OF BOTH FORAGE AND SILAGE, AND PREDICTED MILK YIELDS

5.1 ABSTRACT

This study evaluated the yield, chemical composition, in vitro total-tract NDF digestibility (TTNDFD) using rumen fluid and ensiling characteristics of a mixture of perennial forages (alfalfa, clover and tall fescue; control) seeded alone or with an annual companion crop [sudangrass (SG), sudangrass brown midrib (BMR) gene 12 (BSG), sorghum-sudangrass BMR gene 6 (BSSG), oat or wheat]. Experimental plots (8 replicates per treatment) were harvested on d 60 (1st cut) and d 90 (2nd cut) corresponding to bud stage of alfalfa. Forages harvested at d 90 (control, SG, BSG and BSSG) were ensiled in laboratory silos over 42 days. The TTNDFD was estimated from potentially digestible NDF (pdNDF), digestion rate (kd) and passage rate (kp) while forage indigestible NDF (iNDF) was calculated from 240 h in vitro incubation. Potentially degradable NDF was calculated by subtracting iNDF from total NDF whereas digestion rate of pdNDF was estimated by in vitro incubation at 24, 30 and 48 h. Data were analyzed as repeated measures using the MIXED procedure of SAS with fixed effects of treatment and cut. Results showed that total forage yield (2 cuts) was higher (P < 0.0001) with SG, BSSG and BSG than control. Relative to control, companion crops reduced (P < 0.0001) the yield of perennial legumes, weed biomass, and forage concentrations of acid detergent lignin (ADL) and crude protein (CP). However, companion crops increased (P < 0.0001) NDF and ADF concentrations in forages. Water-soluble carbohydrate (WSC) concentration was highest (P < 0.0001) for oat treatment,

intermediate for SG, BSG and BSSG and lowest ($P < 0.0001$) for control. In vitro iNDF was lower ($P < 0.0001$) for BSSG than control and oat treatments. In vitro TTNDFD of second cut followed the order ($P < 0.0001$): BSG and BSSG (average 62.2%) > SG (58.5%) > oat (53.3%) > control (52.3%). For experimental silages, chemical analysis showed that SG, BSG and BSSG reduced ($P < 0.0006$) ADL and CP concentrations but increased ($P < 0.0001$) NDF and ADF levels compared with control. In vitro iNDF fractions of silages were lower ($P = 0.002$) for SG, BSG and BSSG (average 19.5%) than control (28.9%). In vitro TTNDFD of silages followed the order ($P < 0.0001$): BSG and BSSG (average 64.7%) > SG (62.8%) > control (56.4%). Finally, estimated milk yield was higher ($P < 0.0001$) for SG, BSG and BSSG than control and oat treatment. In conclusion, establishing perennial forages with SG, BSG or BSSG may improve forage yields, and nutritive value and fiber digestibility of both forages and silages therefore increasing milk yields.
Key words: cows, forages, silage, fiber digestibility, sudangrass

5.2 INTRODUCTION

In Canada, perennial forages are mostly seeded as mixtures of legumes (i.e. alfalfa) and gramineaes (i.e. tall fescue and timothy) in order to increase forage yields and quality. However, in mixed cropping, yield contribution of perennial grasses to total forage yields are extremely low in the establishment year because physiological growth and development rate of perennial grasses are slow (Canevari, 2000). Consequently, in the seeding year, harvested forages comprise mostly of legumes. Improper ratio of grasses and legumes affects both forage and silage quality (Xue et al., 2020). Indeed, when compared to gramineaes, legume forages contain less water-soluble carbohydrates (WSC) and possess high pH buffering capacity (Buxton and O'Kiely, 2003). The slow reduction in pH compromises silage fermentation and quality (Dewhurst et al., 2003), leading to nutrient losses such as protein (Xue et al., 2020). Moreover, feeding cows with high proportions of legume silage increases the risks for bloating (Sheaffer et al., 1992; Mourino et al., 2003; Seguin, 2007) and reduces the efficiency of nitrogen utilization (Dewhurst et al., 2003). On the other hand, grasses provide adequate fiber for cud chewing and rumination, and are therefore important in cow's diets to avoid health problems such as acidosis and bloating. Adequate grass:legume ratio in silages improves rumen fermentation (Auldist et al., 1999) and in vitro dry matter digestibility (Xue et al., 2020). Therefore, in the establishment year, it is critical to increase gramineae proportions in harvested forages.

For decades, several annual companion crops have been utilized in the establishment of perennial forages. For example, small-grain cereals such as oat have extensively been used to increase forage yield (oat as green chops), suppress weed growth (without the use of herbicides) and reduce risks of soil erosion (Klebesadel and Smith, 1959; Johnston et al., 1998). However, the use of oat to establish perennial forages has been shown to significantly reduce alfalfa yields both in the establishment and following year of production (Lanini et al., 1991). More recent studies have suggested potential use of other annual grasses such as ryegrass, festulolium (Wiersma et al., 1999) and sudangrass (Matteau et al., 2020) as companion crops for alfalfa establishment. To the best of our knowledge, there are no data regarding the use of different sudangrass (i.e. regular and brown midrib [BMR]) as companion crops for the establishment of perennial forages, and more specifically on forage yield and quality. Moreover, in mixed cropping systems, the effects of sudangrass on silage quality and cow's performance are still unknown.

Therefore, the objectives of this study were to compare the effects of new (sudangrass, BMR sudangrass and BMR sorghum-sudangrass) and traditional (oat and wheat) annual companion crops in the establishment of a mixture of perennial forages on forage yields, nutritive values and in vitro total-tract NDF digestibility for both forages and silages, and estimated milk yields.

5.3 MATERIALS AND METHODS

5.3.1 Experimental design and treatments

Forage experimental plots were established at McGill University Agronomy Research Centre in Sainte-Anne-de-Bellevue, QC (45° 26' N, 73° 55' W). Treatments included a mixture of alfalfa (11 kg/ha), red clover (2 kg/ha), white clover (1 kg/ha) and tall fescue (5 kg/ha) seeded solely (control) or with 1 of 5 annual companion crops [sudangrass (20 kg/ha; SG), sudangrass brown midrib (BMR) gene 12 (20 kg/ha; BSG), sorghum-sudangrass BMR gene 6 (46 kg/ha; BSSG), oat (90 kg/ha) or wheat (160 kg/ha)] in late May 2019. Seeding rate for BSSG was adjusted to contain equal number of seeds as SG or BSG. All forage seeds were provided by Belisle Solution Nutrition Inc. (Saint-Mathias, Quebec, Canada). Experimental plots were assigned to a randomized complete block design with eight blocks and established in Chateauguay clay-loam soil and Wendover clay. Each plot, a total area of 5 x 1.5 m^2, was composed of seven rows separated by 18 cm and seeded at a depth of 2 cm using an experimental seeder (Fabro ltd, Swift Current, SK, Canada).

Experimental plots were fertilized according to soil analysis and CRAAQ guidelines for alfalfa establishment (CRAAQ, 2010). No herbicides or pesticides were applied during the establishment and growing periods. All plots (except for the wheat treatment) were harvested two times: at 60 days (first cut) and 90 days (second cut) corresponding to the budding stage of alfalfa whereas wheat was manually harvested for grains (no forage in the establishment year).

5.3.2 Field data collection

Experimental plots (area of 4 x 0.6 m^2) were individually harvested using an experimental flail mower (Swift Machine and Welding ltd, Swift Current, SK, Canada) and fresh forage yield was recorded per plot. For each plot, one representative forage subsample (500 g) was dried in a forced-air oven at 55°C for 48 h for determination of dry matter (**DM**) yield and subsequently ground to pass through a 1-mm screen using a Wiley mill (Standard model 4, Arthur H. Thomas Co., Swedesboro, NJ).

Another area of 0.35 m^2 was manually harvested and plants were separated by species (i.e. alfalfa, clover, tall fescue, companion crops and weeds). Plant components were dried in a forced-air oven at 55°C for 48 h to determine the DM yield contribution of each species to total forage yields. Alfalfa stem density was evaluated by counting the number of stems in a 0.35 m^2 representative area in the fall of the establishment year and in spring of post-establishment year to assess any impact of companion crops on alfalfa establishment and winter survival (Palmer and Wynn-Williams, 1972).

5.3.3 Chemical analyses

Ground forage samples were analyzed for DM, ash, acid detergent lignin (**ADL**) and ether extract (**EE**, using Ankom^{XT15} Extractor; Ankom Technology Corp., Macedon, NY) following standard procedures (AOAC, 1990). Crude protein (**CP** = N x 6.25) was analyzed using a Leco Nitrogen Analyzer (TruSpec Nitrogen Determinator System; Leco Corp., St Joseph, MI). Acid (**ADF**; AOAC, 1990) and neutral (**NDF**; Van Soest et al., 1991) detergent fiber were determined using an Ankom fiber Analyzer (Ankom Technology, Macedon, NY. USA). Neutral detergent fiber was analyzed with the use of heat stable α-amylase and without the use of sodium sulfite (Van Soest et al., 1991). Acid (**ADICP**) and neutral (**NDICP**) detergent insoluble CP were determined by analyzing ADF and NDF residues for CP, respectively.

Total digestible nutrients (**TDN**; Weiss et al., 1992) and net energy of lactation (**NE_L**; NRC, 2001) were calculated from chemical composition using standard equations. Estimated milk yield was calculated using alfalfa-grass spreadsheet of Milk2016 (Undersander et al., 2016).

For determination of water-soluble carbohydrates (**WSC**), 0.2 g of dried samples were extracted in 35 mL of deionized water for 1 h at 40^{o}C and centrifuged at 12,000 xg for 10 min at ambient temperature (Hall, 2014). Following extraction, WSC was determined using phenol-sulfuric acid method (Dubois et al., 1956).

5.3.4 In vitro fiber digestibility of experimental forages

All animal procedures were approved by the Animal Care Committee of the Faculty of Agriculture and Environmental Sciences of McGill University. Total-tract NDF digestibility (**TTNDFD**) of dried forages was estimated using a Daisy^{II} incubator (Ankom Technology, Fairport, NY) as described by Lopes et al. (2015). The method was based on in vitro incubations at multiple time points using buffered rumen fluid followed by NDF analysis. Rumen fluid was collected from a fistulated cow in the mid lactation stage (62% forages: 38% concentrates). Forage indigestible NDF (**iNDF**) was determined by in vitro incubation in rumen fluid for 240 h (Goeser and Combs, 2009) whereas potentially degradable NDF (**pdNDF**) was estimated as the difference between total NDF and total iNDF (NRC, 2001). Degradation rate (**kd**) of pdNDF was calculated from NDF residues of forages incubated for 24, 30 and 48 h (Goeser and Combs, 2009) using a first order kinetics model (Mertens, 1993). The passage rate of potentially digestible NDF (**kp** = 2.38% /h) was estimated from standard equation (Sniffen et al., 1992). Total-tract NDF digestibility was calculated using the following equation (Lopes et al., 2015):

$$TTNDFD = 100 \times \{pdNDF \times [kd / (kd + kp)]\}/0.9$$

5.3.5 Silage preparation and analyses

Silage quality was evaluated by using the second harvest of control, SG, BSG and BSSG treatments. Harvested forages of uniform particle size were sundried to reduce moisture contents to approximately 80% and then firmly packed into laboratory silos (PVC tubing, 25 cm height and 7.6 cm diameter) using a hydraulic press to ensure anaerobic conditions. Silos were immediately sealed with plastic lids and stored at room temperature over 42 days. After the fermentation period, silos were individually opened and silages were thoroughly mixed. Then, a 25 g silage sub-sample was thoroughly mixed with 250 mL of distilled water for 1 minute and pH of the extract was measured using a pH meter (Mettler-Toledo, Switzerland). From each silo, another subsample (500 g) of ensiled material was dried in a forced air oven at 55^{o}C for 48 h, ground through a 1 mm screen using a Wiley mill and analyzed for chemical composition and in vitro fiber digestibility as previously described for fresh forage analyses.

5.3.6 Statistical analysis

Data were analyzed as a randomized complete block design using PROC MIXED procedure of SAS (SAS Institute, 2014). Differences between treatments means were tested using Scheffe's multiple comparison *t*-test and statistical significance was declared at $P < 0.05$ level.

5.4 RESULTS AND DISCUSSION

5.4.1 Forage yield in the establishment year

5.4.1.1 *Total forage yield*

The effects of underseeding a mixture of perennial forages with annual companion crops on forage yields are presented in Table 5.1. Total forage yields (two cuts) were higher for SG and BSSG than control and oat treatments. There were no differences in forage yields among the warm-season companion forages (i.e. SG, BSG and BSSG). Our findings are in agreement with previous studies which showed that seeding alfalfa or clover with sudangrass or sorghum-sudangrass increased total forage yield compared with solo-seeded alfalfa or clover (La Vallie, 2019; Matteau et al., 2020). Total forage yield was not different between control and oat treatments. However, oat did produce significantly higher forage yield in the first cut only. Indeed, oat regrows poorly after harvest which explains its low forage yield in the second cut. However, when compared to solo-seeded alfalfa, seeding alfalfa together with oat significantly increased both total forage yields and forage yield in the first than subsequent harvests (Lanini et al., 1991; Matteau et al., 2020). Therefore, when considering forage yields, sudangrass is a better companion crop than oat.

Our findings also show differences in total forage yields between harvests (Table 5.1). For instance, forage yields were greater ($P < 0.0001$) with all companion crops than control in the first cut. However, in the second cut, SG and BSG produced higher ($P < 0.0001$) total yields than control and oat treatments. Among the different companion crops, oat treatment produced higher ($P < 0.0001$) forage yield than SG and BSG in the first cut but the same oat treatment had the lowest ($P < 0.0001$) yield in the second cut. For control, SG, BSG, BSSG and oat treatments, total forage yield decreased by 13.6, 5.7, 17.7, 40.5 and 87% respectively in the second cut compared to the first cut. This reduction in forage yield might have been the consequence of low soil nitrogen levels after the first cut. Despite addition of nitrogen fertilizer after the first cut, physiological growth of forages was improved but yields were still low probably because soil fertilization was delayed too much after harvest. Among all companion crops evaluated in this study, SG and BSSG performed better in that to produce higher forage yields compared to control and oat treatments.

5.4.1.2 ***Yields of perennial and companion forages***

All companion forages reduced ($P < 0.0001$) alfalfa, clover and tall fescue yields in the first cut whereas only alfalfa and clover yields were reduced ($P < 0.0001$) in the second cut (Table 5.1). These yield reductions were probably due to competitive growth exerted by companion crops on perennial forages. However, according to Matteau et al. (2020), alfalfa yield was higher with sudangrass than oat. The authors explained that sudangrass exerted less competition on alfalfa than oat. Lower alfalfa and clover yields when using oat (Lanini et al., 1991; Sheaffer et al., 2014) or sorghum-sudangrass (La Vallie, 2019) as companion crops have previously been reported.

Among all of the companion treatments, yield of oat was higher ($P < 0.0001$) than SG and BSG in the first cut whereas yields of all warm-season companion forages were higher ($P < 0.0001$) than oat in the second cut. Indeed, oat yield was reduced by 98.8% in the second cut because oat regrew poorly after the first cut. In agreement with our findings, Matteau et al. (2020) and La Vallie (2019) observed higher proportions of sudangrass and sorghum-sudangrass at time of harvest (first and second), which resulted in higher total forage yields.

5.4.1.3 ***Weed control***

In the establishment year, all companion crops significantly reduced ($P < 0.0001$) weed biomass (Table 5.1). This effect of weed control was similar between the different companion crops. However, when seeded with alfalfa, sudangrass had lower capacity to suppress weeds than oat probably due to selectivity of certain weed species (Matteau et al., 2020). The efficacy of oat to suppress weed growth in alfalfa stands has previously been demonstrated (Lanini et al., 1991; Hurley, 1994; Sheaffer et al., 2014). On the other hand, in alfalfa and clover stands, sorghum-sudangrass significantly reduced weed growth by more than 50% compared to solo-seeded legumes whether these were weeded manually or not (La Vallie, 2019).

5.4.2 Chemical composition of forages

The control forage mix had the highest ($P < 0.0001$) CP concentration in the first cut (Table 5.2). In the second cut, CP concentrations were higher ($P < 0.0001$) for control and oat treatments than SG and BSSG. In general, legumes have higher CP concentrations than grasses (Ball et al., 2001). In fact, there is a positive correlation between CP concentration and the proportion of legumes in forage mixtures (Wiersma et al., 1999). For each treatment, we observed higher CP concentrations in the second than first cut. The unfavorable weather conditions (i.e. heavy rain and cold temperatures) that prevailed after planting may have favored legumes to establish more rapidly than the warm-season forages. In the second cut, the higher ($P < 0.0001$) CP concentration in the oat treatment, which was equivalent to control, was due to poor regrowth of oat causing predominant growth of legumes.

Companion crops significantly increased ($P < 0.0001$) both NDF and ADF concentrations in the first cut when compared to control (Table 5.2). However, ADL concentration (first cut) was lowest ($P < 0.0001$) with BSSG but not different between remaining treatments. In the second cut, warm-season forage treatments had higher ($P < 0.0001$) NDF and ADF levels but significantly lower ($P < 0.0001$) ADL concentrations when compared to control and oat treatments. Reports indicate that BMR sundangrass hybrids contain approximately 9% lower lignin (Casler et al., 2003). Among all companion crops tested, oat treatment had lower NDF and ADF levels in the second cut due to its higher legume proportions (poor regrowth of oat). Conversely, in the first cut, NDF level was similar between companion treatments but oat treatment had higher ADF level compared to BSG. Our results showing lower fiber concentrations in the second cut of oat companion is in accordance with Matteau et al. (2020). In contrast, when alfalfa was established with oat, Wiersma et al. (1999) reported higher fiber concentrations in the second than first cut. In the seeding year, our NDF and ADF results are within the range reported by Matteau et al. (2020)

when sudangrass was seeded with alfalfa at different locations (NDF: 37 to 60% and ADF: 24 to 38%). However, Matteau et al. (2020) observed lower fiber concentrations when sudangrass was seeded with alfalfa in the second cut whereas our findings indicate no difference in fiber concentration between both cuts. Therefore, based on our findings, seeding perennial forages with annual companion crops may increase fiber concentrations and reduce lignin concentration in the establishment year.

Neutral detergent insoluble CP was lower ($P < 0.0001$) in the oat than remaining treatments in the first cut but was similar across treatments in the second cut (Table 5.2). Acid detergent insoluble CP was higher ($P = 0.0118$) in control than oat treatment (first cut) but lower ($P < 0.0001$) in SG, BSG and BSSG than control and oat treatments (second cut).

The concentrations of WSC were higher ($P < 0.0001$) in SG, BSSG and oat treatments compared to control in the first cut, but higher ($P < 0.0001$) in SG and BSG treatments than control in the second cut (Table 5.2). Our WSC values for SG and BSSG were similar to the range (9-10.2%) reported for solo-seeded sudangrass and sorghum-sudangrass (Kondo et al. 2004; Han et al., 2015). In general, WSC concentrations vary according to forage species, maturity stage, harvesting time and environmental conditions (Buxton and O'Kiely, 2003). High WSC concentrations in forages are desired because these produce lactic acid during fermentation thereby ensuring rapid ensiling rate (Adesogan et al., 2004) and high silage quality.

5.4.3 Chemical composition of experimental silages

The chemical compositions of experimental silages are depicted in Table 5.3. Concentrations of CP ($P = 0.0006$), ADL ($P < 0.0001$) and ADICP ($P < 0.0001$) were higher for control than remaining treatments. But BSSG had higher EE content than SG. Silage concentrations of ADF and NDF were higher ($P < 0.0001$) with warm-season forages than control.

Generally, legume forages are rich in protein and lignin while grasses contain high levels of fiber (Ball et al., 2001). There were no treatment differences in NDICP (P = 0.628), WSC (P = 0.081), TDN (P = 0.358) and NE_L (P = 0.357). In contrast to forages, WSC concentrations were lower in respective silages thereby indicating WSC utilization during ensiling as a result of high microbial activity which is desirable to minimize nutrient losses and to preserve silages for longer periods (Jaster, 1995).

5.4.4 Fiber digestibility of experimental forages and silages

The effects of annual companion crops on in vitro fiber digestibility of fresh forages and silages are presented in Table 5.4 and Table 5.5, respectively. When considering sudangrass or sorghum-sudangrass, there were no treatment differences in any of the digestibility parameters in the first cut of experimental forages. However, SG, BSG and BSSG had higher (P < 0.0001) pdNDF but lower (P < 0.0001) iNDF than control and oat treatment in the second cut. Degradation rate of forages was higher (P = 0.0107) for control than oat treatment in the first cut, but there were no differences in the second cut. Warm-season companion forages increased (P < 0.0001) TTNDFD compared with control and oat treatments in the second cut whereas control had higher (P = 0.0207) TTNDFD than that of oat treatment in the first cut. Forages with higher fractions of pdNDF usually have higher TTNDFD (Lopes et al., 2015). Fiber digestibility is usually affected by lignin concentration. For example, legumes tend to have higher iNDF and lower fiber digestibility than grasses (Buxton and Redfearn, 1997). However, fiber in legumes can be digested at a faster rate (Buxton and Redfearn, 1997), which might result in higher digestibility (i.e. higher TTNDFD). Lignification also depends on forage variety. For example, BMR lines are less lignified (Cherney et al., 1991; Oba and Allen, 1999a; Beck et al., 2007; Beck et al., 2013) and therefore have higher fiber digestibility (Casler et al., 2003). However, our findings show similar TTNDFD between SG, BSG and BSSG probably because sudangrass or sorghum-sudangrass were harvested at vegetative than more mature stages.

Silages from SG, BSG and BSSG had higher (P = 0.0019) pdNDF, lower (P = 0.0019) iNDF and faster (P < 0.0001) rate of digestion, and therefore greater (P < 0.0001) TTNDFD compared with control (Table 5.5). Lopes et al. (2015) reported that higher TTNDFD can be attainable with less indigestible fiber content and higher digestion rate of forages. In the nutrition of dairy cows, diets with high fiber digestibility are desirable because these increase DM intake and milk production (Dado and Allen, 1996; Oba and Allen, 1999b; Kendall et al., 2009). Therefore, warm-season forages (SG, BSG and BSSG) with high fiber digestibility are better than control because these can potentially increase both feed intake and cow's productivity.

5.4.5 Total digestible nutrients (TDN), net energy of lactation (NE_L) and estimated milk yield

In the first cut of experimental forages, TDN (P = 0.0131) and NE_L (P = 0.0165) were higher for control than oat treatment while no differences were observed between SG, BSG and BSSG (Table 5.2). In the second cut, no differences were observed across treatments. The TDN values are estimated using the chemical composition of forages such as CP, NDF, lignin, EE, ash, ADICP and NDICP (Weiss, 1992). Control had higher CP, ADICP and NDICP and lower NDF than oat, which positively increased TDN content of forages. Forages with higher TDN are reported to increase milk production (Hoffman et al., 2001). The fact that NE_L was calculated using TDN values, NE_L followed a similar trend as TDN across treatments.

All companion crops significantly increased (P < 0.0001) estimated milk yield in the first cut when compared to control (Table 5.6). However, in the second cut, milk yield was higher (P < 0.0001) with SG, BSG and BSSG compared to control and oat treatments. Milk yields were similar between the three warm-season forages in both cuts. Our estimated milk yield results show different treatment outcomes than TDN and NE_L. Indeed, unlike TDN and NE_L, milk yield calculations also included forage yield for each treatment (Kilcer et al., 2003; Ketterings et al., 2004, 2005; MILK, 2016). Therefore, the higher predicted milk yield for SG, BSG and BSSG is attributable to their higher forage yields. Moreover, as demonstrated in previous feeding trials with cows, silages with higher fiber digestibility increased both DM intake (Grant et al., 1995; Dado and Allen, 1996; Oba and Allen, 1999a; Oba and Allen, 1999b) and milk yields (Miller et al., 1990; Dado and Allen, 1996; Oba and Allen, 1999b; Kendall et al., 2009). Therefore, in addition to increasing the yields of highly digestible forages, we believe that inclusion of SG, BSG and BSSG in cow's rations will also increase DM intake and milk yields. However, BSG and BSSG had no advantage over SG when considering forage yield, fiber digestibility and predicted milk yields. The lack of difference in fiber digestibility and predicted milk yields of BMR hybrids of sudangrass or sorghum-sudangrass may probably be explained by the fact that these annual companion forages were mostly harvested at their early vegetative stages (at budding stage of alfalfa). Indeed, there is strong evidence that effective fiber degradability of BMR hybrids of sudangrass or sorghum-sudangrass is not improved when harvested at vegetative (boot) stage, but only at more mature (dough) stages of maturity (Kilcer et al., 2003; Beck et al., 2013). However, harvesting SG, BSG and BSSG at more mature maturity stage is not an option in our companion strategy for establishment of perennial forages.

5.5 CONCLUSIONS

Findings of this study show that SG, BSG and BSSG significantly increased total forage yield, total-tract fiber digestibility (second cut) and predicted milk yields when compared to control and oat companion crop. All companion crops were successful in controlling weeds. Silage quality was also improved with SG, BSG and BSSG in that these contained higher fiber levels, less lignin and higher total-tract fiber digestibility than control. However, with regards to our companion strategy for perennial forages, there was no benefit of using BMR than regular hybrids of sudangrass. Therefore, it is recommended to establish perennial forages with sudangrass or sorghum-sudangrass so as to increase yields of highly digestible forages and milk production.

Table 5.1 Effects of seeding perennial forages with different annual companion crops on the yields of perennial forages, companion crops and weeds in the establishment year (T/ha: DM basis)

		Forage treatments[1]						
		Control	Control + SG	Control + BSG	Control + BSSG	Control + Oat	SEM[2]	*P*-value
Cut 1	Alfalfa	1.2^{a}	0.2^{bc}	0.5^{b}	0.2^{bc}	0.1^{c}	0.073	<0.0001
	Clover	0.18^{a}	0.03^{b}	0.07^{b}	0.02^{b}	0.01^{b}	0.014	<0.0001
	Tall fescue	0.006^{a}	0.001^{b}	0.0004^{b}	0.0004^{b}	0.0002^{b}	0.0009	<0.0001
	Companion	-	2.8^{bc}	2.2^{c}	3.7^{ab}	4.2^{a}	0.268	<0.0001
	Weeds	0.9^{a}	0.5^{ab}	0.6^{ab}	0.4^{b}	0.3^{b}	0.098	0.0006
	Total	2.2^{c}	3.5^{b}	3.4^{b}	4.2^{ab}	4.6^{a}	0.196	<0.0001
Cut 2	Alfalfa	0.9^{a}	0.5^{b}	0.4^{b}	0.3^{b}	0.4^{b}	0.054	<0.0001
	Clover	0.4^{a}	0.1^{b}	0.2^{b}	0.1^{b}	0.1^{b}	0.041	<0.0001
	Tall fescue	0.02	0.01	0.01	0.004	0.01	0.005	0.5866
	Companion	-	2.5^{a}	2.1^{a}	2.0^{a}	0.05^{b}	0.183	<0.0001
	Weeds	0.6^{a}	0.3^{b}	0.2^{bc}	0.1^{bc}	0.1^{c}	0.039	<0.0001
	Total	1.9^{c}	3.3^{a}	2.8^{ab}	2.5^{bc}	0.6^{d}	0.139	<0.0001
Total	Alfalfa	2.0^{a}	0.6^{b}	0.9^{b}	0.5^{b}	0.5^{b}	0.108	<0.0001
	Clover	0.6^{a}	0.2^{b}	0.3^{b}	0.1^{b}	0.1^{b}	0.045	<0.0001
	Tall fescue	0.02	0.01	0.01	0.01	0.01	0.006	0.2193
	Companion	-	5.3	4.3	5.7	4.3	0.421	0.0456
	Weeds	1.5^{a}	0.7^{b}	0.8^{b}	0.5^{b}	0.4^{b}	0.119	<0.0001
	Total	4.1^{c}	6.8^{a}	6.3^{ab}	6.7^{a}	5.2^{bc}	0.304	<0.0001

[a-d]Means in the same row with different superscripts are different ($P < 0.05$).

[1]Forage treatments: sudangrass (SG), BMR sudangrass (BSSG) or BMR sorghum-sudangrass (BSSG).

[2]SEM: Pooled standard error of the mean.

Table 5.2 Effects of seeding perennial forages together with different annual companion crops on chemical composition of fresh harvested forages (% DM)

		Forage treatments[1]					SEM[2]	*P*-value
		Control	Control + SG	Control + BSG	Control + BSSG	Control + Oat		
Cut 1	Ash	12.0	10.3	11.1	10.4	10.6	0.454	0.0561
	CP[3]	16.1^{a}	11.9^{b}	12.5^{b}	10.0^{c}	11.0^{bc}	0.343	<0.0001
	NDF[3]	45.4^{b}	56.2^{a}	53.7^{a}	57.8^{a}	57.6^{a}	0.915	<0.0001
	ADF[3]	26.6^{c}	32.5^{ab}	31.6^{b}	33.5^{ab}	34.7^{a}	0.565	<0.0001
	ADL[3]	3.2^{a}	3.0^{a}	3.0^{a}	2.5^{b}	3.0^{a}	0.099	0.0005
	NDICP[3]	4.3^{a}	4.0^{a}	3.9^{a}	3.8^{a}	2.4^{b}	0.127	<0.0001
	ADICP[3]	0.8^{a}	0.7^{ab}	0.7^{ab}	0.7^{ab}	0.6^{b}	0.035	0.0118
	EE[3]	2.8^{a}	2.3^{b}	2.5^{ab}	2.4^{b}	2.5^{ab}	0.072	0.001
	WSC[3]	7.7^{c}	10.9^{ab}	10.1^{bc}	12.5^{ab}	13.1^{a}	0.651	<0.0001
	TDN[3]	64.4^{a}	62.7^{ab}	62.7^{ab}	63.1^{ab}	61.7^{b}	0.523	0.0131
	NE_L[3] (Mcal/kg)	1.46^{a}	1.42^{ab}	1.42^{ab}	1.43^{ab}	1.40^{b}	0.013	0.0165
Cut 2	Ash	12.6^{ab}	11.3^{b}	11.3^{b}	12.6^{ab}	14.2^{a}	0.494	0.0009
	CP[3]	18.8^{ab}	15.0^{c}	16.3^{bc}	15.5^{c}	20.7^{a}	0.677	<0.0001
	NDF[3]	43.6^{b}	55.4^{a}	52.8^{a}	55.3^{a}	40.2^{b}	0.999	<0.0001
	ADF[3]	28.6^{b}	33.7^{a}	32.1^{a}	33.2^{a}	27.7^{b}	0.635	<0.0001
	ADL[3]	4.8^{a}	3.6^{b}	3.6^{b}	2.8^{c}	4.4^{a}	0.136	<0.0001
	NDICP[3]	4.7	3.8	4.5	4.4	4.7	0.296	0.1981
	ADICP[3]	1.4^{a}	1.0^{b}	1.0^{b}	0.9^{b}	1.3^{a}	0.062	<0.0001
	EE[3]	2.6^{a}	2.2^{b}	2.4^{ab}	2.4^{ab}	2.5^{ab}	0.061	0.0182
	WSC[3]	6.0^{b}	7.9^{a}	7.8^{a}	7.6^{ab}	7.2^{ab}	0.354	0.0041
	TDN[3]	62.1	60.9	62.2	61.5	62.2	0.576	0.4432
	NE_L[3] (Mcal/kg)	1.40	1.37	1.40	1.39	1.40	0.014	0.4346
Average	Ash	12.3^{a}	10.7^{b}	11.2^{ab}	11.2^{ab}	11.0^{ab}	0.311	0.0118
	CP[3]	17.3^{a}	13.4^{bc}	14.3^{b}	12.0^{c}	12.2^{c}	0.363	<0.0001
	NDF[3]	44.6^{b}	55.8^{a}	53.3^{a}	56.9^{a}	55.6^{a}	0.859	<0.0001
	ADF[3]	27.5^{b}	33.1^{a}	31.9^{a}	33.4^{a}	33.9^{a}	0.505	<0.0001
	ADL[3]	3.9^{a}	3.3^{b}	3.2^{b}	2.6^{c}	3.2^{b}	0.071	<0.0001
	NDICP[3]	4.5^{a}	3.9^{a}	4.2^{a}	4.0^{a}	2.7^{b}	0.134	<0.0001
	ADICP[3]	1.04^{a}	0.84^{b}	0.85^{b}	0.80^{bc}	0.68^{c}	0.033	<0.0001
	EE[3]	2.7^{a}	2.3^{b}	2.4^{b}	2.4^{b}	2.5^{ab}	0.047	<0.0001

WSC[3]	7.0^{c}	9.5^{b}	9.1^{b}	10.7^{b}	12.4^{a}	0.362	<0.0001
TDN[3]	63.4^{a}	61.8^{ab}	62.5^{ab}	62.5^{ab}	61.7^{b}	0.353	0.0181
NE_L[3] (Mcal/kg)	1.43	1.39	1.41	1.41	1.39	0.009	0.0241

[a-c]Means in the same row with different superscripts are different ($P < 0.05$).

[1]Forage treatments: sudangrass (SG), BMR sudangrass (BSSG) or BMR sorghum-sudangrass (BSSG).

[2]SEM: Pooled standard error of the mean.

[3]CP: crude protein; NDF: neutral detergent fiber; ADF: acid detergent fiber; ADL: acid detergent lignin; NDICP: neutral detergent insoluble protein; ADICP: acid detergent insoluble protein; EE: ether extract; WSC: water-soluble carbohydrate; TDN: total digestible nutrient; NE_L: net energy of lactation.

Table 5.3 Effects of seeding perennial forages together with different annual companion crops on chemical composition of silages (% DM)

	Forage treatments[1]				SEM[2]	*P*-value
	Control	Control + SG	Control + BSG	Control + BSSG		
Ash	11.8^{ab}	10.9^{b}	11.0^{b}	12.5^{a}	0.292	0.0037
CP[3]	18.8^{a}	14.7^{b}	15.7^{b}	15.1^{b}	0.580	0.0006
NDF[3]	41.9^{b}	54.6^{a}	51.1^{a}	53.5^{a}	1.057	<0.0001
ADF[3]	28.8^{b}	35.7^{a}	33.8^{a}	34.9^{a}	0.631	<0.0001
ADL[3]	5.6^{a}	3.7^{b}	3.3^{b}	2.4^{b}	0.330	<0.0001
NDICP[3]	2.9	3.1	3.2	3.1	0.142	0.628
ADICP[3]	1.1^{a}	0.8^{b}	0.9^{b}	0.9^{b}	0.036	<0.0001
EE[3]	3.0^{ab}	2.8^{b}	3.0^{ab}	3.1^{a}	0.071	0.0449
WSC[3]	1.4	1.7	1.6	1.4	0.096	0.0812
TDN[3]	62.2	61.9	63.7	63.3	0.811	0.3589
NE_L[3]	1.40	1.40	1.44	1.43	0.019	0.357

[a-b]Means in the same row with different superscripts are different ($P < 0.05$).

[1]Forage treatments: sudangrass (SG), BMR sudangrass (BSSG) or BMR sorghum-sudangrass (BSSG).

[2]SEM: Pooled standard error of the mean.

[3]CP: crude protein; NDF: neutral detergent fiber; ADF: acid detergent fiber; ADL: acid detergent lignin; NDICP: neutral detergent insoluble protein; ADICP: acid detergent insoluble protein; EE: ether extract; WSC: water-soluble carbohydrate; TDN: total digestible nutrient; NE_L: net energy of lactation.

Table 5.4 Effects of seeding perennial forages together with different annual companion crops on in vitro digestibility of fresh harvested forages

		Forage treatments[1]					SEM[2]	*P*-value
		Control	Control + SG	Control + BSG	Control+ BSSG	Control + Oat		
Cut 1	NDFD24h[3], %	65.6	59.1	64.3	61.1	59.8	2.068	0.1381
	NDFD30h[3], %	67.8[a]	64.9[ab]	65.5[ab]	65.1[ab]	61.1[b]	1.037	0.0017
	NDFD48h[3], %	75.4[a]	71.9[ab]	73.4[a]	72.9[a]	68.6[b]	0.851	<0.0001
	pdNDF[3], % of NDF	79.8	79.1	80.04	82.1	78.3	1.114	0.1866
	iNDF[3], % of NDF	20.2	20.9	20.0	17.9	21.7	1.114	0.1866
	kd[3], %/h	6.1[a]	5.4[ab]	5.8[ab]	5.0[ab]	4.7[b]	0.301	0.0107
	TTNDFD[3], % of NDF	64.2[a]	61.1[ab]	62.7[ab]	61.7[ab]	59.6[b]	0.914	0.0207
Cut 2	NDFD24h[3], %	51.2[c]	56.9[b]	60.7[ab]	63.1[a]	51.9[c]	0.893	<0.0001
	NDFD30h[3], %	52.8[b]	60.0[a]	64.4[a]	65.2[a]	51.8[b]	1.226	<0.0001
	NDFD48h[3], %	61.9[b]	69.3[a]	71.9[a]	73.6[a]	62.5[b]	0.95	<0.0001
	pdNDF[3], % of NDF	68.7[c]	75.9[b]	78.1[ab]	81.2[a]	67.7[c]	1.111	<0.0001
	iNDF[3], % of NDF	31.3[a]	24.1[b]	21.9[bc]	18.8[c]	32.3[a]	1.111	<0.0001
	kd[3], %/h	5.3	5.3	6.3	5.4	6.0	0.439	0.3788
	TTNDFD[3], % of NDF	52.3[b]	58.5[a]	62.1[a]	62.3[a]	53.3[b]	1.012	<0.0001

[a-c]Means in the same row with different superscripts are different ($P < 0.05$).
[1]Forage treatments: sudangrass (SG), BMR sudangrass (BSSG) or BMR sorghum-sudangrass (BSSG).
[2]SEM: Pooled standard error of the mean.
[3]NDFD: neutral detergent fiber digestibility; pdNDF: potentially digestible neutral detergent fiber; iNDF: indigestible fiber; kd: digestion rate; TTNDFD: total-tract neutral detergent fiber digestibility.

Table 5.5 Effects of seeding perennial forages together with different annual companion crops on in vitro digestibility of silages

	Forage treatments[1]				SEM[2]	*P*-value
	Control	Control + SG	Control + BSG	Control + BSSG		
NDFD24h[3], %	53.4^{b}	60.9^{a}	62.3^{a}	63.5^{a}	1.207	<0.0001
NDFD30h[3], %	53.3^{b}	63.3^{a}	67.2^{a}	65.5^{a}	0.922	<0.0001
NDFD48h[3], %	65.8^{b}	73.8^{a}	75.6^{a}	76.7^{a}	0.803	<0.0001
pdNDF[3], % of NDF	71.1^{b}	79.8^{a}	80.6^{a}	81.2^{a}	1.691	0.0019
iNDF[3], % of NDF	28.9^{a}	20.2^{b}	19.4^{b}	18.8^{b}	1.691	0.0019
kd[3], %/h	2.5^{b}	3.1^{a}	3.3^{a}	3.4^{a}	0.064	<0.0001
TTNDFD[3], % of NDF	56.4^{b}	62.8^{a}	64.5^{a}	64.9^{a}	0.589	<0.0001

[a-b]Means in the same row with different superscripts are different ($P < 0.05$).
[1]Forage treatments: sudangrass (SG), BMR sudangrass (BSSG) or BMR sorghum-sudangrass (BSSG).
[2]SEM: Pooled standard error of the mean.
[3]NDFD: neutral detergent fiber digestibility; pdNDF: potentially digestible neutral detergent fiber; iNDF: indigestible fiber; kd: digestion rate; TTNDFD: total-tract neutral detergent fiber digestibility.

Table 5.6 Effects of seeding perennial forages together with different annual companion crops on estimated milk yield per hectare of cultivated land (T/ha)

	Forage treatments[1]					SEM[2]	*P*-value
	Control	Control + SG	Control + BSG	Control + BSSG	Control + Oat		
Cut 1	3.3^{b}	5.1^{a}	4.9^{a}	5.9^{a}	6.1^{a}	0.278	<0.0001
Cut 2	2.4^{b}	4.2^{a}	4.1^{a}	3.4^{a}	0.8^{c}	0.183	<0.0001
Total	5.3^{b}	8.1^{a}	8.9^{a}	9.3^{a}	6.9^{b}	0.549	<0.0001

[a-c]Means in the same row with different superscripts are different ($P < 0.05$).
[1]Forage treatments: sudangrass (SG), BMR sudangrass (BSSG) or BMR sorghum-sudangrass (BSSG) in the seeding year.
[2]SEM: Pooled standard error of the mean.

CHAPTER VI. GENERAL DISCUSSION AND CONCLUSION

Two studies were conducted to investigate a novel approach of establishing perennial forages with sudangrass and sorghum-sudangrass as new companion crops. In both Experiments 1 and 2, we evaluated the effects of establishing perennial forages with different annual companion crops (i.e. SG, BSG, BSSG or oat) on forage yields and quality, in vitro total-tract fiber digestibility and predicted milk yields. Experiment 2 was conducted to confirm findings of Experiment 1 (i.e. different climatic conditions and soil types). Furthermore, in Experiment 2, all harvested forages were ensiled in laboratory silos in order to evaluate the effects of companion crops on silage quality.

In both Experiment 1 (Table 3.1) and Experiment 2 (Table 5.1), establishing perennial forages together with SG, BSG and BSSG significantly increased total forage yields and reduced weed population in the establishment year. Our findings are in agreement with previous studies by La Vallie (2019) and Matteau et al. (2020) who reported that seeding alfalfa or clover together with sorghum-sudangrass or sudangrass increased total forage yield and reduced weed population compared with solo-seeded alfalfa or clover. In both Experiments 1 and 2, we observed that oat effectively controlled weeds similar to SG, BSG and BSSG. However, in another study, sudangrass was found to be less effective in controlling weeds than oat probably due to selectivity of certain weed species (Matteau et al., 2020). It was also reported that sorghum-sudangrass reduced more than 50% of weeds when seeded with alfalfa or clover relative to solo-seeded legumes (La Vallie, 2019). Therefore, we strongly believe that establishment of perennial forages with sudangrass and sorghum-sudangrass as companion crops is an effective strategy to increase forage yields and control weeds.

However, we also observed that SG, BSG and BSSG reduced the yield of perennial legumes (i.e. alfalfa and clover) in both Experiment 1 (Table 3.1) and Experiment 2 (Table 5.1) probably due to competitive effects between the various forage types. Our findings are in accordance with La Vallie (2019) who found reduction in alfalfa or clover yields when seeded with sorghum-sudangrass. In contrast to our results, Matteau et al. (2020) reported higher alfalfa yields with sudangrass. Our findings of Experiment 1 show that BSG and BSSG reduced the yield of tall fescue. However, in Experiment 2, tall fescue yield was not affected by any of the warm-season companion forages (i.e. SG, BSG and BSSG). Differences in the yield of perennial legumes or Gramineae may be attributed to variations in environmental conditions (Sheaffer et al., 2014).

In the post-establishment year, none of the companion crops (SG, BSG, BSSG and oat) affected total forage yield (4 cuts; Table 3.2). However, wheat treatment produced higher forage yield compared with BSSG. Weed growth was also similar across treatments. In accordance with previous findings, Matteau et al. (2020) reported similar alfalfa yield when seeded with sudangrass, oat or wheat.

In the establishment year, in both Experiments 1 and 2, all companion forages (SG, BSG, BSSG and oat) reduced protein and lignin concentrations of harvested forages whereas NDF and ADF levels were increased (Tables 3.5 and 5.2). Higher fiber concentrations in harvested forages may be due to high proportions of companion forages at the time of harvest. In agreement with our findings, Matteau et al. (2020) and La Vallie (2019) reported higher percentage of companion forage yields (first and second harvest) when alfalfa was seeded with sudangrass or sorghum-sudangrass.

In both Experiments 1 and 2 (second harvest), SG, BSG and BSSG significantly improved TTNDFD when compared with control and oat treatments. Higher TTNDFD for warm-season companion forages may be due to less iNDF content (Lopes et al., 2015). In general, legumes contain higher levels of indigestible fiber than grasses, which negatively affects fiber digestibility (Buxton and Redfearn, 1997). Moreover, in Experiment 1 (second cut), our findings show higher

TTNDFD with BSG and BSSG than SG likely due to expression of the BMR genes (*bmr 12* and *bmr 6*) which reduced lignification of plant cells (Table 3.6). However, the same benefit of BMR gene was not evidenced in Experiment 2. Indeed, SG, BSG and BSSG had similar TTNDFD (Table 5.4). In contrast, it is well documented that BMR varieties may increase fiber digestibility due to less lignin deposition (Cherney et al., 1991; Oba and Allen, 1999a; Casler et al., 2003; Beck et al., 2007; Beck et al., 2013).

In Experiment 2, we observed that silages of SG, BSG and BSSG had higher fiber but lower CP and lignin contents than control (Table 5.3). Generally, legumes contain higher levels of protein (Wiersma et al., 1999; Ball et al., 2001) and lignin than grasses (Buxton and Redfearn, 1997). Lignin deposition also depends on forage variety. For instance, BMR sudangrass contains 9% lower lignin than regular sudangrass (Casler et al., 2003). Silages from SG, BSG and BSSG had higher TTNDFD than control (Table 5.5) due to their higher pdNDF content and faster digestion rate (Lopes et al., 2015).

In both Experiments 1 and 2, total estimated milk yields were greater with SG, BSG and BSSG than control and oat treatment (Tables 3.7 and 5.6). However, predicted milk yield was not different between SG, BSG and BSSG. The later result occurred despite the higher TTNDFD of BSG and BSSG than SG as observed in Experiment 2. However, our findings show that higher milk yield was mostly influenced by forage yields rather than chemical compositions of harvested forages. Furthermore, SG, BSG and BSSG produced higher predicted milk yields although TDN and NE_L were similar compared to control or oat treatment. The strong correlation between milk yield and forage yield has previously been demonstrated (Kilcer et al., 2003; Ketterings et al., 2004, 2005). According to previous studies, feeding cows silages with high fiber digestibility significantly increased DM intake (Grant et al., 1995; Dado and Allen, 1996; Oba and Allen, 1999a; Oba and Allen, 1999b) and milk yields (Miller et al., 1990; Dado and Allen, 1996; Oba and

Allen, 1999b; Kendall et al., 2009). Therefore, we believe that inclusion of SG, BSG and BSSG in the diets of lactating cows may increase both feed intake and milk yields.

Based on findings of Experiments 1 and 2, it can be concluded that establishment of perennial forages with SG, BSG or BSSG as companion crops may improve forage yields, nutritive value and fiber digestibility of both forages and silages, and therefore increase milk yields. Finally, SG, BSG and BSSG are better companion crops than oat.

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