

## Illinois Bundleflower Forage Potential in the Upper Midwestern USA: II. Forage Quality

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### ABSTRACT

Illinois bundleflower [*Desmanthus illinoensis* (Mich.) MacMill] is a warm-season perennial legume native to the central USA. Little is known about its forage quality in the upper midwestern USA. Two experiments were established at four Minnesota locations in 2000 to evaluate the effects of N fertilization, maturity at harvest, and residual height of cutting on the acid detergent fiber (ADF), neutral detergent fiber (NDF), crude protein (CP), in vitro true digestibility (IVTD), in vitro dry matter digestibility (IVDMD), and leaf proportion of three Illinois bundleflower (IBF) ecotypes. Whole-herbage forage quality was greatest ( $P < 0.05$ ) at early flower in mid-July with average ADF, NDF, and CP concentrations of 315, 352, and 180 g kg<sup>-1</sup>, respectively. Fiber values increased to 412 and 479 g kg<sup>-1</sup>, respectively, while CP decreased to 129 g kg<sup>-1</sup> at late pod in mid-August. Leaf proportion decreased ( $P < 0.05$ ) from 618 g kg<sup>-1</sup> at early flower to 335 g kg<sup>-1</sup> at late pod while leaf CP decreased ( $P < 0.05$ ) from 216 to 147 g kg<sup>-1</sup>. Whole-herbage IVDMD and IVTD concentrations decreased ( $P < 0.05$ ) from 470 and 648 g kg<sup>-1</sup> at early flower to 390 and 560 g kg<sup>-1</sup> at late pod, respectively. Increasing residual cutting height from 15 to 35 cm decreased ( $P < 0.05$ ) ADF and NDF concentrations by an average of 50 g kg<sup>-1</sup> and increased ( $P < 0.05$ ) IVTD, IVDMD, and CP concentrations by 43, 39, and 20 g kg<sup>-1</sup>, respectively. Illinois bundleflower can provide good quality summer forage in the upper midwestern USA, but more research is needed to ascertain the implications of its low IVDMD on animal performance.

ILLINOIS BUNDLEFLOWER is a warm-season legume native to the central plains of the USA. There is interest in mixing IBF with native warm-season grasses in the upper midwestern USA because of its potential for supplying fixed N to ruminants. Research in the grasslands of Texas, Oklahoma, and Kansas has indicated that IBF has significant potential as a forage legume. It has been successfully introduced into existing grass swards and increased dry matter (DM) yield (Dovel et al., 1990; Posler et al., 1993). In addition, IBF has a vigorous seedling that establishes well in monoculture and binary mixtures when planted into prepared seedbeds (Piper, 1998; Beran et al., 2000).

Despite a growing body of research evaluating IBF's agronomic potential, little is known about its forage quality potential or how management practices affect it, particularly in northern regions of adaptation such as Minnesota. In Kansas, IBF in binary mixture with sideoats gramma [*Bouteloua curtipendula* (Michx.) Torr.], indiagrass [*Sorghastrum nutans* (L.) Nash], or switchgrass (*Panicum virgatum* L.) increased CP concentration

of the forage mixture over grass alone (Posler et al., 1993). However, the addition of IBF decreased the IVDMD concentration of the forage compared with grass alone. Adjei and Pitman (1993) reported leaf CP concentration of 230 g kg<sup>-1</sup> for IBF grown on a phosphatic clay mine-spoil in Florida. Despite relatively high leaf CP concentration, in vitro organic matter digestibility (IVOMD) concentrations averaged across three harvest frequencies were quite low, with leaf IVOMD of only 400 g kg<sup>-1</sup> and stem IVOMD of 350 g kg<sup>-1</sup>. Interestingly, the IVOMD of IBF actually increased with plant maturity, which the authors suggest was due to dilution of antiquality components, possibly tannins. In Texas, despite an ADF concentration of only 214 g kg<sup>-1</sup>, IBF in sacco DM and N degradability averaged only 422 and 450 g kg<sup>-1</sup>, respectively, considerably less than five other forages with which it was compared (Packard et al., 2003).

Little is known about the forage quality of northern accessions of IBF grown in the upper midwestern USA. DeHaan et al. (2003) determined the ADF, NDF, and CP concentrations of 20 accessions of IBF at three locations in Minnesota. Averaged across locations and accessions, whole-herbage IBF forage quality was favorable compared with that reported for other more common forages (Sanderson and Wedin, 1989; Sheaffer et al., 2003) with 329, 367, and 180 g kg<sup>-1</sup> ADF, NDF, and CP, respectively. However, in the DeHaan et al. (2003) study, forage quality was determined on spaced plants with an average density of 0.9 plants m<sup>-2</sup> to characterize genotypic diversity. In forage production systems, IBF will likely be grown at much greater densities in monoculture or in mixture with warm-season grasses. Greater plant density is likely to affect the growth form of IBF and, consequently, its forage quality (Volencic et al., 1987). In addition, understanding the effects of N fertilization, plant maturity, and cutting height on the forage quality of IBF is essential to designing productive forage systems for the upper midwestern USA.

Although IBF nodulates with indigenous and applied *Rhizobium* spp., a positive yield response to N fertilization indicates the symbiosis may not be fully effective in satisfying the N needs of the plant (Byun et al., 2004; Fischbach et al., 2005). Thus, in some environments, N fertilization may be required to maximize total forage production of IBF grown in monoculture or in mixture with warm-season grasses. Nitrogen fertilization can increase the height of cool-season legumes, resulting in lower leaf proportions and greater fiber concentration.

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**Abbreviations:** ADF, acid detergent fiber; CP, crude protein; DM, dry matter; IBF, Illinois bundleflower; IVDMD, in vitro dry matter digestibility; IVOMD, in vitro organic matter digestibility; IVTD, in vitro true digestibility; NDF, neutral detergent fiber.

It can also increase the N content of the leaves, resulting in greater CP levels (Fishbeck and Phillips, 1981; Redfearn et al., 2001). It is unknown how N fertilization may affect forage quality of IBF.

Forage quality of most temperate legumes is affected by cutting height and maturity at harvest (Sheaffer et al., 2003). Cool-season legumes harvested before flowering or at early flower have greater forage quality than when harvested at late pod (Sanderson and Wedin, 1989). A higher cutting height tends to increase the leaf-to-stem ratio of the harvested forage and, thus, results in greater forage quality (Buxton et al., 1985). The magnitude of the increase depends on basal leaf retention and growth form of the forage species. Because of IBF's tall growth in dense stands (Fischbach et al., 2005), a higher residual cutting height should increase forage quality by increasing the leaf proportion of harvested forage.

The objective of this study was to evaluate the effects of N fertilization, maturity at harvest, and cutting height on forage quality of three northern ecotypes of IBF grown in monospecific stands in the upper midwestern USA.

## MATERIALS AND METHODS

### Field Experiments

Experiment 1 was established at three University of Minnesota agricultural experiment stations at Rosemount, St. Paul, and Becker, MN, in spring 2000. Locations were selected to represent a range of potential environments in which IBF might be grown in the upper midwestern USA. The trials were seeded 7 June 2000 at Rosemount (44°53' N, 93°13' W) on a Tallula silt loam (coarse-silty, mixed, mesic Typic Hapludoll) with pH 6.9, 30 mg kg<sup>-1</sup> P, and 67 mg kg<sup>-1</sup> K; 8 June 2000 at St. Paul (44°99' N, 93°18' W) on a Waukegan silt loam (fine-silty over sandy, mixed Typic Hapludoll) with pH 6.8, 288 mg kg<sup>-1</sup> P, and 445 mg kg<sup>-1</sup> K; and 25 May 2000 at Becker (45°38' N, 93°89' W) on a Hubbard loamy sand (fine-silty over sandy, mixed Typic Hapludoll) with pH 7.1, 35 mg kg<sup>-1</sup> P, and 90 mg kg<sup>-1</sup> K.

The experiment was designed as a randomized complete block in a split-split plot arrangement with four replications. Treatments were imposed beginning in 2001, the year after establishment. Whole-plot treatments were either no N added or 110 kg N ha<sup>-1</sup> yr<sup>-1</sup>. Subplot treatments were two stages of maturity at harvest. In 2001, an early-flower harvest was made around 17 July and a late-pod harvest around 17 August. Harvest treatments were repeated in 2002 at all locations; however, the mid-August 2001 treatment harvested at late pod was not repeated at St. Paul or Rosemount in 2002 due to a lack of persistence. Sub-subplot ecotype treatments were three relatively persistent northern ecotypes of IBF selected from the University of Minnesota Native Perennial Legume Collection by DeHaan et al. (2003). Accession 3 (Ecotype 3), from Gordner Lake, Stevens County, MN (45°30'49" N, 96°00'38" W), has early to midseason maturity with low to moderate seed yield and short plant stature. Accession 8 (Ecotype 8), from Spirit Lake, Dickinson County, IA (43°28'31" N, 95°41'40" W), has midseason maturity with moderate to high seed yield and tall height. Accession 10 (Ecotype 10), from Cottonwood Lake, Spink County, SD (44°46'41" N, 98°41'40" W), has early maturity with high seed yield and moderate height. All three accessions mapped to Cluster 1 in

the genetic analysis of DeHaan et al. (2003). See Fischbach et al. (2005) for a complete description of the experimental design and treatments.

Experiment 2 was established in spring 2000 at three University of Minnesota agricultural experiment stations in southern Minnesota representing a range of potential environments in which IBF might be grown in the upper midwestern USA. Trials were seeded 7 June 2000 at Rosemount (same soil as in Experiment 1), 25 May 2000 at Becker (same soil as in Experiment 1), and 8 June 2000 at Lamberton (44°15' N, 95°19' W) on a Normania loam (fine-loamy mixed, mesic Aquic Hapludoll) with pH 7.5, 20 mg kg<sup>-1</sup> P, and 189 mg kg<sup>-1</sup> K. The experiment was designed as a randomized complete block in a split-split plot arrangement with three replications. Treatments were imposed in 2002, 2 yr after seeding. Whole-plot treatments were three stages of maturity at harvest. An early-flower harvest was made around 15 July, an early-pod harvest around 22 July, and a late-pod harvest around 13 August. Subplot ecotype treatments were the same three northern ecotypes of IBF used in Experiment 1, selected from the University of Minnesota Native Perennial Legume Collection by DeHaan et al. (2003). Sub-subplot cutting height treatments were either a 15- or 35-cm residual height and were selected to represent a range of potential residual heights to which IBF might be cut or grazed. See Fischbach et al. (2005) for a complete description of the experimental design and treatments.

### Forage Quality Analysis

In Experiment 1, random 1-kg samples were hand-clipped from each sub-subplot at all locations immediately before early-flower and late-pod harvests and dried at 60°C for 96 h. Since leaves would most likely be grazed preferentially where IBF is used as pasture (Muir, 2002), selected locations and treatments were separated into leaf and stem components to gain information on leaf proportion and leaf quality with what resources were available. In 2001, samples from Rosemount were analyzed as whole herbage. All samples from St. Paul were separated into leaf and stem components following determination of total dry weight to quantify leaf proportion. Forage quality analysis was performed on only the leaf component. At Becker, all samples were separated into leaf and stem components to determine leaf proportion, recombined, and analyzed as whole herbage. In addition, at Becker, a sample taken at the late-pod harvest was separated into leaf and stem components, and forage quality analysis was performed on the leaf component. In 2002, harvested samples from all locations were analyzed as whole herbage.

In Experiment 2, random 1-kg samples were hand-clipped from each sub-subplot at all locations immediately before harvest and dried at 60°C for 96 h. All samples were left intact and analyzed as whole herbage except at Lamberton where Ecotype 3 samples were separated into leaf and stem components and forage quality analysis was performed on only the leaf component.

Samples from both experiments were ground with a Thomas-Wiley Laboratory Mill Model 4 (Thomas Scientific,<sup>1</sup> Swedesboro, NJ) to pass a 10-mm screen, hand-mixed, and then ground to 1 mm with a Cyclotec 1093 Sample Mill (Foss Tecator,<sup>1</sup> Eden Prairie, MN). All samples were scanned with

<sup>1</sup> Names are necessary to report factually on available data; however, the University of Minnesota neither guarantees nor warrants the standard of the product, and the use of the name by the University of Minnesota implies no approval of the product to the exclusion of others that may be suitable.

a NIRSystems 6500 scanning monochrometer with a range of 400 to 2500 nm (NIRSystems Inc.,<sup>1</sup> Silver Springs, MD) to determine DM, NDF, ADF, CP, IVTD, and IVDMD concentrations. In vitro true digestibility was determined in addition to IVDMD to provide an alternative measure of digestibility and to begin to gain information about IBF's fiber digestibility. To develop calibration equations, 50 samples from Experiment 1 and 10 samples from Experiment 2 were chosen using WINSI II software (Infrasoft International,<sup>1</sup> Port Matilda, PA). Samples were selected based on spectral differences and represented leaves and whole herbage from harvests across four locations, three maturities, and 2 yr. Prediction equations were developed from the calibration sets by performing a modified partial least squares regression using the "Global Calibration" function of the WINSI II software. Equation statistics are shown in Table 1.

Neutral detergent fiber and ADF were determined using the methods of Goering and Van Soest (1970) as modified by Mertens (2002). Sodium sulfite and heat-stable  $\alpha$ -amylase were used in the NDF procedure. Crude protein was measured using the micro-Kjeldahl procedure and multiplying Kjeldahl N by 6.25 (AOAC, 1975). In vitro dry matter digestibility of calibration samples was measured using the Marten and Barnes (1980) procedure as modified for the ANKOM Daisy II incubator (Ankom Technol. Corp.,<sup>1</sup> Fairport, NY). In vitro true digestibility was analyzed using the procedure of Tilley and Terry (1963) as modified for the ANKOM system. The first stage of the IVTD procedure consisted of a 48-h incubation with rumen fluid in an ANKOM Daisy II incubator. The second stage was the NDF procedure for the ANKOM-200 Fiber Analyzer (Ankom Technol. Corp.,<sup>1</sup> Fairport, NY).

### Statistical Analysis

Analyses of variance (ANOVA) of forage quality data were performed using the PROC GLM procedure of SAS (SAS Inst., 2001) using a split-split plot model (Gomez and Gomez, 1984). In Experiment 1, N fertilization, maturity at harvest, and ecotype were the whole-plot, split-plot, and split-split plot factors, respectively. In Experiment 2, maturity at harvest was the whole-plot factor, ecotype was the split-plot factor, and cutting height was the split-split plot factor. Results from each experiment, location, and year were analyzed separately due to heterogeneity among environments. Significant interaction effects were sporadic and inconsistent across locations and

**Table 1. Equation statistics for near infrared reflectance spectroscopy prediction of Illinois bundleflower herbage concentrations of crude protein (CP), acid detergent fiber (ADF), neutral detergent fiber (NDF), dry matter (DM), in vitro dry matter digestibility (IVDMD), and in vitro true digestibility (IVTD) for Experiments 1 and 2 in Minnesota. Equations were developed with 60 whole-herbage and leaf samples from four locations and 3 yr.**

Constituent	N	SEC <sup>†</sup>	SECV	R <sup>2</sup>	1 - VR
CP	58	0.959	1.091	0.952	0.938
ADF	59	1.004	1.175	0.990	0.986
NDF	57	0.908	1.127	0.994	0.990
DM	59	0.153	0.210	0.941	0.888
IVDMD	54	2.706	3.145	0.916	0.885
IVTD	52	1.810	2.754	0.968	0.926

<sup>†</sup> SEC, standard error of calibration; SECV, standard error of cross-validation in modified partial least squares regression; R<sup>2</sup>, coefficient of determination of calibration; 1 - VR, 1 minus the variance ratio calculated in cross validation during modified partial least squares regression.

years; they are presented only in the text where relevant response variables are discussed and footnoted in tables. Main-effect means were separated with the Least Significant Differences test with a significance level of  $P = 0.05$ . Where treatment effects are discussed in the following sections, they are at the  $P = 0.05$  level of significance.

## RESULTS

### Experiment 1

#### Leaf Proportion

Of all factors studied, maturity at harvest had the greatest effect on leaf proportion of harvested IBF forage (Table 2). Averaged for St. Paul and Becker, IBF leaf proportion decreased from 618 g kg<sup>-1</sup> at early flower to 335 g kg<sup>-1</sup> at late pod, an 85% decrease in leaf proportion from mid-July to mid-August. The influence of N fertilization on leaf proportion was biologically insignificant (i.e., statistically significant at St. Paul 2001 but not practically significant overall). Leaf proportion among IBF ecotypes was similar at Becker with an average of 551 g kg<sup>-1</sup>. At St. Paul, a maturity at harvest  $\times$  ecotype crossover interaction occurred because Ecotype 3 had

**Table 2. Crude protein (CP) concentration and leaf proportion of Illinois bundleflower as influenced by N fertilization, harvest maturity, and ecotype in Experiment 1 at three Minnesota locations over 2 yr.**

	Whole-herbage CP						Leaves			
	Becker		St. Paul	Rosemount		St. Paul, 2001		Becker, 2001		
	2001	2002	2002	2001	2002	%	CP	%	CP	
	g kg <sup>-1</sup>									
N (kg ha <sup>-1</sup> yr <sup>-1</sup> )										
0	177	181	199	101	180	405	168	570	205	
110	161	162	216	124	158	398	195	532	204	
LSD (0.05) <sup>†</sup>	NS <sup>‡</sup>	NS	NS	7	9	6	13	NS	NS	
Maturity at harvest										
Early flower	185	179	209	120	169	581	216	655	-	
Late pod	151	148	-	105	-	222	147	447	205	
LSD (0.05)	11	9	-	7	-	33	5	38	-	
Ecotype										
3	174	172	219	118	188	410	175	553	212	
8	168	174	203	111	166	404	189	549	192	
10	184	167	202	109	153	391	181	551	212	
LSD (0.05)	8	NS	NS	6	11	NS	7	NS	NS	

<sup>†</sup> LSD, least significant difference ( $P = 0.05$ ); maturity  $\times$  ecotype ( $P < 0.05$ ) for leaf proportion at St. Paul 2001; N  $\times$  maturity ( $P < 0.01$ ) for CP concentration at Rosemount 2001.

<sup>‡</sup> NS, not significant ( $P > 0.05$ ).

the greatest leaf proportion at early flower, but Ecotype 8 had the greatest at late pod (data not shown).

### Fiber Concentration

Illinois bundleflower forage averaged 332 g kg<sup>-1</sup> ADF and 378 g kg<sup>-1</sup> NDF. Leaves averaged 170 g kg<sup>-1</sup> ADF and 210 g kg<sup>-1</sup> NDF. Both ADF and NDF concentrations increased with advancing maturity (Table 3). Averaged across locations in 2001, whole-herbage NDF and ADF concentrations increased by 96 and 74 g kg<sup>-1</sup> from early flower to late pod, respectively. At St. Paul in 2001, NDF concentration of leaves increased 16 g kg<sup>-1</sup> from early flower to late pod. The influence of N fertilization on fiber concentrations was biologically insignificant.

There were inconsistent ecotype differences in whole-herbage ADF and NDF concentration across locations and years. At Becker in 2001, there was a maturity × ecotype crossover interaction for whole-herbage NDF concentration. Ecotype 10 had the lowest NDF concentration at early flower but the greatest NDF concentration at late pod. At Rosemount in 2001, Ecotype 3 had a lower NDF concentration than Ecotypes 8 and 10. In 2002, there were no ecotype differences in whole-herbage ADF or NDF concentration at any location. There were slight differences in leaf ADF and NDF concentration among ecotypes at St. Paul in 2001. Ecotype 8 had lower ADF and NDF concentrations than Ecotypes 3 and 10.

### Crude Protein

Whole-herbage CP concentration of IBF averaged 160 g kg<sup>-1</sup> while leaf CP concentration averaged 190 g kg<sup>-1</sup>. Whole-herbage CP concentration decreased by an average of 27 g kg<sup>-1</sup> from early flower to late pod at Rosemount and Becker (Table 2). Over the same period, leaf CP concentration decreased by 69 g kg<sup>-1</sup> at St. Paul in 2001.

Nitrogen fertilization had inconsistent effects on CP concentration of IBF (Table 2). There was no effect on whole-herbage CP at Becker in 2001 and 2002. At Rosemount in 2001, there was an N × maturity interaction for CP concentration (data not shown); at early flower, N fertilization increased CP concentration by 38 g kg<sup>-1</sup>, but there was no N effect at late pod. In 2002 at Rosemount, N fertilization decreased CP concentration by 22 g kg<sup>-1</sup>. Leaf CP concentration increased 27 g kg<sup>-1</sup> at St. Paul in 2001 in response to N fertilization.

Ecotypes of IBF did not consistently differ in CP concentration across locations (Table 2). Averaged across all treatments in 2001, Ecotype 10 had greater whole-herbage CP concentration than Ecotypes 3 and 8 at Becker, but at Rosemount, Ecotype 3 had greater CP concentration than Ecotype 10. In 2002, Ecotype 3 had the greatest CP concentration at Rosemount, but there was no difference among ecotypes at Becker or St. Paul. Leaf CP concentration differed among ecotypes at St. Paul where Ecotype 8 had the greatest CP concentration

**Table 3. Neutral detergent fiber (NDF) and acid detergent fiber (ADF) concentrations in Illinois bundleflower whole herbage and leaves as influenced by N fertilization, harvest maturity, and ecotype in Experiment 1 at three Minnesota locations over 2 yr.**

	Whole herbage						Leaves	
	Becker		St. Paul	Rosemount		St. Paul	Becker	
	2001	2002	2002	2001	2002	2001	2001	
	g kg <sup>-1</sup>							
	<b>NDF</b>							
N (kg ha <sup>-1</sup> yr <sup>-1</sup> )								
0	359	439	331	396	314	211	212	
110	388	483	317	406	342	202	202	
LSD (0.05)†	NS‡	NS	NS	NS	NS	4	NS	
Maturity at harvest								
Early flower	311	426	320	368	328	199	–	
Late pod	437	496	–	434	–	215	207	
LSD (0.05)	37	29	–	18	–	10	–	
Ecotype								
3	361	455	313	383	317	214	213	
8	346	424	315	416	339	198	206	
10	326	462	335	404	328	208	203	
LSD (0.05)	NS	NS	NS	21	NS	9	NS	
	<b>ADF</b>							
N (kg ha <sup>-1</sup> yr <sup>-1</sup> )								
0	303	385	306	334	288	174	170	
110	329	423	292	344	316	167	157	
LSD (0.05)	NS	NS	NS	NS	NS	4	NS	
Maturity at harvest								
Early flower	265	380	295	316	302	169	–	
Late pod	366	429	–	363	–	176	164	
LSD (0.05)	33	25	–	16	–	NS	–	
Ecotype								
3	304	399	290	321	292	175	171	
8	295	377	289	354	313	164	162	
10	280	408	308	343	302	173	161	
LSD (0.05)	NS	NS	NS	18	NS	8	NS	

† LSD, least significant difference ( $P = 0.05$ ); N × ecotype ( $P < 0.05$ ) for ADF at Becker 2001; maturity × ecotype ( $P < 0.05$ ) for NDF at Becker 2001.

‡ NS, not significant ( $P > 0.05$ ).

and Ecotype 3 had the lowest. There were no ecotype differences in leaf CP concentration at Becker.

In 2001, nearly 75% of total harvested N was in IBF leaves. At Becker, an average of 3.9 Mg DM ha<sup>-1</sup> was harvested at late pod (Fischbach et al., 2005). This biomass had whole-herbage CP concentration of 151 g kg<sup>-1</sup> (Table 2). Therefore, a single harvest at late pod contained 94 kg N ha<sup>-1</sup>. Harvested herbage had an average leaf proportion of 447 g kg<sup>-1</sup> with a CP concentration of 205 g kg<sup>-1</sup>. Thus, of the 94 kg N ha<sup>-1</sup> harvested at late pod, 57 kg N ha<sup>-1</sup> was contained in the leaves. This value is similar to N yield at St. Paul at early flower where harvested leaves yielded an average of 62 kg N ha<sup>-1</sup>.

### Digestibility

Whole-herbage IVTD and IVDMD concentrations of IBF averaged 630 and 475 g kg<sup>-1</sup>, respectively. In 2001, whole-herbage IVTD decreased by an average of 100 g kg<sup>-1</sup> from early flower to late pod at Becker (Table 4). At Rosemount in 2001, IVTD decreased by 31 g kg<sup>-1</sup>. Leaf IVTD and IVDMD decreased by an average of 31 g kg<sup>-1</sup> from early flower to late pod at St. Paul in 2001.

Nitrogen fertilization had slight and inconsistent effects on digestibility (Table 4). In 2001, N fertilization increased whole-herbage IVDMD of IBF by 14 g kg<sup>-1</sup> at Rosemount but decreased IVTD by 15 g kg<sup>-1</sup>. At St.

Paul, N fertilization increased leaf IVDMD and IVTD concentrations by an average of 17 g kg<sup>-1</sup>.

There were slight digestibility differences among ecotypes (Table 4). Ecotype 3 had greater whole-herbage IVTD than Ecotypes 8 and 10 at Rosemount in 2001. Ecotype 8 had greater leaf IVTD and IVDMD than Ecotypes 3 and 10 at St. Paul. There was a three-way N × maturity × ecotype interaction for whole-herbage IVTD and IVDMD at Becker in 2001. Ecotype 10 had greater digestibility than Ecotypes 3 and 8 at early flower in unfertilized plots, but there were no differences among ecotypes at early flower in fertilized plots (data not shown). At late pod, Ecotype 10 was less digestible than Ecotypes 3 and 8 in unfertilized plots, but in N-fertilized plots, Ecotypes 10 and 8 had similar digestibility.

## Experiment 2

### Fiber Concentration

Whole herbage of IBF averaged 398 and 452 g kg<sup>-1</sup> ADF and NDF, respectively. Whole-herbage fiber concentrations increased from early flower to late pod at all locations (Table 5). Acid detergent fiber increased from 340 g kg<sup>-1</sup> at early flower to 450 g kg<sup>-1</sup> at late pod while NDF increased from 370 g kg<sup>-1</sup> at early flower to 520 g kg<sup>-1</sup> at late pod. A 35-cm residual cutting height decreased ADF and NDF concentrations at all locations

**Table 4. In vitro true digestibility (IVTD) and in vitro dry matter digestibility (IVDMD) of Illinois bundleflower whole herbage and leaves as influenced by N fertilization, harvest maturity, and ecotype in Experiment 1 at three Minnesota locations over 2 yr.**

	Whole herbage					Leaves	
	Becker		St. Paul	Rosemount		St. Paul	Becker
	2001	2002	2002	2001	2002	2001	2001
	g kg <sup>-1</sup>						
	IVTD						
N (kg ha <sup>-1</sup> yr <sup>-1</sup> )							
0	618	611	671	592	700	774	724
110	593	576	691	577	669	782	727
LSD (0.05)†	NS‡	NS	NS	11	NS	4	NS
Maturity at harvest							
Early flower	655	614	686	600	685	790	—
Late pod	555	572	—	569	—	766	725
LSD (0.05)	72	NS	—	18	—	13	—
Ecotype							
3	626	592	693	602	695	774	718
8	591	606	687	568	670	786	729
10	599	585	675	583	689	773	725
LSD (0.05)	22	NS	NS	19	NS	11	NS
	IVDMD						
Nitrogen (kg ha <sup>-1</sup> yr <sup>-1</sup> )							
0	547	419	503	473	459	625	645
110	515	391	530	487	423	651	653
LSD (0.05)	NS	NS	NS	13	NS	8	NS
Maturity at harvest							
Early flower	562	433	522	484	441	657	—
Late pod	500	377	—	476	—	619	648
LSD (0.05)	NS	31	—	NS	—	6	—
Ecotype							
3	550	405	529	490	460	633	642
8	516	421	521	475	431	644	640
10	528	400	513	475	432	636	660
LSD (0.05)	20	NS	NS	NS	NS	9	NS

† LSD, least significant difference ( $P = 0.05$ ); at Becker 2001, N × maturity × ecotype ( $P < 0.05$ ) for both IVTD and IVDMD, and N × ecotype and maturity × ecotype ( $P < 0.01$ ) for both IVTD and IVDMD.

‡ NS, not significant ( $P > 0.05$ ).

**Table 5. Acid detergent fiber (ADF) and neutral detergent fiber (NDF) concentrations in Illinois bundleflower whole herbage and leaves as influenced by harvest maturity, ecotype, and residual height of cutting in Experiment 2 at three Minnesota locations in 2002.**

	Whole herbage						Leaves	
	Becker		Rosemount		Lamberton		Lamberton	
	ADF	NDF	ADF	NDF	ADF	NDF	ADF	NDF
	$\text{g kg}^{-1}$							
<b>Maturity at harvest</b>								
Early flower	356	395	312	347	341	379	187	210
Early pod	461	526	384	439	371	419	192	216
Late pod	512	586	402	457	446	518	246	288
LSD (0.05)†	31	37	16	25	53	62	NS‡	NS
<b>Ecotype</b>								
3	435	494	367	414	–	–	210	240
8	448	509	363	410	401	457	–	–
10	447	505	370	420	367	415	–	–
LSD (0.05)	NS	NS	NS	NS	14	17	–	–
<b>Cutting height</b>								
15 cm	469	532	388	439	408	464	186	210
35 cm	418	473	345	392	362	410	232	267
LSD (0.05)	14	17	15	18	19	21	NS	NS

† LSD, least significant difference ( $P = 0.05$ ); no significant interactions ( $P > 0.05$ ).‡ NS, not significant ( $P > 0.05$ ).

by an average of  $50 \text{ g kg}^{-1}$  compared with the 15-cm cutting height. There were no ecotype differences in fiber concentration except at Lamberton where Ecotype 10 had lower ADF and NDF concentrations than Ecotype 8. Leaf fiber concentrations, analyzed only for Lamberton, were unaffected by any treatment factor.

### Crude Protein

Whole-herbage CP concentration of IBF averaged  $155 \text{ g kg}^{-1}$  and decreased with advancing maturity by an average of  $61 \text{ g kg}^{-1}$  from early flower to late pod (Table 6). Cutting height consistently affected CP concentration. Averaged across locations, whole-herbage CP concentration was  $20 \text{ g kg}^{-1}$  greater when harvests were made to a 35-cm residual cutting height compared with a 15-cm residual cutting height. There was no ecotype effect except at Lamberton where Ecotype 10 had 9% greater CP concentration than Ecotype 8.

Whole-herbage N yield (data not presented) at Becker

**Table 6. Crude protein (CP) concentration in Illinois bundleflower whole herbage and leaves as influenced by harvest maturity, ecotype, and residual height of cutting in Experiment 2 at three Minnesota locations in 2002.**

	Whole herbage			Leaves
	Becker	Rosemount	Lamberton	Lamberton
	$\text{g kg}^{-1}$			
<b>Maturity at harvest</b>				
Early flower	200	198	162	229
Early pod	158	144	154	206
Late pod	115	149	113	173
LSD (0.05)†	13	20	20	NS‡
<b>Ecotype</b>				
3	162	167	–	201
8	152	168	138	–
10	159	156	150	–
LSD (0.05)	NS	NS	7	–
<b>Cutting height</b>				
15 cm	147	154	134	211
35 cm	168	173	153	192
LSD (0.05)	5	8	6	NS

† LSD, least significant difference ( $P = 0.05$ ); no significant interactions ( $P > 0.05$ ).‡ NS, not significant ( $P > 0.05$ ).

varied among harvest treatments and was affected primarily by CP concentration rather than biomass yield. At Becker, first-harvest yield of IBF at early flower averaged  $3.8 \text{ Mg DM ha}^{-1}$  (Fischbach et al., 2005) with a CP concentration of  $200 \text{ g kg}^{-1}$  (Table 6). Therefore, harvested biomass contained nearly  $122 \text{ kg N ha}^{-1}$ . At late pod,  $4.1 \text{ Mg DM herbage ha}^{-1}$  with a CP concentration of  $115 \text{ g kg}^{-1}$  yielded  $75 \text{ kg N ha}^{-1}$ . Thus, over a 1-mo period, the N yield of harvestable herbage declined by 63%, likely due largely to leaf drop.

### Digestibility

Whole-herbage IVTD and IVDMD concentrations of IBF averaged 604 and  $386 \text{ g kg}^{-1}$ , respectively, across locations, and decreased by an average of  $107 \text{ g kg}^{-1}$  from early flower to late pod (Table 7). The largest decline in digestibility occurred at Becker where IVDMD concentration declined by  $170 \text{ g kg}^{-1}$  from early flower to late pod. There was no significant change in leaf digestibility despite a trend toward lower digestibility with advancing maturity (Table 7). Both measures of digestibility were affected by cutting height. Leaving a higher residual cutting height increased whole-herbage IVTD and IVDMD at all locations by an average of  $41 \text{ g kg}^{-1}$ . There were no differences in ecotypes except at Lamberton where whole-herbage IVTD and IVDMD were greater for Ecotype 10 than for 8.

## DISCUSSION AND CONCLUSIONS

Two experiments, spanning nine site-years, demonstrate that IBF can produce good quality forage during the summer slump in cool-season legume growth in the upper midwestern USA. With low fiber and high CP concentration, IBF has the potential to enhance the forage quality of warm-season grass pastures. However, despite low fiber and high CP concentrations, in vitro measures of digestibility indicate IBF digestibility is low and may be limited by antiquality components, possibly tannins (Miller and Ehlke, 1994) and/or lignin (Packard et al., 2003).

**Table 7. In vitro true digestibility (IVTD) and in vitro dry matter digestibility (IVDMD) of Illinois bundleflower whole herbage and leaves as influenced by harvest maturity, ecotype, and residual height of cutting in Experiment 2 at three Minnesota locations in 2002.**

	Whole herbage						Leaves	
	Becker		Rosemount		Lamberton		Lamberton	
	IVTD	IVDMD	IVTD	IVDMD	IVTD	IVDMD	IVTD	IVDMD
	g kg <sup>-1</sup>							
<b>Maturity at harvest</b>								
Early flower	638	471	680	437	671	401	795	557
Early pod	530	377	617	396	628	398	773	527
Late pod	508	303	610	381	547	311	703	470
LSD (0.05)†	33	27	25	13	54	40	NS‡	NS
<b>Ecotype</b>								
3	570	390	637	399	–	–	754	515
8	553	377	637	410	602	358	–	–
10	556	384	632	403	634	386	–	–
LSD (0.05)	NS	NS	NS	NS	20	14	–	–
<b>Cutting height</b>								
15 cm	535	361	617	385	595	354	778	533
35 cm	582	406	654	423	639	388	733	499
LSD (0.05)	14	9	20	13	18	17	NS	NS

† LSD, least significant difference ( $P = 0.05$ ); no significant interactions ( $P > 0.05$ ).

‡ NS, not significant ( $P > 0.05$ ).

Whole-herbage and leaf fiber concentrations in IBF compared favorably with cool-season legumes. The experiment average of 360 g kg<sup>-1</sup> whole-herbage ADF concentration is similar to a season average of 370 g kg<sup>-1</sup> for alfalfa (*Medicago sativa* L.) in Minnesota (Sheaffer et al., 2000) and 350 g kg<sup>-1</sup> for stockpiled birdsfoot trefoil (*Lotus corniculatus* L.) harvested in mid-August in Wisconsin (Collins, 1982). The NDF concentration is also similar, if not less, with 410 g kg<sup>-1</sup> for IBF compared with 440 g kg<sup>-1</sup> for alfalfa.

In mid-July, whole-herbage CP concentration of IBF averaged 180 g kg<sup>-1</sup> but dropped to 130 g kg<sup>-1</sup> by mid-August. The mid-July CP concentration at early flower is about 20 g kg<sup>-1</sup> less than that measured in alfalfa cut at early flower in Minnesota (Sheaffer et al., 2000) but similar to the season-average CP concentration of cool-season legumes in a two-cut system (Sheaffer and Marten, 1991).

Whole-herbage and leaf IVDMD of IBF were low compared with reports for cool-season legumes. Whole-herbage IVDMD of cool-season legumes in a two-cut system in Minnesota ranged from 672 g kg<sup>-1</sup> for kura clover (*Trifolium ambiguum* M. Bieb.) to 554 g kg<sup>-1</sup> for birdsfoot trefoil (Sheaffer et al., 2003). In this study, IBF whole-herbage IVDMD averaged only 450 g kg<sup>-1</sup> at early flower in mid-July to 350 g kg<sup>-1</sup> at late pod in mid-August. Although both measures of digestibility were positively correlated with leaf concentration (IVDMD  $r = 0.89$ ,  $P < 0.05$ ; IVTD  $r = 0.93$ ,  $P < 0.05$ ), even the IVDMD of leaf samples was only 650 g kg<sup>-1</sup> compared with an IVDMD of alfalfa leaf of 800 g kg<sup>-1</sup>.

As with other legumes (Sanderson and Wedin, 1989), IBF whole-herbage quality declined as plants matured from early flower to late pod. Crude protein concentration decreased by 38% from 180 to 130 g kg<sup>-1</sup> while ADF and NDF concentration each increased by about 34%. In vitro dry matter digestibility and IVTD decreased by averages of 29 and 17%, respectively. Between mid-July and mid-August, leaf proportion decreased 85%. The overall decline in forage quality with advancing maturity is most likely due to the precipitous

decline in leaf proportion and concomitant increase in stem concentration as has been reported for a diversity of legumes (Sanderson and Wedin, 1989; Sheaffer et al., 2003).

The decline in leaf proportion was partly a result of normal leaf senescence associated with flowering and seed set. However, leaf proportion of harvested biomass was also affected by the variable and poorly understood occurrence of leaf drop. In both experiments, leaf drop from the lower two-thirds of the stem was sporadic across environments, occurring at any stage of maturity. The cause is unknown but may be due to micronutrient deficiencies, plant density, drought stress, leaf maturity, or foliar disease. A better understanding of the causes of leaf drop is needed to develop management schemes that limit leaf drop and, thus, maintain forage quality through July and August.

Adding N increased whole-herbage CP concentration at only one of five site-years and had no effect or decreased whole-herbage CP at four of five site-years. Nitrogen fertilization had no consistent, biologically significant affect on whole-herbage fiber concentration or digestibility. The lack of a consistent increase in CP concentration in response to N may be due to the relative unresponsiveness of IBF to N fertilization or the utilization of supplemental N for stem growth rather than leaf growth. The positive yield response to N fertilization (Fischbach et al., 2005) coupled with a decrease in leaf proportion in response to N seen in Experiment 1 supports the latter explanation.

A higher residual cutting height improved forage quality at all three site-years. Acid detergent fiber and NDF concentrations decreased by about 50 g kg<sup>-1</sup>, CP increased by 20 g kg<sup>-1</sup>, and IVTD and IVDMD both increased by 40 g kg<sup>-1</sup>. In the high-density stands of our study, leaves senesced and dropped from the basal 30 cm of IBF stems by mid-July, perhaps due to shading. The higher residual cutting height did not include these leafless stems and, thus, effectively increased leaf proportion and whole-herbage quality. In Experiment 2, Ecotype 3 had greater fiber concentration and digestibility

responses to cutting height than did Ecotypes 8 and 10. As the shortest of the three ecotypes, Ecotype 3 may have benefited more from having its leafless stem bases excluded from harvested forage. Since Ecotypes 8 and 10 were taller, the 35-cm cutting height did not alter the leaf proportion as in Ecotype 3. Given the resulting greater forage quality, greater regrowth, and the lack of a reduction in first-harvest forage yield associated with a 35-cm cutting height (Fischbach et al., 2005), the data suggest that IBF should be harvested with a relatively high residual cutting height to optimize yield and forage quality. Indeed, a 35-cm residual height may more closely approximate the height to which grazing animals are apt to defoliate the plant than a 15-cm residual.

The low IVDMD of IBF leaves and whole herbage measured in this study is consistent with existing literature. Posler et al. (1993) found that addition of IBF to pure grass forage decreased digestibility compared with grass alone. Adjei and Pitman (1993) reported IBF whole-herbage IVOMD of only 35%. Interestingly, in contrast to our study, digestibility increased with advancing maturity, which the authors suggest was due to dilution of antiquality components. Packard et al. (2003) reported only 422 g kg<sup>-1</sup> in sacco degradability for IBF. Percentage and species of legume in the diet of cattle in/from which digestibility estimates were made influenced IBF in sacco degradability, but to a limited extent.

Neutral detergent fiber digestibility (NDFD) of IBF forage computed from IVTD and NDF measurements ( $NDFD = \{1 - [(100 - IVTD)/NDF]\} \times 100$ ) was consistently less than 5 g kg<sup>-1</sup> for Experiment 2 and negative for Experiment 1 (data not shown). These low levels further suggest that little digestion occurred in the rumen fluid incubation stage of the IVTD procedure and that IVTD values were determined primarily by the second-stage NDF extraction. These results agree with those of Packard et al. (2003), who reported 0 g kg<sup>-1</sup> ADF degradability of IBF measured in sacco.

It is unclear what caused low IBF digestibility in vitro; however, there is evidence that tannins may be responsible. Preliminary tannin analysis of IBF leaves in our laboratory, using the procedure of Terrill et al. (1990) as modified by Miller and Ehlke (1994), revealed 63 g catechin equivalent (CE) tannin kg<sup>-1</sup> DM compared with leaf values of 1 and 139 g CE kg<sup>-1</sup> DM for alfalfa and sericea lespedeza (*Lespedeza cuneata* G. Don 'Interstate-76'), respectively. The presence of tannins in IBF, especially in leaves, is consistent with reports by Adjei and Pitman (1993) and W.R. Ocumpaugh (personal communication, 2003). However, the effect of tannins on digestibility of IBF forage remains unknown. Condensed tannin concentration is negatively related to IVDMD in birdsfoot trefoil (Miller and Ehlke, 1994). Lignin may also limit IBF digestibility (Packard et al., 2003). Additional research is needed to identify and quantify any other antiquality or antimicrobial compounds that may interfere with ruminal digestion of IBF herbage.

Forage quality for the three ecotypes in our study was slightly less than values reported for the same eco-

types in a spaced-plant study by DeHaan et al. (2003). In their study, ADF and NDF concentrations were greater by about 40 g kg<sup>-1</sup> at both early flower and late pod while CP concentration was 13 g kg<sup>-1</sup> less at early flower but nearly 40 g kg<sup>-1</sup> less at late pod. Growing IBF in the high-density monocultures of our study tended to increase the height of IBF plants, decrease the number of stems per plant, and decrease leaf concentration (Fischbach et al., 2005) compared with spaced plants in the DeHaan et al. (2003) study. These differences in IBF growth form may explain the differences in forage quality, especially the much larger decline in CP concentration between early flower and late pod in our study. Taller, more closely spaced plants may have shaded basal nodes and had relatively greater leaf senescence/drop, which by mid-August affected leaf and CP concentrations. However, environmental differences between the two studies may have contributed to forage quality differences as well.

In any one site-year in our study, differences among ecotypes were small. When averaged across all environments, however, Ecotype 3 tended to have the greatest CP and lowest fiber concentrations. Ecotypes 8 and 10 generally had similar forage quality. In spaced planting, Ecotype 3 tended to have the greatest CP and lowest ADF and NDF concentrations while Ecotype 10 had the lowest CP and greatest fiber concentrations (DeHaan et al., 2003). Differences among ecotypes, although small, can be explained by differences in growth form. As the shortest of the three ecotypes, Ecotype 3 tended to have the greatest leaf proportion in both studies and, thus, the greatest forage quality.

This study demonstrates that IBF can be a source of high-protein, low-fiber herbage during the summer slump in cool-season legume growth in the upper mid-western USA. However, the impact of IBF's limited in vitro digestibility on the performance potential of ruminants is unknown and needs further investigation.

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