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Intercropping Winter Cereals in Kura Clover for Spring Forage Production

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ABSTRACT

Intercropping winter cereals into Kura clover (*Trifolium ambiguum* M. Bieb.) has potential to improve forage nutritive value without reducing yields. The objective of this research was to compare forage yield and nutritive value of cereal-legume mixtures to cereals or Kura clover grown in monoculture, and harvested at two different maturity stages in spring. Winter rye (*Secale cereale* L.), winter wheat (*Triticum aestivum* L.), and winter triticale ($\times$ *Triticosecale* Wittmack) were sown in autumn at two locations as monocultures and into herbicide suppressed and non-suppressed Kura clover, and harvested for forage the following spring. Harvested at cereal crop boot stage, at Arlington, forage yields averaged 4.7, 4.8, 5.3 and 1.7 Mg ha⁻¹ and at Lancaster 3.5, 3.7, 4.1 and 2.6 Mg ha⁻¹ for mixtures with non-suppressed Kura clover, suppressed Kura clover, monoculture cereals, and monoculture clover, respectively. At milk stage, yields across all mixture treatments increased by 46 to 115% compared to boot stage at Arlington and by 88 to 101% at Lancaster. Kura clover in mixtures increased crude protein by 34 to 46% and in vitro true digestibility by 3 to 6%, neutral detergent fiber digestibility by 0 to 6% and reduced neutral detergent fiber concentration by 8 to 18% relative to cereal monocultures. The nutritive value of mixtures and monoculture Kura clover was always greater than that of monoculture cereals. Winter cereals can be successfully managed with Kura clover for forage production by maximizing nutritive value with boot stage harvests or achieving higher yields by harvesting at the milk stage.

Winter cereals are an important source of forage in rations of ruminant livestock in Europe and North America. For example, in California, 243,000 ha of cereal crops are produced annually, mostly through the winter, as feed for dairy cows and other livestock (Collar et al. 2006). They are commonly grown as forage through the winter in the Mediterranean region of Europe (Royo et al. 1993). In the UK, cereal crops are grown alone or intercropped with legumes during summer for forage production (Anil et al. 1998). On the northern prairies of Canada, cereals are important silage crops (Juskiw et al. 2000), even though their nutritive value is relatively low (Chapko et al. 1991; Juskiw et al. 2000). Due to relatively high fiber and low crude protein (CP) concentrations, cereal crops grown in summer have not played an important role as dairy forage in the Upper Midwest. Instead forage production is based on rotations of alfalfa (*Medicago sativa* L.) and corn (*Zea mays* L.) silage (NASS, 2019). Simultaneous production of winter cereals with crops that are able to increase the nutritive value of forage may be an alternative, providing a consistent forage early in the spring.

Differences among cereal crops for forage nutritive value and agronomic characteristics exist. Forage crop potential differs among cereals, winter cereals often having greater nutritive value (Baron et al. 1992; Maloney et al. 1999), and yield than spring cereals (Entz and Fowler, 1991). However, summer sown oat (*Avena sativa* L.) is recommended as a source of autumn forage because of high nonstructural carbohydrate concentrations and high fiber digestibility (Contreras-Govea and Albrecht 2006). Among cereals, winter rye is the most winter hardy and begins regrowth earliest in the spring (Stoskopf 1985). Moreover, rye generally has a greater proportion of stems and less leaves because of its tall stems, (Edmisten et al. 1998), and this contributes to its competitiveness for available resources compared to

winter wheat (White and Scott 1991; Beres et al. 2010). No matter the stage of maturity at harvest, monoculture winter rye usually yields more than wheat and triticale, which have similar yield (Poysa 1985; Maloney et al. 1999). Maloney et al. (1999) recommended planting winter wheat, rye and triticale when need for emergency spring forage is anticipated in the north central USA, and pointed out that rye and wheat have the greatest yields while triticale and wheat forage have potential to support greater milk production per hectare.

Stage of maturity at harvest affects nutritive value and yield of cereal forages. When harvested at boot stage, cereal crop forage can have high energy density, but can be low in protein (Favre et al. 2019). In order to maximize milk production, dairy cows require high energy feed and thus winter cereals harvested at the boot stage are recommended (Collar et al. 2006). As maturity progresses, stem elongation results in greater forage yield, but also reduced forage nutritive value (Stoskopf 1985; Fohner 2002). Previous research has shown differences in nutritive value among winter cereal species. Winter triticale, grown in the Mediterranean region of Europe, is considered a better energy and protein source than wheat or rye (Royo et al. 1993). In Canada, Juskiw et al. (2000) reported that winter triticale harvested at soft-dough stage had greater protein but also had greater neutral detergent fiber (NDF) and acid detergent fiber (ADF) concentrations than winter rye. When harvested at late boot stage, winter wheat had lower fiber and greater relative feed value than other winter cereal grasses (Maloney et al. 1999). Although management and species affect nutritive value of cereal crop forage, when the goal is to satisfy nutritional requirements of high producing dairy cattle, these forages may need to be supplemented with costly concentrate (Anil et al. 1998). Intercropping cereals with legumes that can increase nutritive value of the total forage may be another practical option (Anil et al. 1998).

Intercropping winter cereals with legumes has been accomplished, but can be challenging. While numerous European studies focused on grain production have reported that intercropping cereals with legumes results in grain yield reductions (Hiltbrunner et al. 2007; Thorsted et al. 2006a), this usually does not take place when they are harvested for forage (Anil et al. 1998) because the total aboveground biomass of all crops is of interest. Instead, major difficulties are associated with establishing the most appropriate time of harvest, optimizing yields of two different species without losing nutritive value, together with seed proportion and species choice (Salawu et al. 2001). In a system developed to grow corn in living Kura clover, it is advantageous to suppress the clover before planting corn (Zemenchik et al. 2000) but the role of legume suppression in cereal crop-legume intercropping has not been thoroughly investigated.

Intercropping cereals with legumes, which are richer in protein and often more digestible, and with greater intake potential (Sheaffer and Seguin 2003), may improve overall performance of a forage-livestock production system. Research has shown that legumes improve forage nutritive value of mixtures and that cereal-legume mixtures have significantly greater in vitro true digestibility (IVTD), CP, and lower NDF concentrations compared to grass monocultures (Ghanbari-Bonjar and Lee 2003; Anil et al. 1998; Contreas-Govea and Albrecht 2005; Contreas-Govea et al. 2006). Salawu et al. (2002) reported that feeding a cereal-legume forage mixture resulted in greater milk production than moderate-quality cereal silage.

In addition to improving nutritive value of mixtures compared to grasses alone, annual and perennial legumes contribute to the overall nitrogen economy of crop-livestock systems. Zemenchik et al. (2001) reported that nitrogen replacement value of birdsfoot trefoil

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(*Lotus corniculatus* L.) mixed with cool-season grasses ranged from 147 to 295 kg N ha⁻¹ yr⁻¹, while with Kura clover ranged from 74 to 325 kg N ha⁻¹ yr⁻¹. Ghanbari-Bonjar and Lee (2003) reported that bean (*Vicia faba* L.) intercropped with spring wheat without any additional fertilization produced more dry matter yield than monoculture crops. In a Wisconsin study, winter wheat was successfully intercropped with Kura clover for silage with mixture yield nearly equal to wheat with nitrogen fertilizer and double that of Kura clover grown in monoculture (Contreras-Govea and Albrecht 2005). Kosinski et al. (2011) reported that suppressing Kura clover before sowing barley (*Hordeum vulgare* L.) or triticale in spring increased the proportion of cereal crop in mixtures, but decreased total forage yield, and concluded more research was needed to understand cereal crop-Kura clover interactions. Intercropped triticale and berseem clover (*Trifolium alexandrinum* L.) yielded 9.9 Mg ha⁻¹ when harvested at the soft dough stage in Alberta, slightly more than similar intercrops of oat or barley (Ross et al. 2004). Because legumes fix atmospheric nitrogen but also assimilate nitrate from the soil, they serve as a buffer in forage-livestock systems; fixing nitrogen when soil N is limiting and extracting soil N when it is in abundance, for example after manure application. Niches exist for intercropping winter cereal crops with perennial legumes for forage, however very little research has been done in this area.

The objectives of this research were (i) to compare spring yield and forage nutritive value of cereal crop mixtures with Kura clover to monoculture cereals at two stages of maturity and (ii) to determine if suppressing Kura clover before sowing the cereal crop affects mixture productivity or composition.

MATERIALS AND METHODS

Experimental Design and Stand Establishment

Field research was conducted at the University of Wisconsin-Madison Agricultural Research Stations near Arlington (43°18'N, 89°21'W) on Plano silt loam soil (well-drained, fine-silty, mixed, superactive, mesic Typic Argiudoll) (Orthic Grey Brown Luvisol, approximate Canadian classification) and Lancaster (42°18'N, 90°47'W) on Fayette silt loam soil (fine-silty, mixed, superactive, mesic Typic Hapludalf) (Orthic Grey Brown Luvisol, approximate Canadian classification). Kura clover experimental line KTA202 was established 3 years before the experiment was initiated and harvested three or four times per year during that time. Kura clover stands were dense and uniform at both locations. One week before sowing cereals, when Kura clover was approximately 15 cm tall, plots were prepared by killing Kura clover, suppressing Kura clover, or leaving areas untreated within the Kura clover fields. To kill Kura clover, a mixture of clopyalid (Stinger, 0.16 kg a.i. ha⁻¹ (Corteva Agriscience, Indianapolis, IN, USA) and glyphosate (Roundup WeatherMax, 1.12 kg a.e. ha⁻¹, Monsanto, St. Louis, MO, USA) was used to create sites for small grain monocultures. For suppressed plots, only glyphosate (0.28 kg a.e. ha⁻¹) was applied. Spray volume for all applications was 190 l ha⁻¹ applied at 200 kPa with a backpack sprayer equipped with 8003 flat fan nozzles. The entire area was clipped to a height of 8 cm the day of cereal sowing.

Nine cereal treatments consisted of three winter cereal crops: 'Spooner' winter rye, 'Kaskaskia' winter wheat, and '815' winter triticale grown in monoculture and in binary mixtures with suppressed or non-suppressed Kura clover. There were also two control treatments of suppressed and non-suppressed Kura clover in monoculture. Each plot was 2.0 m by 7.6 m and divided in half where one side was harvested at Feekes growth stage 10

(boot, flag leaf sheath opening) and second Feekes 11.1 growth stage (medium milk) of cereal development, according to Large (1954). The experimental design was a randomized complete block with four replications in a split-plot structure, where treatment (cereal crop × Kura clover suppression level combination) was the main plot and growth stage at harvest was the split factor.

Cereal crops were sown at Arlington on 24 September and Lancaster on 28 September 2009 at densities of 450, 350, and 300 seeds m⁻² for winter wheat, winter triticale and winter rye, respectively, with a Tye Pasture Pleaser no-till drill (Tye Mfg., Lockney, TX) at 17.8 cm row spacing and 2.5 cm depth. Soil tests indicated that Arlington had 19 mg P kg⁻¹, 69 mg K kg⁻¹, a pH of 6.3, and organic matter levels of 4.3% and that Lancaster had 12 mg P kg⁻¹, 73 mg K kg⁻¹, a pH of 7.7, and organic matter levels of 2.7%. Consequently, 41 kg P ha⁻¹ and 234 kg K ha⁻¹ were surface applied before sowing at both locations to account for deficiencies and no N fertilizer was applied.

Harvest and Sample Handling

Forage was harvested in spring when cereals were in boot and milk stages of development. On the day of harvest, canopy height was measured in four random locations in each subplot and tiller density determined by counting tillers in one meter of row. Winter rye was the first cereal to reach boot stage and was harvested on 5 May at Arlington and 10 May at Lancaster. Winter wheat and winter triticale reached the boot stage on 17 May at Arlington and 19 May at Lancaster. Milk stage harvest occurred on 11 and 16 of June at Arlington and Lancaster, respectively. Plots with monoculture Kura clover were harvested at the same time as winter cereal boot and milk stage harvests. Kura clover was in vegetative stage of development at all harvests in mixtures and monoculture.

After the alleys were cut, the harvested area of subplots was 0.9 m by 6.1 m. Only the center five of seven rows were collected for yield. All forages were harvested to an 8-cm stubble height using a small-plot flail harvester. The wet weight of harvested vegetation was recorded, and a subsample of approximately 0.5 kg of fresh material was oven-dried at 60°C for dry matter yield calculation.

Just before harvest, forage from 15 cm by 80 cm quadrats was sampled for separation into components of Kura clover, cereals and weeds. Components were oven-dried at 60°C and weighed to determine their proportions on a dry matter basis. Samples were then recombined and ground in a laboratory mill to pass through a 1.0-mm screen before laboratory analysis of nutritive value.

Laboratory Analysis

The dried, milled samples were analyzed for total N by the Dumas combustion method with a LECO model FP-528 (LECO Corporation), and crude protein (CP) was calculated as $N \times 6.25$. Neutral detergent fiber was analyzed by the batch procedures outlined by the ANKOM Technology Corporation (Macedon, New York) with an ANKOM²⁰⁰ fiber analyzer and addition of heat-stable α -amylase and sodium sulfite to the neutral detergent solution (Hintz *et al.*, 1996). A modified Goering and Van Soest (1970) procedure was used to determine 48 h IVTD with buffered rumen fluid followed by a neutral detergent wash of post-digestive residues. The rumen fluid incubation was performed with ANKOM F57 filter bags and an ANKOM Daisy II incubator using the batch incubation procedure (ANKOM Technology, Macedon, New York). Neutral detergent fiber digestibility was calculated from NDF and IVTD values as $1000\{[NDF - (1000 - IVTD)]/NDF\}$.

Statistical Analysis

All data were subjected to analysis of variance using the PROC MIXED procedure of SAS (SAS Inst., 2013). For determining the expected mean squares, appropriate *F*-tests and *T*-tests in the analysis with random effects of replications as blocks and fixed effects of location, treatment, and harvest stage were used. Variable effects were tested for the interactions and responses of yield, botanical composition, canopy height, tiller density, and nutritive value analysis (CP, IVTD, NDF, and NDFD).

The variance at each location was plotted and assessed with a scatterplot tool in the GLM procedure of SAS Institute (2013) revealing no evidence of deviation from the assumption of equal variance. The residuals were tested for normality using the Shapiro-Wilk test using the PROC UNIVARIATE procedure of SAS (SAS Inst, 2013). Only yield, canopy height, IVTD and NDFD residuals were found to be non-normal; the original data were then ranked and analyzed in a non-parametric manner. Treatment means were compared using Fisher's protected LSD at $P = 0.05$ for each harvest stage separately, except where otherwise stated. Single degree of freedom contrasts were used to compare meaningful treatment effects on forage yield and nutritive value data.

RESULTS AND DISCUSSION

Climate Data

Temperature was near the 30-yr normal over the period of the experiments at both locations (Table 1). The month of planting, September, was relatively dry, with precipitation levels under the 30-yr mean at both locations. However, timely substantial rainfall occurred just before and after sowing at both locations, resulting in excellent germination and emergence. The cereal crops established well and no apparent winter damage was observed

in spring. April through June, a crucial period for forage growth, received above average precipitation, with June being the wettest month at both locations.

Total Forage Yield

There was a highly significant location $\times$ treatment $\times$ stage interaction detected (not shown), so forage yield data were analyzed separately for each location. There were significant effects of treatment, maturity stage at harvest, and treatment $\times$ stage interaction at each location (Table 2).

Forage yield of all cereal crops, with or without suppression of Kura clover, was greater at Arlington than at Lancaster (Table 3). At Arlington, mixture forage yields at boot stage ranged from 4.02 Mg ha⁻¹ for rye to 5.26 Mg ha⁻¹ for triticale in non-suppressed Kura clover treatments, and among monoculture cereal grasses ranged from 4.51 to 6.02 Mg ha⁻¹ (Table 3). Mixtures yielded equally to respective monoculture cereals, except triticale in the suppressed Kura clover treatment, which had lower forage yield. There was no Kura clover suppression effect on mixture yields at the boot stage. Winter rye yields were the lowest ($P < 0.05$) across non-suppressed, suppressed, and monoculture cereal treatments, whereas no yield differences were detected between winter triticale and winter wheat. At cereal milk stage, yield averages across all mixture treatments increased by 46 to 115%, with greatest increases in winter rye (Table 3). At cereal milk stage, mixtures with suppressed Kura clover out-yielded non-suppressed mixture treatments and produced yields equal to respective monoculture grasses, which caused a significant treatment $\times$ stage interaction. When averaged across harvest stages, only mixtures of rye and triticale with suppressed Kura clover yielded equally to respective monocultures.

At Lancaster, at boot stage, yields among all forage mixtures were not different, ranging from 3.27 Mg ha⁻¹ to 3.80 Mg ha⁻¹, and among monoculture cereal grasses yield ranged from 3.94 to 4.29 Mg ha⁻¹ (Table 3). Mixtures yielded equally to their respective monoculture cereals, except wheat and triticale in non-suppressed Kura clover, which had lower forage yield. At milk stage, yield averages across all mixture treatments were more consistent than at Arlington and increased from 88 to 101% (Table 3) over boot stage yields. At both locations, these increases suggest a potential harvest strategy for greater forage yield. Mixtures with suppressed Kura clover had similar yields to both non-suppressed respective mixtures and monocultures. However, monoculture rye and winter wheat had significantly greater yields than non-suppressed Kura clover mixtures, which caused a significant treatment × stage interaction. When averaged across harvest stages, all suppressed Kura clover, and rye in non-suppressed mixtures yielded equally to their respective monocultures.

In an evaluation of spring-sown barley and triticale into either suppressed or non-suppressed Kura clover living mulch in Alberta, mixtures produced lower forage yield than monoculture cereals (Kosinski et al. 2011) at soft-dough stage of cereal development. Mixture yields in the current study were 80 to 100% greater than in the Canadian research, likely a function of greater competitive ability with Kura clover of winter cereals sown in autumn compared to spring cereals sown in spring. However, these cropping systems described by Kosinski et al. (2011) provided additional benefits that can compensate for the yield loss in a form of weed suppression, improved forage quality and reduced disease pressure. Forage yields, in the current study, were slightly lower than those reported by Ross et al. (2004) for a spring triticale-berseem clover mixture (9.9 Mg ha⁻¹) and Edmisten et al. (1998) for monoculture winter wheat and winter rye. It is important to point out that the

former only had a 17% legume component while Kura clover proportion averaged 40% in the current research (Table 4). Moreover, unlike Edmisten et al. (1998), no nitrogen fertilizer was applied in the current research; cereal crops utilized N from pools in the soil, some of this N likely derived from decaying Kura clover residues. Generally, intercropping winter cereals with legumes has been reported to increase forage yield when compared to monoculture grasses (Ghanbari-Bonjar and Lee 2003; Buyukburc and Karadag 2002), which is often due to improved light or water use efficiency of the mixture (Anil et al. 1998; Thorsted et al. 2006b).

The main cause of yield difference between locations was the rye response at boot stage. At Arlington, winter rye mixtures and monoculture treatments had the lowest yields and at Lancaster rye mixture yields were similar to winter wheat or triticale combinations (Table 3). Lauriault and Kirksey (2004) also reported that, in New Mexico, even though winter rye yield was more stable than triticale and wheat over years, its yields were always lower. This was most likely due to earlier date of maturation of rye, and in their study, relatively cool temperatures and low amounts of precipitation may have limited rye growth.

Monoculture Kura clover yields were the lowest among crops, and averaged 1.7 and 4.91 Mg ha⁻¹ at Arlington and 2.59 and 3.54 Mg ha⁻¹ at Lancaster when harvested at the cereal crop boot and milk stages, respectively (Table 3). Addition of cereal crops approximately doubled yields compared to monoculture Kura clover, demonstrating the benefits of this intercropping system. Contreras-Govea et al. (2006) reported similar increase in yield of Kura clover over-sown with wheat compared to monoculture clover. There is no information describing nitrogen credits from killed Kura clover to monoculture winter cereals, however Kelling et al. (2002) reported that wheat planted into killed alfalfa did not

respond to additional N fertilizer. Furthermore, Berkevich (2008) reported that corn planted into suppressed or killed Kura clover did not respond to additional N fertilizer. Therefore, although no nitrogen fertilizer was applied to the monoculture cereals sown into killed Kura clover, nitrogen supply was assumed to be not limiting.

Botanical Composition and Grass Performance

Cereal and clover proportions varied with clover suppression level, cereal crop species, and maturity stage at harvest. Also, there were location $\times$ treatment and treatment $\times$ stage (cereal only) interactions detected (Table 2). Contributing to the location $\times$ treatment interaction was a greater Kura clover suppression effect at Arlington resulting in an average of 26% greater proportion of a cereal grasses in mixtures compared to Lancaster (Table 4). Also, as a result of spring recovery after incomplete kill the previous autumn, there was a greater proportion of residual Kura clover in monoculture cereals at Arlington. In suppressed Kura clover treatments, cereals predominated, averaging 57% across all treatments and stages (Table 4).

The significant treatment $\times$ stage interaction on cereal grass proportion was because winter wheat proportion in mixtures with suppressed Kura clover was on average 18% lower relative to rye and no significant differences existed among species at milk stage (data not shown). Harvest stage had an effect on all mixture components (Table 4) where, at milk stage, cereal proportion increased by 36% and 11% across Kura clover non-suppressed and suppressed mixtures, respectively (data not shown). This concurs with earlier research showing that legumes in mixture with grasses compete more effectively for light (Martens et al. 2001) and water (Queen et al. 2009) in early rather than later stages of annual grass growth, and they would again be more competitive if grasses were allowed to fully mature

and senesce (Steiner and Snelling 1994). Martens et al. (2001) documented that because wheat develops later than rye, it is less competitive for light early in the season but becomes more competitive later in the season with stem elongation.

The decrease in Kura clover and increase in cereal proportions at milk stage is also associated with change in tiller height during maturation, even though tiller density decreased (Table 4). There was a significant location $\times$ stage interaction, but no significant location $\times$ treatment, or location $\times$ treatment $\times$ stage interaction was detected for cereal crop tiller density or height (Table 2). The former resulted primarily from changes in magnitude of differences among tiller density and tiller height between stages and locations rather than changes in their rankings. At Lancaster, tiller density and height at the milk stage were lower than at Arlington (data not shown). On average, at milk stage, winter cereals had 18% lower tiller density, across treatments and locations compared to boot stage (Table 4). Compared to monoculture cereals, mixtures had lower tiller densities across maturity stages ($P < 0.05$) and Kura clover suppression had little or no influence on tiller density at either stage ($P > 0.05$) (Table 4). Similar tiller densities and mortality rates have been reported by previous research with monoculture cereals (Sharma 1995; Bulman and Hunt 1988). Tiller development is controlled by phytochrome (Kasperbauer and Karlen 1986) and the change in red/far red light ratio associated with the presence of Kura clover may have contributed to reduced tiller density of cereal crops in mixtures. Although plant density was not monitored in the current study, Pridham and Entz (2008) tested several different intercrops for organic wheat production and reported that wheat plant densities with most intercropped treatments were similar to monoculture wheat.

There was a significant treatment $\times$ stage interaction for canopy height response (Table 2). This is because the average cereal height was similar, 65 to 74 cm, across all treatments at the boot stage (Table 4) but at milk stage, distinct differences in height among the cereals was observed. Winter wheat was always the shortest, winter triticale was intermediate, and winter rye was the tallest. Earlier research reported that legume competition at later stages of cereal development is minimized due to rapid stem elongation (Steiner and Snelling 1994). Stem elongation patterns help to explain the detected forage quality differences between stages and among cereal species.

Forage Nutritive Value

There were significant effects of location, treatment and maturity stage at harvest for all forage quality parameters and location $\times$ treatment interaction for IVTD, NDFD and CP, and there was no location $\times$ treatment $\times$ stage interaction detected for any nutritive value parameter (Table 2). Overall, the presence of Kura clover in mixtures increased CP and IVTD and reduced NDF concentration compared to monoculture cereals. Our findings are consistent with other research on winter cereals reporting declining forage CP, IVTD, and NDFD and increasing NDF with maturity (Andrzejewska et al. 2014; Kantar et al. 2011; Contreras-Govea et al. 2006).

Crude protein concentration of monoculture Kura clover was greater than mixtures or monoculture cereals at both stages ($P < 0.05$) (Table 5). Overall, CP decreased at milk stage in all mixtures due to increased proportion of cereal and cereal stem elongation. A significant stage $\times$ treatment interaction was caused by differences among cereal species. At boot stage, monoculture winter rye contained 21 to 25 g kg⁻¹ more CP than winter wheat and winter triticale; however, at milk stage CP concentrations were not different among

monoculture cereals. Crude protein concentrations were greater in all mixtures with non-suppressed Kura clover than with suppressed Kura clover at boot stage and only in rye mixtures at milk stage ($P < 0.05$). This difference is associated with clover proportions (Table 3). Maturity effects on winter rye forage yield and quality were reported in previous research (Andrzejewska et al. 2014; Kantar et al. 2011) indicating that producers may choose to maximize forage yield by harvesting at more mature stages or increase forage nutritive value by harvesting earlier at the boot stage. Our results are consistent with these studies as well as research that documented lower CP in monoculture cereal crops compared to mixtures with legumes (Contreras-Govea and Albrecht 2005; Chapko et al. 1991). Crude protein concentrations were greater at Arlington than at Lancaster, and at Arlington mixtures with suppressed Kura clover had significantly lower CP relative to non-suppressed Kura clover mixtures. At Lancaster, no differences were detected among mixtures causing a significant location $\times$ treatment interaction (data not shown). Crude protein was the only nutritive value parameter that was affected by location $\times$ stage interaction ($P < 0.05$) (Table 2). This interaction was a result of changes in magnitude of differences due to maturity stage between locations, where at both stages CP levels at Arlington were greater than at Lancaster (data not shown).

All forages had very high IVTD at boot stage, ranging between 873 and 945 g kg⁻¹, indicating that clover proportion had rather minor effect on IVTD at this stage (Table 5). Mixtures with cereal crops at boot stage were always more digestible than 892 g kg⁻¹, and at milk stage were always less than 780 g kg⁻¹ (Table 5). Furthermore, all mixtures were more digestible than monoculture cereals at both stages ($P < 0.05$). Kura clover suppression did not decrease IVTD at either stage despite reducing the proportion of clover in mixtures.

Within all mixture treatment combinations, winter rye had the greatest IVTD ($P < 0.05$) at boot and the lowest at milk stage compared to the two other cereals, and this is associated with a stage $\times$ treatment interaction ($P < 0.05$) (Table 2). The IVTD data together with high CP concentrations indicate that when winter rye is harvested at the boot stage it produces the best quality forage among the winter cereals tested. Other studies on nutritive value also acknowledged that cereals decrease in IVTD with maturation (Brink and Marten 1986; Kantar et al. 2011), and that intercropping cereals with annual or perennial legumes results in improved mixture nutritive value compared to monoculture cereal forage (Contreras-Govea and Albrecht 2005; Ross et al. 2004). A significant location $\times$ treatment interaction ($P = 0.018$) for IVTD was detected, primarily as a result of changes in magnitude of differences among treatments between locations rather than changes in ranking of treatments, where at Lancaster IVTD values were slightly lower than at Arlington (data not shown).

Maturity at harvest, the amount of cereal in a mixture and grass species played an important role in NDF concentration (Table 5). With one exception, the mixture of non-suppressed Kura clover with wheat at milk stage, monoculture cereals consistently had greater NDF concentrations than mixtures or monoculture Kura clover. This demonstrates again that NDF concentrations are lower in grass-legume mixtures than in grasses, increasing their potential intake by ruminants (Kosinski et al. 2011; Ross et al. 2004; Sleugh et al. 2000; Chapko et al. 1991). All treatments had greater NDF concentrations at cereal milk stage compared to boot, with maturity stage having greatest impact on NDF in winter rye. Treatments with winter wheat always had lower NDF values compared to other mixtures in cereal milk stage, while at boot stage no differences were detected, resulting in a significant stage $\times$ treatment interaction (Table 2).

Kura clover in mixtures had a different effect on NDFD, depending on grass maturity stage. Mixtures did not differ at boot stage relative to their monoculture cereals. However, at milk stage, mixtures of non-suppressed Kura clover in rye and suppressed Kura clover in rye and wheat had significantly higher NDFD values than monocultures. Monoculture rye and rye-Kura clover mixtures values at the boot stage harvest were never under 818 g kg⁻¹; whereas, those of other cereals never exceeded 758 g kg⁻¹, confirming winter rye's superior nutritive value at this stage (Table 5). Conversely, at milk stage, winter rye lost its advantage, with NDFD value at nearly half its boot stage level ranging from 415 to 474 g kg⁻¹ across all rye treatments. This occurrence was the reason for the detected stage × treatment interaction ($P < 0.05$) (Table 2) and can be explained by rapid stem elongation of winter rye. Stem elongation increases the total dry matter yield (Edmisten et al. 1998), and in our study also greatly affected NDFD and IVTD nutritive value parameters. A significant location × treatment interaction ($P = 0.032$) for NDFD was due to the fact that at Arlington non-suppressed Kura clover mixtures with either rye or wheat had slightly greater NDFD relative to monocultures, and at Lancaster all mixtures were equal to respective monoculture cereals (data not shown).

CONCLUSIONS

Results of this research demonstrate that winter cereals can be successfully established in autumn into an existing Kura clover sward for spring forage production. Although none of the mixtures increased spring forage yield compared to monoculture cereals, this production was accomplished with no N fertilizer. Furthermore, mixture yields were almost double that of monoculture Kura clover. Kura clover suppression before sowing

cereal crops increased mixture yields to nearly those achieved by monoculture cereals, but numerical differences were relatively small compared to non-suppressed treatments, therefore, suppression may be not economical. Because of the relatively high nutritive value of cereal crop-Kura clover mixtures, this forage is suitable for inclusion in dairy cattle rations. Harvest of mixtures when cereal crops, especially the relatively earlier maturing rye, would allow timely planting of corn into the same Kura clover field (Affeldt et al. 2004) and cereals could be again sown after corn silage harvest in humid continental climates. This type of double cropping allows exploiting parts of the growing season not occupied by corn silage production.

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Table 1. Total precipitation and mean monthly temperature data collected at the Arlington and Lancaster, WI Agricultural Research Stations from September 2009 to June 2010 (the entire experimental period) with the departure from the 1981-2010 normal.

Month	Arlington		Lancaster	
	Growth period	Departure	Growth Period	Departure
Precipitation, mm				
September	60	-30	20	-60
October	117	52	148	83
November	33	-28	21	-42
December	70	33	97	57
January	43	14	46	23
February	28	-5	17	-12
March	26	-22	22	-29
April	93	4	119	26
May	105	11	117	12
June	193	74	215	81
Total	768	104	822	140
Temperature, °C				
September	15.6	0.4	16.3	0.3
October	5.5	-3.1	5.8	-3.4
November	3.4	2.4	5.0	3.3
December	-7.9	-1.5	-7.0	-0.9
January	-9.9	-0.9	-10.9	-2.2
February	-6.9	-0.4	-7.8	-0.2
March	2.0	2.3	3.8	3.1
April	9.2	2.1	12.3	4.2
May	14.0	0.8	15.8	1.8
June	18.9	0.2	20.7	1.3

Table 2. Analyses of variance of the effects of location, treatment (cereal-Kura clover suppression combination), maturity stage at harvest, and their interactions on forage yield, forage mixture components, and forage quality parameters at locations in Wisconsin.

Effect	Forage yield ^a		Cereal		Mixture component			Forage quality parameter ^b			
	Arlington	Lancaster	Tillers	Height	Grass	Kura	Weeds	CP	IVTD	NDF	NDFD
Location (L)			<0.001	ns	ns	ns	ns	0.004	<0.001	0.018	<0.001
Treatment (T)	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
L × T			ns	ns	<0.001	<0.001	ns	<0.001	0.018	ns	0.032
Stage (S)	<0.001	<0.001	<0.001	<0.001	<0.001	0.009	<0.001	<0.001	<0.001	<0.001	<0.001
L × S			<0.001	<0.001	ns	ns	0.002	<0.001	ns	ns	ns
T × S	<0.001	<0.001	ns	<0.001	0.026	ns	0.001	<0.001	<0.001	<0.001	<0.001
L × T × S			ns	ns	ns	ns	0.003	ns	ns	ns	ns

Note: ns means that p-values not significant with *F*-test.

^aForage yield data were analyzed at each location separately due to highly significant L × T × S interaction (not shown).

^bForage quality parameters: CP, crude protein; IVTD, in vitro true digestibility; NDF, neutral detergent fiber; NDFD, and NDF digestibility.

Table 3. Interaction effect of treatment (forage yield of winter cereals grown alone or in binary mixtures with suppressed or non-suppressed Kura clover) and maturity stage (Boot, Milk) at harvest at Arlington and Lancaster, WI.

Management factor	Arlington			Lancaster		
	Boot	Milk	Mean	Boot	Milk	Mean
<hr/> Mg ha ⁻¹ <hr/>						
<u>Crop</u>						
Cereal-Kura Clover (KC)						
Rye-KC	4.02 _j	8.06 _{cd}	6.04 _D	3.73 _{def}	7.03 _{bc}	5.38 <i>BC</i>
Wheat-KC	4.91 _{f-i}	8.00 _{cd}	6.45 _C	3.27 _{fg}	6.35 _c	4.81 <i>C</i>
Triticale-KC	5.26 _{ef}	7.69 _d	6.48 _C	3.63 _{ef}	7.23 _{bc}	5.43 <i>BC</i>
Cereal-Supressed KC (SKC)						
Rye-SKC	4.46 _{ij}	9.59 _{ab}	7.02 _{BC}	3.80 _{def}	7.19 _{abc}	5.49 <i>ABC</i>
Wheat-SKC	4.99 _{fgh}	8.70 _{abc}	6.84 _{BC}	3.63 _{ef}	7.29 _{abc}	5.46 <i>BC</i>
Triticale-SKC	5.09 _{fg}	8.80 _{abc}	6.95 _{BC}	3.76 _{def}	7.32 _{abc}	5.54 <i>ABC</i>
Monoculture Cereal						
Rye	4.51 _{hij}	9.95 _{ab}	7.23 _{BC}	3.95 _{de}	8.28 _a	6.11 <i>AB</i>
Wheat	5.34 _{ef}	10.21 _a	7.77 _A	3.94 _{de}	7.82 _{ab}	5.88 <i>AB</i>
Triticale	6.02 _e	8.53 _{bcd}	7.28 _{AB}	4.29 _d	7.99 _{ab}	6.14 <i>A</i>
Monoculture KC						
KC	1.75 _k	4.72 _{ghi}	3.23 _E	2.63 _g	3.35 _{efg}	2.99 <i>D</i>
SKC	1.66 _k	5.11 _{fg}	3.38 _E	2.55 _g	3.74 _{def}	3.14 <i>D</i>
<u>Maturity stage</u>						
Mean	4.36 _A	8.12 _B		3.56 _A	6.69 _B	

Note: Values within or across maturity stage, within a location, followed by different lowercase italic letters are different at $P < 0.05$.

Means across maturity stages within a location followed by different uppercase italic letters are different at $P < 0.05$.

Maturity stage main effect means across crop type, within location, followed by different uppercase italic letters are different at $P < 0.05$.

Table 4. Canopy height, tiller density and forage mixture components of winter cereals grown in monoculture or with suppressed or non-suppressed Kura clover (treatment × stage interaction effect) at two locations in Wisconsin.

Management factor	Cereal			Mixture Component				
	Height ^a		Tillers ^b	Cereal ^c		Kura clover ^c		Weeds ^b
	Boot	Milk		Arlington	Lancaster	Arlington	Lancaster	
	-----cm -----		no. tillers m ⁻²	----- g kg ⁻¹ -----				
<u>Crop</u>								
Cereal-Kura Clover (KC)								
Rye-KC	65 <i>h</i>	149 <i>b</i>	279 <i>d</i>	52.4	44.9 <i>fg</i>	43.5 <i>bcd</i>	47.7 <i>b</i>	5.7 <i>ab</i>
Wheat-KC	68 <i>gh</i>	108 <i>d</i>	360 <i>c</i>	52.6	39.8 <i>g</i>	41.9 <i>bcd</i>	58.6 <i>a</i>	6.1 <i>a</i>
Triticale-KC	67 <i>h</i>	123 <i>c</i>	354 <i>c</i>	53.4	47.8 <i>fg</i>	42.4 <i>bcd</i>	46.2 <i>bc</i>	5.2 <i>ab</i>
Cereal-Suppressed KC (SKC)								
Rye-SKC	70 <i>fg</i>	159 <i>ab</i>	335 <i>cd</i>	69.1	51.8 <i>ef</i>	28.0 <i>ef</i>	46.8 <i>bc</i>	2.1 <i>cd</i>
Wheat-SKC	71 <i>fg</i>	108 <i>d</i>	355 <i>c</i>	60.7	47.3 <i>fg</i>	34.9 <i>de</i>	49.0 <i>ab</i>	4.0 <i>bc</i>
Triticale-SKC	67 <i>fgh</i>	122 <i>c</i>	350 <i>c</i>	60.7	51.6 <i>ef</i>	37.7 <i>cde</i>	47.1 <i>bc</i>	1.5 <i>d</i>
Monoculture Cereal								
Rye	74 <i>e</i>	171 <i>a</i>	437 <i>b</i>	83.3	96.3 <i>a</i>	15.5 <i>g</i>	2.4 <i>h</i>	1.2 <i>d</i>
Wheat	70 <i>ef</i>	113 <i>d</i>	573 <i>a</i>	79.3	95.4 <i>a</i>	19.3 <i>fg</i>	0.7 <i>h</i>	2.6 <i>cd</i>
Triticale	69 <i>fg</i>	126 <i>c</i>	486 <i>b</i>	82.1	96.5 <i>a</i>	16.8 <i>g</i>	2.7 <i>h</i>	1.0 <i>d</i>
<u>Maturity stage</u>								
Boot	69 <i>B</i>	430 <i>A</i>	61.4 <i>B</i>	34.4 <i>A</i>	4.2 <i>A</i>			
Milk	131 <i>A</i>	354 <i>B</i>	68.0 <i>A</i>	30.2 <i>B</i>	2.4 <i>B</i>			

Note: Means for cereal height, tiller number, and proportions of cereal, Kura clover, and weeds followed by different lowercase italic letters are different at *P* < 0.05. Maturity stage main effect means followed by different uppercase italic letters are different at *P* < 0.05.

^aMeans represent average values across locations.
^bMeans represent average values across maturity stages and locations.
^cMeans represent average values across maturity stages.

Table 5. Crude protein (CP), in vitro true digestibility (IVTD), neutral detergent fiber (NDF), and neutral detergent fiber digestibility (NDFD) of monoculture winter cereals and mixtures of winter cereals with suppressed or non-suppressed Kura clover separately or across maturity stage (Boot, Milk) at harvest across two locations in Wisconsin.

across maturity stage (Boot, Milk) at harvest across two locations in Wisconsin.								
Management factor	CP		IVTD		NDF		NDFD	
	Boot	Milk	Boot	Milk	Boot	Milk	Boot	Milk
	g kg ⁻¹						g kg ⁻¹ NDF	
<u>Crop</u>								
Cereal-Kura Clover (KC)								
Rye-KC	217 <i>b</i>	114 <i>g</i>	937 <i>ab</i>	718 <i>lm</i>	382 <i>l</i>	536 <i>cd</i>	837 <i>a</i>	474 <i>g</i>
Wheat-KC	202 <i>bc</i>	118 <i>g</i>	900 <i>de</i>	761 <i>ij</i>	380 <i>l</i>	506 <i>ef</i>	736 <i>b</i>	531 <i>ef</i>
Triticale-KC	200 <i>c</i>	116 <i>g</i>	904 <i>cd</i>	746 <i>jk</i>	392 <i>jkl</i>	534 <i>cde</i>	756 <i>b</i>	523 <i>ef</i>
Cereal-Suppressed KC (SKC)								
Rye-SKC	179 <i>d</i>	95 <i>h</i>	929 <i>b</i>	708 <i>m</i>	419 <i>ij</i>	544 <i>c</i>	831 <i>a</i>	467 <i>g</i>
Wheat-SKC	183 <i>d</i>	107 <i>gh</i>	900 <i>de</i>	780 <i>hi</i>	391 <i>kl</i>	480 <i>fg</i>	744 <i>b</i>	537 <i>de</i>
Triticale-SKC	185 <i>d</i>	113 <i>g</i>	892 <i>e</i>	749 <i>jk</i>	415 <i>ijk</i>	523 <i>cde</i>	758 <i>b</i>	521 <i>ef</i>
Monoculture Cereal								
Rye	160 <i>e</i>	68 <i>i</i>	913 <i>c</i>	648 <i>n</i>	474 <i>g</i>	603 <i>a</i>	818 <i>a</i>	415 <i>h</i>
Wheat	139 <i>f</i>	80 <i>i</i>	876 <i>f</i>	739 <i>kl</i>	469 <i>gh</i>	525 <i>cde</i>	737 <i>b</i>	503 <i>fg</i>
Triticale	135 <i>f</i>	80 <i>i</i>	873 <i>f</i>	722 <i>lm</i>	509 <i>de</i>	574 <i>b</i>	752 <i>b</i>	517 <i>ef</i>
Monoculture KC								
KC	269 <i>a</i>	189 <i>cd</i>	942 <i>a</i>	827 <i>g</i>	352 <i>m</i>	435 <i>i</i>	835 <i>a</i>	607 <i>c</i>
SKC	280 <i>a</i>	183 <i>d</i>	945 <i>a</i>	825 <i>gh</i>	318 <i>n</i>	442 <i>hi</i>	825 <i>a</i>	610 <i>cd</i>
<u>Maturity stage</u>								
Boot	195 <i>A</i>		910 <i>A</i>		409 <i>B</i>		784 <i>A</i>	
Milk	115 <i>B</i>		747 <i>B</i>		518 <i>A</i>		519 <i>B</i>	

Note: Means within a nutritive value parameter within and across columns followed by different lowercase italic letters are different at $P < 0.05$.

Maturity stage main effect means within a nutritive value parameter followed by different uppercase italic letters are different at $P < 0.05$.