

## Illinois Bundleflower Forage Potential in the Upper Midwestern USA: I. Yield, Regrowth, and Persistence

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### ABSTRACT

Illinois bundleflower [*Desmanthus illinoensis* (Michx.) MacMillan] is a warm-season perennial legume native to the central plains of the USA with potential as both a forage and grain crop. The effects of management variables on Illinois bundleflower (IBF) forage production in the upper midwestern USA have not been evaluated. We evaluated the effects of maturity at harvest, cutting height, and N fertilization on forage yield, regrowth, and persistence of three northern ecotypes of IBF. Field experiments were established at four Minnesota locations in 2000. Total-season forage yields in postestablishment years ranged from 2.5 to 5.3 Mg dry matter (DM) ha<sup>-1</sup> across environments. First-harvest forage yield increased ( $P < 0.05$ ) from 2.8 Mg DM ha<sup>-1</sup> at early flower in mid-July to 4.2 Mg DM ha<sup>-1</sup> at late pod in mid-August. In mid-September, within-season regrowth averaged 1.7 Mg ha<sup>-1</sup> from plants previously cut at early flower and 0.6 Mg ha<sup>-1</sup> from plants cut at late pod. A 35-cm cutting height resulted in 60% more regrowth yield ( $P < 0.05$ ) than a 15-cm cutting height, but only in plants harvested at early flower. Plants cut at late pod in 2001 did not persist into 2002. October root total nonstructural carbohydrate (TNC) concentration ranged ( $P < 0.05$ ) from 244 g kg<sup>-1</sup> in plants cut at late pod to 280 g kg<sup>-1</sup> in plants left uncut. Complete winterkill of all treatments at all locations between 2002 and 2003, regardless of October TNC level, may have been caused by below-average snow cover. Illinois bundleflower can provide summer forage in the upper midwestern USA, but persistence in monoculture is limited, especially in harsh winters.

AGRICULTURE in the upper midwestern USA is currently dominated by the production of corn (*Zea mays* L.) and soybean [*Glycine max* (L.) Merr.]. Though highly productive, this system is proving unsustainable (Rabalais et al., 2002; Kirschenmann, 2002). An alternative is greater adoption of perennial forage systems for feeding beef and dairy cattle. Historically, however, forage-based systems have been plagued by a “summer slump” during which cool-season legumes and grasses produce little forage.

A forage-based system for feeding dairy and beef cattle can be facilitated by developing warm-season pastures that can be used during the summer slump. Warm-season perennial grasses native to the upper midwestern USA produce high forage yields during July and August. However, forage quality is low, and supplemental N is required for sustained high levels of grass production (Posler et al., 1993; Vogel et al., 2002). It is clear from research on cool-season pastures that legumes are an

essential pasture component (Haynes, 1980). They increase crude protein concentration and ruminant intake potential of the forage mixture and provide a source of organic N for grass growth through their symbiotic relationship with N<sub>2</sub>-fixing bacteria (Sleugh et al., 2000). In theory, warm-season perennial legumes should be better suited for mixtures with warm-season perennial grasses than cool-season perennial legumes due to similar emergence times and period of active growth (Posler et al., 1993). Currently, however, there are no domesticated, native, warm-season legumes available for warm-season pasture mixtures in the northern USA. Illinois bundleflower has been proposed as one possible choice for domestication (DeHaan, 2002).

Illinois bundleflower is a perennial legume native to the central plains of the USA. It is an upright herbaceous plant with multiple stems growing from a woody caudex (Great Plains Flora Association, 1986). Its current range extends from Florida north to Minnesota and west to Colorado. Its historical distribution and abundance are unknown due to the lack of records and almost complete disappearance of the prairie ecosystem.

Forage yield potential and persistence of IBF have not been adequately evaluated in the upper midwestern USA. DeHaan et al. (2003) extrapolated from single-plant harvests in a spaced-plant study in northern Iowa to estimate IBF single-harvest forage yield potential of up to 4.9 Mg DM ha<sup>-1</sup> in mid-August. Southern accessions had poor persistence while northern accessions had good persistence (DeHaan et al., 2003). There are currently no reports evaluating yield potential or management strategies for IBF in solid or mixed stands in the upper midwestern USA.

There have been several studies evaluating the agronomic potential of IBF in the southern and central USA. In Florida, a single forage harvest in September yielded 7.1 Mg DM ha<sup>-1</sup>; however, two harvests (mid-July and September) yielded 23.8 Mg ha<sup>-1</sup> (Adjei and Pitman, 1993). Yield data were extrapolated from 0.5-m<sup>2</sup> quadrats from transplants with 0.5-m row spacing and 25-cm plant spacing. In Texas, yields of IBF interseeded into existing kleingrass (*Panicum coloratum* L.) stands ranged from 0.3 Mg ha<sup>-1</sup> in the establishment year to 2.9 Mg ha<sup>-1</sup> 3 yr after seeding (Dovel et al., 1990). Springer et al. (2001) transplanted IBF on 15-cm centers in monoculture plots in Arkansas. A single harvest in June averaged 5.5 Mg ha<sup>-1</sup> in 1996 and 8.1 Mg ha<sup>-1</sup> in 1997. In Kansas, total forage yield of IBF grown in mixture with switchgrass (*Panicum virgatum* L.), sideoats gramma [*Bouteloua curtipendula* (Michx.) Torr.], and indian-

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**Abbreviations:** IBF, Illinois bundleflower; TNC, total nonstructural carbohydrates.

grass [*Sorghastrum nutans* (L.) Nash] was 2.1, 1.3, and 1.7 Mg ha<sup>-1</sup> yr<sup>-1</sup>, respectively (Posler et al., 1993). In Nebraska, IBF in mixture with big bluestem [*Andropogon gerardii* (Vitman)] produced 3.5, 2.2, and 1.6 Mg ha<sup>-1</sup> in a single harvest at 97, 57, and 50 plants m<sup>-2</sup> (Beran et al., 2000). Despite the significant yield potential of IBF in Florida and on the southern plains of the USA, it is unknown how much forage can be produced by northern ecotypes of IBF in the cooler, shorter growing season of the upper midwestern USA. In addition, the influence of cutting management and N fertilization on forage yield and persistence of IBF is unknown.

Domesticated perennial forage legumes persist under the intense defoliation of grazing or cutting because of their ability to regrow following defoliation. For example, the vigorous regrowth of alfalfa (*Medicago sativa* L.) allows three harvests per year in the upper midwestern USA with good persistence (Sheaffer et al., 2000). Adequate regrowth increases TNC levels in root and crown tissue, which are strongly associated with winter survival and persistence. Harvests in autumn can decrease TNC levels and result in poor persistence (Dhont et al., 2002). Because of the importance of regrowth to total-season yield, TNC levels, winter survival, and persistence, it is important to understand how individual species regrow and the nodal origin of regrowth. For example, following defoliation during the growing season, alfalfa regrowth originates predominantly from basal axillary buds and becomes less basal as cutting height increases (Sheaffer et al., 1988; Frame et al., 1998).

Little is known about the regrowth characteristics of IBF. In Florida, Adjei and Pitman (1993) found that IBF regrowth depended on height and growth stage due to the strong acropetal gradient of axillary bud viability. Harvesting in the midbloom stage, before basal bud viability was lost, resulted in significant regrowth yields with high forage quality. However, the origin of regrowth was not reported. There are reports that IBF does not persist well following defoliation, especially when defoliation occurs later in the season (Latting, 1961; McGinnies and Townsend, 1983; Michaud et al., 1989). However, it is unknown whether the lack of persistence was due to poor regrowth. Given the reports suggesting IBF may not persist well following defoliation, it is important to characterize and subsequently maximize IBF regrowth in the short season of the upper midwestern USA.

Yield and persistence of IBF may be influenced by soil N. Adding N to cool-season legumes such as alfalfa rarely occurs because of the potential for inhibiting nodulation and effectively decreasing forage yield (Streeter, 1988). It is unclear, however, how N may affect the growth and performance of warm-season legumes such as IBF. The occasionally low N<sub>2</sub> fixation rates of IBF (Byun et al., 2004) and the positive N yield response of warm-season grasses (Vogel et al., 2002) may necessitate N fertilization of IBF or IBF-grass mixtures. However, it is possible that N added to boost the production of warm-season grasses or offset the low N<sub>2</sub> fixation rates of IBF may be detrimental to the growth and persistence of IBF in competition with grasses.

The objective of this study was to evaluate the effects of maturity at harvest, N fertilization, and cutting height on forage yield, patterns of regrowth, and persistence of three northern IBF ecotypes in the upper midwestern USA.

## MATERIALS AND METHODS

### Experiment 1

A field experiment was established at three University of Minnesota agricultural experiment stations in southern Minnesota in spring of 2000. Locations were chosen to represent a range of potential environments in which IBF might be grown. The trials were seeded on 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°09' 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 replicates. Whole-plot treatments were either no N added or 110 kg N ha<sup>-1</sup> yr<sup>-1</sup>. In 2000, five equal applications of ammonium nitrate were made at 28, 42, 56, 70, and 84 d after planting for a total of 110 kg N ha<sup>-1</sup> yr<sup>-1</sup>. In 2001 and 2002, two equal applications of ammonium nitrate were applied at emergence and immediately following harvest for a total of 110 kg N ha<sup>-1</sup> yr<sup>-1</sup>. Subplot treatments were 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 late-pod (mid-August) cutting treatment was not repeated at St. Paul or Rosemount in 2002 due to the lack of persistence of plants cut in mid-August 2001. In mid-September 2001, vegetative regrowth was harvested from all plots at St. Paul. There was insufficient regrowth to harvest at Rosemount and Becker. Sub-subplot ecotype treatments were three 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.

Seed was mechanically scarified, inoculated with 5 g kg<sup>-1</sup> seed of *Desmanthus* spp. inoculant (Nitragin strains 43A1 and 43C2, Liphatech, Milwaukee, WI), and seeded at 179 pure live seed m<sup>-2</sup> with a 10-row plot seeder and 15-cm row spacing (Wintersteiger, Inc., Salt Lake City, UT). Weeds were controlled in 2000 with 52 mL a.i. ha<sup>-1</sup> imazapic {± 2-[4,5-dihydro-4-methyl-4-(1-methylethyl)-5-oxo-1H-imidazol-2-yl]-5-methyl-3-pyridinecarboxylic acid; Plateau,<sup>1</sup> American Cyanamid Co., Parsippany, NJ} applied pre-emergence. In 2001, 68 mL a.i.

<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.

ha<sup>-1</sup> imazapic and 0.65 mL a.i. ha<sup>-1</sup> pendimethalin [*N*-(1-ethyl-propyl)-3,4-dimethyl-2,6-dinitrobenzenamine; Prowl,<sup>1</sup> American Cyanamid Co.] with 1.17 L ha<sup>-1</sup> of a N-surfactant blend (Class<sup>1</sup> Prefer-28, Cenex/Land O'Lakes Agron. Co., Winona, MN) were applied pre-emergence. In 2002 and 2003, weeds were controlled with a pre-emergent application of 0.65 mL a.i. ha<sup>-1</sup> pendimethalin and 3.8 L ha<sup>-1</sup> glyphosate [*N*-(phosphonomethyl)glycine; Roundup UltraMax,<sup>1</sup> Monsanto Corp., St. Louis, MO].

Forage yield was measured in 2001 and 2002 by harvesting a 3.7- by 0.9-m strip from the center of each plot with a flail harvester set to a 15-cm residual height. Random 0.5-kg samples from each plot were clipped immediately before harvest, weighed, dried at 60°C for 48 h, weighed again, and used to convert fresh yield to DM yield. Stand density was determined at harvests in 2001, in mid-June 2002, and again in mid-June 2003 by counting the number of crowns within two 0.25-m<sup>2</sup> quadrats per sub-subplot. Plant height and maturity were measured at harvest on five random plants within each sub-subplot. Growth stage was quantified using the approach of Lancashire et al. (1991) as modified by DeHaan (2002).

Analyses of variance (ANOVA) for DM yield, plant height, plant maturity, and stand density data were performed using the PROC GLM procedure of SAS (SAS Inst., 2001). Data were analyzed within locations and years due to the heterogeneity of environments. Significant interaction effects were sporadic and inconsistent across locations and are presented only in the text. Main effect means were separated with the least significant differences (LSD) test ( $P < 0.05$ ). Where significant effects are presented in sections that follow, they are at the  $P < 0.05$  level of significance.

## Experiment 2

Experiment 2 was established at three University of Minnesota agricultural experiment stations in southern Minnesota representing a range of potential environments in which IBF might be grown. It was seeded 7 June 2000 in Rosemount (44°53' N, 93°13' W) on a Tallula silt loam with pH 7.0, 31 mg kg<sup>-1</sup> P, and 87 mg kg<sup>-1</sup> K; 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; and 25 May 2000 at Becker (45°38' N, 93°89' W) on a Hubbard loamy sand with pH 7.0, 57 mg kg<sup>-1</sup> P, and 98 mg kg<sup>-1</sup> K.

The experiment was designed as a randomized complete block in a split-split plot arrangement with three replications. Whole-plot treatments were three stages of maturity at first harvest in 2002. Early-flower, early-pod, and late-pod harvests were made on 15 July, 22 July, and 13 August, respectively. In mid-September, regrowth of all first-harvest treatments was harvested to determine total-season forage yield for each treatment. Subplot ecotype treatments were three northern ecotypes of IBF selected from the University of Minnesota Native Perennial Legume Collection by DeHaan et al. (2003). Ecotype accessions were the same as in Experiment 1. Sub-subplot cutting height treatments were either a 15- or 35-cm residual height, representing two potential levels of defoliation severity by mowing or grazing.

Planting and weed control procedures were the same as in Experiment 1. No herbicide application was made in 2002 at Lamberton, resulting in significant populations of common ragweed (*Ambrosia artemisiifolia* L.) in two of three early-pod whole plots.

Forage yield was measured in 2002 by harvesting a 3.0- by 0.9-m strip from the center of each plot with a flail harvester. Random 1-kg samples were hand-clipped immediately before

harvest from each sub-subplot, weighed, dried at 60°C for 48 h, weighed again, and used to calculate DM concentration. Stand density was determined at harvest in 2002 and again in spring 2003 as in Experiment 1. Stand densities before the application of treatments in 2002 averaged 40, 35, and 22 plants m<sup>-2</sup> at Becker, Rosemount, and Lamberton, respectively. Illinois bundleflower did not persist into 2003 in any treatments at any locations, which may have been due to a lack of snow cover that also damaged other perennial forages in Minnesota (Sheaffer et al., 2003).

Plant height and maturity were measured at harvest on five random plants within each sub-subplot. Biomass regrowth was determined by hand-clipping plants at the soil surface in two 0.1-m<sup>2</sup> quadrats per sub-subplot in mid-September 2002. Regrowth tissue originating from below the soil surface was separated from regrowth tissue originating from residual stem tissue, both dried at 60°C for 48 h, and weighed. Before clipping plants within the quadrats, positional analysis of regrowth was performed. For each residual stem, the number of nodes was counted, and the presence or absence of axillary bud development at each node was recorded. An axillary bud was considered developed if at least 1 cm of regrowth tissue was present at the time of sampling.

Total nonstructural carbohydrate concentration of crown and root samples was determined using a modified procedure of Smith (1969). Crown and root samples were collected at Rosemount on 18 Oct. 2002 and at Becker on 25 Oct. 2002. An average of eight samples were collected from within two 0.1-m<sup>2</sup> quadrats per sub-subplot by loosening the soil to a depth of 0.3 m using a spade and gently pulling the plants from the ground. This method allowed extraction of the entire crown, the uppermost 15 cm of the taproot, and varying amounts of lateral root tissue. Samples were placed in a plastic bag and transported on ice. After 54 h of storage at 1°C, each plant was processed in the following manner: All residual stem material was trimmed to 15 cm in length, all taproots were trimmed to 15 cm, and all lateral roots were trimmed to 15 cm from the point of attachment to the taproot. Each sample was then washed in cold water and divided into three parts: stem, crown, and root. The crown was defined as all belowground plant tissue down to just below the most basally attached stem. All tissue below this point was considered root.

All processed samples were pooled by sub-subplot and dried at 60°C for 1 h and 35°C for 48 h. Samples were then ground to 10 mm with a Thomas-Wiley Laboratory Mill (Model 4, Thomas Scientific, Swedesboro, NJ), hand-mixed, and ground to 1 mm with a Cyclotec Mill (Cyclotec 1093 Sample Mill, Foss Tecator, Eden Prairie, MN).

All samples were scanned with a NIRSystems 6500 scanning monochromator with a range of 400 to 2500 nm (NIRSystems Inc., Silver Springs, MD). Fifty samples (25 crown and 25 root) were chosen as the calibration set using the WINSI II software (Infrasoft Int., Port Matilda, PA). Total nonstructural carbohydrate values for the calibration set were obtained using a modified procedure of Smith (1969). Two hundred milligram samples were incubated with 3 mL of 95% ethyl alcohol and 15 mL H<sub>2</sub>O in a boiling water bath for 8 min to gelatinize the starch. Prediction equations were developed from the calibration set by performing a modified partial least squares regression using the "Global Calibration" function of the WINSI II software. The equation had a strong fit with standard error of calibration of 1.10,  $r^2$  of 0.97, and standard error of cross validation of 1.52.

Analyses of variance for first-harvest, regrowth, and total-season forage yield, plant height, plant maturity, positional regrowth, and TNC data were performed using the PROC GLM procedure of SAS (SAS Inst., 2001). Data were analyzed

**Table 1. Monthly precipitation (mm) and mean air temperature (°C) for 2001–2003 at Becker, Lamberton, Rosemount, and St. Paul, MN, and 30-yr averages (1971–2000).**

Month	Becker				Lamberton				Rosemount				St. Paul			
	2001	2002	2003	Avg.	2001	2002	2003	Avg.	2001	2002	2003	Avg.	2001	2002	2003	Avg.
mm																
Jan.	29	4	7	28	22	1	8	17	32	16	13	31	34	5	4	26
Feb.	41	49	13	22	20	10	9	13	53	19	20	22	23	7	17	20
Mar.	23	68	30	43	12	31	4	45	31	54	50	57	25	38	30	49
Apr.	181	66	98	60	154	51	38	69	145	107	70	73	198	90	60	65
May	85	75	97	82	65	61	75	83	116	59	182	102	127	77	172	94
June	71	103	133	113	49	127	134	98	113	261	81	117	110	249	165	125
July	39	147	131	105	129	82	48	91	26	85	100	119	50	193	52	112
Aug.	73	186		117	39	130		86	67	209		118	76	152		109
Sept.	60	142		73	75	24		65	87	150		89	83	102		82
Oct.	27	114		63	26	79		49	34	133		64	39	113		62
Nov.	86	3		47	91	3		36	78	3		57	58	1		56
Dec.	10	6		23	8	5		15	16	11		29	13	4		26
°C																
Jan.	na	-6	-11	-12	-7	-5	-9	-11	-8	-6	-9	-11	-6	-4	-9	-10
Feb.	na	-5	-11	-8	-13	-2	-10	-7	-13	-3	-9	-7	-10	-1	-8	-6
Mar.	na	-7	-2	-1	-4	-6	-2	-1	-4	-5	0	0	-2	-4	0	0
Apr.	7	6	7	7	-4	7	8	7	8	7	8	8	10	8	9	8
May	14	11	14	15	15	12	13	15	15	12	14	15	16	13	15	15
June	20	21	19	19	20	22	19	20	19	21	19	20	21	22	20	20
July	23	24	22	21	23	24	22	22	23	24	22	22	24	25	23	23
Aug.	23	21		20	22	21		21	22	20		21	23	22		21
Sept.	15	17		15	16	17		16	15	18		16	16	19		16
Oct.	8	5		8	9	5		9	9	6		9	10	6		10
Nov.	7	-1		-1	7	0		0	8	0		0	8	1		0
Dec.	-4	-5		-9	-4	-3		-8	-3	-3		-8	-2	-3		-7

within locations due to the heterogeneity of environments. Significant interaction effects were sporadic and inconsistent across locations and are presented only in the text. Main-effect means were separated with the LSD test ( $P < 0.05$ ). When significant effects are presented in sections that follow, they are always at the  $P < 0.05$  level of significance unless otherwise indicated.

### Temperature and Precipitation

Mean growing season air temperatures were similar to the 30-yr average throughout the study at all four locations (Table 1). In contrast, total precipitation during June, July, and August was 137 mm below average (avg. 345 mm across three locations) in 2001 and 153 mm above average (avg. 327 mm across four locations) in 2002. Mean winter air temperatures during the study were near or slightly above average. However, winter precipitation was 75% of average (117 mm) in winter 2001–2002 and 50% of average in 2002–2003. In January, the coldest month in Minnesota, precipitation was about 30% of normal (avg. 27 mm) in both 2002 and 2003, respectively (Minnesota Climatology Working Group, 2003). This resulted in inadequate snow cover to provide insulation against normal low air temperatures.

## RESULTS

### Experiment 1

#### Stand Density

Before initiation of treatments in 2001, IBF stand density at Becker, Rosemount, and St. Paul was 78, 47, and 36 plants  $m^{-2}$ , respectively. Stand density in 2002 was affected by treatments applied in 2001. Plants cut at late pod in mid-August 2001 experienced winter injury and had very poor stands in spring 2002, averaging 12, 1, and 0 plants  $m^{-2}$  at Becker, Rosemount, and St. Paul, respectively. Stand density in plots cut at early

flower in mid-July 2001 was reduced in 2002 compared with initial stands in 2001, but persistence was better than for mid-August-cut plots and averaged 49, 24, and 4 plants  $m^{-2}$  at Becker, Rosemount, and St. Paul, respectively. Nitrogen applied in 2001 had no effect on persistence at any location. Averaged across treatments at Becker, Ecotype 3 had better persistence than Ecotypes 8 and 10. No IBF plants persisted into 2003 at any location, regardless of harvest treatment in 2002, possibly due to winter weather that damaged many other perennial forage species in Minnesota (Sheaffer et al., 2003).

#### Forage Yield

Averaged across treatments, single-harvest DM yields ranged from 2.3 to 4.1 Mg  $ha^{-1}$  but varied considerably among locations and years (Table 2). At Becker and

**Table 2. Illinois bundleflower single-harvest forage yield as influenced by N fertilization, maturity at harvest, and ecotype in Experiment 1 at three Minnesota locations over 2 yr.**

	Becker		Rosemount		St. Paul	
	2001	2002	2001	2002	2001	2002
Mg dry matter $ha^{-1} yr^{-1}$						
N ( $kg ha^{-1} yr^{-1}$ )						
0	2.0	1.8	2.8	1.2	3.9	0.5
110	2.9	2.0	3.2	1.7	4.3	1.0
LSD (0.05)†	NS‡	NS	NS	0.2	NS	0.3
Maturity at harvest						
Early flower	1.0	3.3	2.9	2.9	3.1	1.4
Late pod	3.9	1.3	3.2	0.0	5.1	0.0
LSD (0.05)	0.8	1.0	NS	0.2	0.4	0.6
Ecotype						
3	2.2	3.0	2.9	1.6	4.4	1.0
8	2.6	1.7	3.2	1.3	3.9	0.5
10	3.6	2.2	2.9	1.6	4.0	0.6
LSD (0.05)	NS	NS	NS	NS	NS	0.3

† LSD, least significant difference ( $P = 0.05$ ).

‡ NS, not significant.

St. Paul in 2001, harvesting at late pod produced 2.9 and 2.0 Mg ha<sup>-1</sup> more DM, respectively, than harvesting at early flower. In contrast, at Rosemount in 2001, DM yield did not increase from early flower to late pod. Neither fertilization nor ecotype influenced DM yield in 2001.

There was no harvestable regrowth following the initial harvest at Becker and Rosemount, probably due to below-average precipitation. However, at St. Paul, an average of 1.6 Mg DM ha<sup>-1</sup> of harvestable regrowth was produced from plots harvested at early flower, resulting in total-season DM yields averaging 4.7 Mg ha<sup>-1</sup>.

In 2002, early-flower harvests yielded an average of 2.1 Mg DM ha<sup>-1</sup> more than late-pod harvests due to winter injury and lack of persistence of plants harvested at late pod in 2001 (Table 2). The addition of N increased 2002 yields of IBF harvested at early flower by 38% at Rosemount and 100% at St. Paul. An ecotype × maturity interaction occurred at St. Paul in 2002 because Ecotype 3 yielded more than Ecotypes 8 and 10 at early flower, but there was no difference among ecotypes at late pod due to the lack of persistence. At Becker and Rosemount, there were no ecotype differences in yield.

### Plant Height and Maturity

In 2001, plants were taller and more mature when harvested at late pod in mid-August than when harvested at early flower in mid-July (Table 3). In 2002, emergence of IBF plants harvested at late pod in 2001 was not uniform and much later than plants harvested at early flower in 2001. Consequently, IBF plants harvested in 2002 were similar in height when harvested at early flower and late pod in 2001. Plant height and growth stage were similar across N treatments.

Ecotype differences for plant maturity and height at harvest were sporadic and inconsistent across locations and years. In 2001, at St. Paul, Ecotype 3 was shorter than Ecotypes 8 and 10 (Table 3). At Becker, there was no difference in height between ecotypes, but Ecotype 8 was less mature at harvest than Ecotypes 3 and 10. Maturity at harvest × ecotype interactions occurred at St. Paul and Rosemount in 2001. At Rosemount, Ecotype 3 was taller and more mature than Ecotypes 8 and 10 at early flower, but at late pod, there was no difference in maturity, and Ecotype 8 was taller than Ecotypes 3 and 10. At St. Paul, there were no ecotype differences in maturity at early flower, but at late pod, Ecotype 8 was less mature than Ecotypes 3 and 10. In 2002, Ecotype 8 was less mature than Ecotypes 3 and 10 at Rosemount, and Ecotype 10 was shorter than Ecotypes 8 and 3 at St. Paul. There were no ecotype differences in height or maturity at Becker in 2002.

## Experiment 2

### First-Harvest Forage Yield

First-harvest forage yields averaged across treatments ranged from 2.8 Mg DM ha<sup>-1</sup> at Lamberton to 4.1 Mg DM ha<sup>-1</sup> at Rosemount (Table 4). Yield did not increase between early flower and late pod at Becker or Rose-

**Table 3. Plant height and growth stage of three ecotypes of Illinois bundleflower at first harvest as influenced by N fertilization in Experiment 1 at three Minnesota locations over 2 yr.†**

	Becker		Rosemount		St. Paul	
	2001	2002	2001	2002	2001	2002
	plant height, cm					
Nitrogen (kg ha <sup>-1</sup> yr <sup>-1</sup> )						
0	64	58	61	37	115	27
110	75	77	64	39	116	25
LSD (0.05)‡	NS§	NS	NS	NS	NS	NS
Maturity at harvest						
Early flower	46	73	46	38	–	29
Late pod	93	62	79	38	116	23
LSD (0.05)	6	NS	10	NS	–	NS
Ecotype						
3	65	63	64	39	108	36
8	73	70	64	38	126	25
10	70	70	60	37	114	18
LSD (0.05)	NS	NS	NS	NS	3	13
	plant maturity, growth stage¶					
N (kg ha <sup>-1</sup> yr <sup>-1</sup> )						
0	64	67	71	60	74	59
110	65	68	72	60	74	59
LSD (0.05)	NS	NS	NS	NS	NS	NS
Maturity at harvest						
Early flower	57	68	63	60	59	59
Late pod	72	–	80	–	83	–
LSD (0.05)	1	–	1	–	1	–
Ecotype						
3	65	68	73	62	75	61
8	63	67	71	57	73	56
10	65	69	71	61	74	60
LSD (0.05)	1	NS	1	1	1	NS

† 2001 first-harvest dates: 24, 20, and 9 July at Becker, Rosemount, and St. Paul, respectively, for early flower; 20, 14, and 15 Aug., respectively, for late pod. 2002 first-harvest dates: 17, 18, and 12 July at Becker, Rosemount, and St. Paul, respectively, for early flower; 12 Aug. at Becker for late pod.

‡ LSD, least significant difference ( $P = 0.05$ ).

§ NS, not significant.

¶ Growth stage determined by method of Lancashire et al. (1991) as modified by DeHaan (2002); 50 = flower buds just visible, 60 = first flower open, 70 = first fruit visible, 80 = beginning of fruit ripening, and 90 = fruit fully ripe and beginning to senesce.

mount, but at Lamberton, there was a 39% increase in DM yield between early flower and late pod. A decline in yield from early flower to early pod at Lamberton was due to weed pressure in two of the three replications of the early-pod treatment (Table 4). At all locations, first-harvest forage DM yields were similar among ecotypes and among cutting height treatments. A maturity × ecotype interaction occurred at Lamberton because Ecotype 10 had the greatest yield at early flower but the least yield at late pod.

### Regrowth Yield

Regrowth yield in mid-September was highly variable at all locations with a range of 0.4 Mg DM ha<sup>-1</sup> for IBF previously harvested in mid-August to 2.4 Mg ha<sup>-1</sup> for IBF previously harvested in mid-July at Lamberton (Table 4). Regrowth yield was affected by maturity at first harvest. At Rosemount, regrowth from plants harvested at early flower yielded 58% more than from plants harvested at late pod. At Becker and Lamberton, there tended to be more regrowth from plants harvested at early flower than at late pod. Ecotype 8 produced more regrowth than Ecotype 3 at Becker and Rosemount.

Although cutting height did not affect first-harvest

**Table 4.** First-harvest,† mid-September regrowth, and total-season forage yields of Illinois bundleflower as influenced by maturity at harvest, ecotype, and cutting height in Experiment 2 at three Minnesota locations in 2002.

	Becker			Rosemount			Lamberton		
	First	Regrowth	Total	First	Regrowth	Total	First	Regrowth	Total
	Mg dry matter ha <sup>-1</sup>								
<b>Maturity at harvest</b>									
Early flower	3.8	1.0	4.8	3.7	1.9	5.6	2.5	2.4	4.9
Early pod	3.5	0.7	4.2	3.6	1.2	4.9	1.8	1.4	3.3
Late pod	4.1	0.7	4.8	4.8	0.8	5.3	4.0	0.4	4.2
LSD (0.05)‡	NS§	NS	NS	NS	0.7	NS	0.7	NS	NS
<b>Ecotype</b>									
3	3.7	0.6	4.3	4.3	0.8	4.7	2.7	1.6	4.3
8	3.8	0.9	4.7	4.4	1.7	6.2	2.8	–	–
10	3.8	0.9	4.7	3.5	1.4	4.9	2.9	1.3	4.1
LSD (0.05)	NS	0.2	NS	NS	0.4	NS	NS	NS	NS
<b>Cutting height</b>									
15 cm	3.8	0.6	4.4	3.9	1.0	4.7	2.9	1.7	4.7
35 cm	3.8	1.0	4.7	4.2	1.6	5.8	2.6	1.1	3.7
LSD (0.05)	NS	0.1	NS	NS	0.3	NS	NS	0.3	NS

† Becker first-harvest dates: 17 July, 25 July, and 12 Aug. for early flower, early pod, and late pod, respectively; Rosemount: 18 July, 26 July, and 13 Aug., respectively; Lamberton: 11 July, 19 July, and 14 Aug., respectively.

‡ LSD, least significant difference ( $P = 0.05$ ).

§ NS, not significant.

yield, it did affect regrowth yield. Harvesting to a 35-cm residual height resulted in 35 and 38% more regrowth than harvesting to 15 cm at early flower at Rosemount and Becker, respectively, but at late pod, cutting height did not influence regrowth yield. In contrast, at Lamberton, a 15-cm residual cutting height resulted in 35% more regrowth than a 35-cm residual height. An ecotype  $\times$  cutting height interaction occurred at Becker. The 35-cm cutting height resulted in more regrowth than the 15-cm cutting height for both Ecotypes 8 and 10, but there was no cutting height effect on Ecotype 3.

### Total-Season Forage Yield

Total-season DM yield averaged across treatments ranged from 4.2 Mg ha<sup>-1</sup> yr<sup>-1</sup> at Lamberton to 5.3 Mg ha<sup>-1</sup> yr<sup>-1</sup> at Rosemount (Table 4). Maturity at first harvest did not affect total-season yield at any location. Total-season yields were also similar for ecotypes at all locations. There was no difference in total-season yield

between cutting height treatments at Becker and Lamberton, but at Rosemount, a maturity at harvest  $\times$  cutting height interaction occurred. The 35-cm cutting height resulted in greater total-season yields than the 15-cm cutting height at early flower, but there was no difference between cutting heights at early pod or late pod.

### Plant Height and Maturity

Plants cut in mid-August at late pod were taller and more mature than plants cut at early flower in mid-July (Table 5). There was a maturity  $\times$  ecotype interaction for plant height at both Becker and Rosemount. There were no ecotype differences at early flower or early pod, but at late pod, Ecotype 8 was taller than Ecotypes 3 and 10. Ecotypes 3 and 10 were more mature than Ecotype 8 at Becker. At Rosemount, Ecotype 3 was more mature than Ecotype 8 at early flower, but there were no ecotype differences at early pod or late pod.

**Table 5.** First-harvest† plant height and growth stage of Illinois bundleflower as influenced by harvest maturity, ecotype, and cutting height in Experiment 2 at three Minnesota locations in 2002.

	Plant height			Growth stage‡		
	Becker	Rosemount	Lamberton	Becker	Rosemount	Lamberton
	cm			stage		
<b>Maturity at harvest</b>						
Early flower	96	81	94	63	62	–
Early pod	117	97	98	70	71	72
Late pod	154	113	108	80	80	80
LSD (0.05)§	11	10	NS¶	2	1	NS
<b>Ecotype</b>						
3	114	92	91	72	72	77
8	131	105	112	70	70	76
10	123	94	97	72	70	76
LSD (0.05)	5	6	NS	1	1	NS
<b>Cutting height</b>						
15 cm	123	97	100	71	71	76
35 cm	122	97	101	71	71	76
LSD (0.05)	NS	NS	NS	NS	NS	NS

† Becker first-harvest dates: 17 July, 25 July, and 12 Aug. for early-flower, early-pod, and late-pod harvests, respectively; Rosemount: 18 July, 26 July, and 13 Aug., respectively; Lamberton: 11 July, 19 July, and 14 Aug., respectively.

‡ Growth stage determined by method of Lancashire et al. (1991) as modified by DeHaan (2002); 50 = flower buds just visible, 60 = first flower open, 70 = first fruit visible, 80 = beginning of fruit ripening, and 90 = fruit fully ripe and beginning to senesce.

§ LSD, least significant difference ( $P = 0.05$ ).

¶ NS, not significant.

**Table 6. Frequency of nodes and axillary shoots on first-harvest† residual stems of Illinois bundleflower in September 2002 as influenced by first-harvest cutting height in Experiment 2 in Minnesota (pooled over three harvest maturities).**

Node	Stems with node present			Nodes with axillary shoots present		
	Cutting height		LSD (0.05)‡	Cutting height		LSD (0.05)
	15 cm	35 cm		15 cm	35 cm	
	stems, %			nodes, %		
	Rosemount§					
1	100	100	NS¶	84	37	16
2	75	100	13	85	55	15
3	15	98	8	78	56	NS
4	4	92	6	8	65	23
5	0	78	13	0	78	12
6	0	58	21	0	79	12
7	0	40	19	0	93	6
	Becker					
1	100	100	NS	88	37	16
2	73	100	8	86	55	15
3	20	99	6	82	72	NS
4	1	90	5	22	79	33
5	0	63	11	0	82	9
6	0	29	11	0	72	14
7	0	14	7	0	73	25

† Becker first-harvest dates: 17 July, 25 July, and 12 Aug. for early-flower, early-pod, and late-pod harvests, respectively; Rosemount: 18 July, 26 July, and 13 Aug., respectively; Lamberton: 11 July, 19 July, and 14 Aug., respectively.

‡ LSD, least significant difference ( $P = 0.05$ ).

§ At Rosemount, only Ecotype 3 was sampled; at Becker, only Ecotype 10 was sampled.

¶ NS, not significant.

### Nodal Origin of Regrowth

At both Becker and Rosemount, neither maturity at harvest nor ecotype affected the number of nodes per residual stem or lateral bud development occurring at those nodes. Cutting height clearly affected the percentage of stems having a particular node (Table 6). Stems cut to 15 cm had, on average, two aboveground nodes, whereas most stems cut to 35 cm had five or six nodes. The development of lateral buds at the two most basal nodes was clearly affected by cutting height (Table 6). Eighty-five percent of Nodes 1 and 2 had lateral bud development on stems cut to 15 cm, whereas 37% of the most basal nodes and 55% of the second nodes had lateral bud development on stems cut to 35 cm. In contrast, with stems cut to 35 cm, the three most apical nodes had lateral bud development more than 70% of the time.

### Total Nonstructural Carbohydrates

Maturity at harvest and cutting height affected October TNC concentration of root and crown tissues (Table 7). At both locations, root and crown TNC levels were greatest in plants that were not cut during the growing season and least in plants that were cut at late pod in mid-August. Total nonstructural carbohydrate levels in the crown tissue of plants cut at early flower were equal to or slightly less than TNC levels in the uncut plants. Concentration of TNC in root and crown tissue was similar among ecotypes. A maturity  $\times$  ecotype crossover interaction occurred for root TNC concentration at Rosemount. Ecotype 3 had greater TNC

**Table 7. October total nonstructural carbohydrate (TNC) concentration in Illinois bundleflower roots and crowns as influenced by first-harvest† maturity, ecotype, and cutting height in Experiment 2 at two Minnesota locations in 2002.**

	Becker		Rosemount	
	Root	Crown	Root	Crown
	g TNC kg <sup>-1</sup>			
<b>Maturity at harvest</b>				
Early flower	263	177	247	145
Early pod	257	150	233	133
Late pod	268	146	221	120
No harvest	288	178	271	157
LSD (0.05)‡	NS§	22	5	8
<b>Ecotype</b>				
3	267	161	242	140
10	265	160	236	132
LSD (0.05)	NS	NS	NS	NS
<b>Cutting height</b>				
15 cm	255	149	218	122
35 cm	270	167	250	143
LSD (0.05)	13	12	11	12

† Becker first-harvest dates: 17 July, 25 July, and 12 Aug. for early-flower, early-pod, and late-pod harvests, respectively; Rosemount: 18 July, 26 July, and 13 Aug., respectively; Lamberton: 11 July, 19 July, and 14 Aug., respectively.

‡ LSD, least significant difference ( $P = 0.05$ ).

§ NS, not significant.

levels than Ecotype 10 when cut at early pod and late pod but less than Ecotype 10 when cut at early flower.

In root and crown tissues, TNC levels were greater in plants cut to 35 cm than in plants cut to 15 cm. Averaged across locations, TNC levels in crown tissue were 153 g kg<sup>-1</sup> for IBF cut to 35 cm and 135 g kg<sup>-1</sup> in plants cut to 15 cm while root tissue had 260 g TNC kg<sup>-1</sup> in IBF cut to 35 cm and only 236 g kg<sup>-1</sup> in plants cut to 15 cm.

## DISCUSSION AND CONCLUSIONS

Two experiments spanning 3 yr and four locations in Minnesota showed a range of IBF single-harvest forage yields from 2.3 to 4.3 Mg DM ha<sup>-1</sup>. DeHaan et al. (2003), using spaced transplants from 20 accessions, reported a single-harvest yield range of 0.5 to 3.4 Mg DM ha<sup>-1</sup> with a maximum average yield of 4.9 Mg ha<sup>-1</sup> in August at Sioux Center, IA. Despite stand densities 60 times greater than in the study of DeHaan et al. (2003), the single-harvest forage yield range in the present study is remarkably similar. The yield range was only slightly lower than single-harvest IBF yield data from Arkansas (Springer et al., 2001). However, the maximum total-season yield measured in the present study was much less than the 23.8 Mg ha<sup>-1</sup> yr<sup>-1</sup> reported in Florida (Adjei and Pitman, 1993), due primarily to limited regrowth in the relatively shorter northern growing season, cooler temperatures, less moisture, and perhaps coarser-textured soils.

Illinois bundleflower is productive during the slump in cool-season forage production in July and August. However, total-season IBF yield, averaging 4.7 Mg DM ha<sup>-1</sup> yr<sup>-1</sup> across four locations and 3 yr, is less than the total-season yield of most cool-season legumes grown in the upper midwestern USA. In Minnesota, three-cut alfalfa, averaged across 2 yr, three locations, and six entries, yielded 10.8 Mg DM ha<sup>-1</sup> yr<sup>-1</sup> (Sheaffer et al.,

2000). Averaged across 2 yr, two-cut kura clover [*Trifolium ambiguum* (M.) Bieb.], cicer milkvetch (*Astragalus cicer* L.), and birdsfoot trefoil (*Lotus corniculatus* L.) yielded 6.3, 9.3, and 9.6 Mg ha<sup>-1</sup> yr<sup>-1</sup>, respectively (Sheaffer and Marten, 1991). Despite lower total-season yield, IBF single harvests made in midsummer can be competitive with stockpiling cool-season legumes. For example, in mid-August in Wisconsin, birdsfoot trefoil stockpiled beginning in late May yielded 5.1 Mg DM ha<sup>-1</sup> (Collins, 1982). Growing IBF in mixture with warm-season grasses may increase total forage biomass in mid-August.

Results from Experiments 1 and 2 suggest that an increase in forage yield between mid-July and mid-August may depend on plant height and stand density. In Experiment 1 in 2001, biomass increased from mid-July to mid-August as IBF matured from early flower to late pod. However, there was no increase in biomass from mid-July to mid-August at two of the three locations of Experiment 2 in 2002. Even though plant heights at early flower averaged 46 cm in 2001 in Experiment 1 and 89 cm in 2002 in Experiment 2, plants in both experiments grew an average of 35 cm between early flower and late pod. It is possible the increase in height in 2002 was offset by leaf drop as the taller plants shaded basal nodes, effectively negating any yield increase. In 2001, more of the basal nodes may have maintained their leaves and, thus, allowed an increase in total yield. It is unclear how an IBF plant density greater than the 81 or less than the 22 plants m<sup>-2</sup> in our experiments would affect basal leaf retention and forage yield. However, similar yields between this study and that of the DeHaan et al. (2003) spaced-plant study suggest that IBF's growth form can adjust to varying plant densities and fully utilize available resources.

The addition of 110 kg N ha<sup>-1</sup> yr<sup>-1</sup> increased first-harvest forage yield in 2002 at two of the three locations. The results suggest that biological N<sub>2</sub> fixation may not be occurring at optimal levels due to poor nodulation and that improvement could be made in inoculation procedures. These results are consistent with those reported by Byun et al. (2004) and ongoing work with *Rhizobium* spp. specificity at the University of Minnesota (E. Beyhaut, personal communication, 2003).

Central to the viability of IBF as a forage crop in the upper midwestern USA is its regrowth potential. In Experiment 2, regrowth yield, averaged across locations, was greater from plants originally cut at early flower. A lack of consistency in cutting height effects across locations may have been due to powdery mildew (*Erysiphe* spp.) that decreased regrowth on the taller residual stems at Lambertson but was not present at Rosemount. It was clear that most regrowth following harvest originated from axillary meristems on aboveground stem tissue. Furthermore, the apical-most axillary buds tended to produce the most regrowth for both cutting heights, suggesting that cutting height may be an effective tool to allow IBF to "forage" for resources when grown in mixture with grasses (de Kroon and Hutchings, 1995). Leaving more residual stem increased the amount of biomass produced. However, this was true only when

plants were cut in mid-July at early flower. When cut at late pod, regrowth was limited by the short growing season, effectively masking cutting height effects.

Results from both experiments suggest that IBF persistence is reduced if defoliation occurs during seed set. In Experiment 1, plants cut at late pod in 2001 did not persist into 2002, except at Becker where persistence was poor, but there was sufficient plant material to harvest. Experiment 2 was designed to further evaluate the effects of maturity at harvest, cutting height, and their interactions on the persistence of IBF. Total nonstructural carbohydrate concentrations, measured in October of the harvest year, showed clear differences among cutting treatments and cutting heights. Plants cut at early flower had TNC concentrations similar to plants that had not been cut. Leaving a higher residual cutting height also increased the TNC concentrations. Plants cut at late pod had lower TNC concentrations than uncut IBF plants.

Based on these data, differences in persistence and plant vigor were expected the following spring. However, due to below-average January precipitation and consequent lack of snow cover statewide during the winter of 2002–2003, no IBF plants at the four locations survived. Even unharvested plants were winterkilled. In a more average winter, the differences in TNC concentrations may have translated into differences in persistence. Low TNC levels in plants harvested at late pod may explain the lack of persistence for the late-pod treatment in Experiment 1.

The survival of IBF grown in mixture with warm-season grasses in neighboring plantings through the winter of 2002–2003 (data not shown) suggests that a lack of insulating ground cover in the monoculture IBF plots may have been the persistence-limiting factor rather than strictly a lack of genotypic winter hardiness. Furthermore, the complete lack of persistence of IBF in monoculture plots was a first in over 8 yr of research with IBF in Minnesota.

Differences among ecotypes were not consistently apparent in Experiment 1 or 2. DeHaan et al. (2003) characterized Ecotype 3 as short with early to midseason maturity, Ecotype 8 as tall with midseason maturity, and Ecotype 10 as having moderate height with early maturity. In some site-years, Ecotype 8 was taller than 3 and 10, but overall, there was too much variability within ecotypes to make conclusions regarding plant height. Ecotype 8 tended to be slower to mature than Ecotypes 3 and 10 but only in mid-July. There were no consistent yield differences among ecotypes in Experiment 1 or 2 as was expected given the lack of height differences. However, genotype × environment interactions for yield suggest that certain ecotypes are better adapted to certain locations. Such diversity will be important to future breeding work.

Illinois bundleflower has demonstrated seed yield potential in Minnesota of 1350 kg ha<sup>-1</sup> yr<sup>-1</sup> (data not shown). Its high seed yield may explain the lack of persistence when cut at late pod during seed fill. High seed yield could be used to build a seedbank as a means of maintaining a perennial stand. Illinois bundleflower's

high seedling vigor and relatively high percentage of hard seed suggest that such a strategy has potential.

Illinois bundleflower has potential as a forage crop in the upper midwestern USA. Although its total-season forage yield is less than most cool-season legumes, a single hay or silage harvest made during the summer slump (mid-July to mid-August) yields enough biomass to make such a harvest a viable option. Alternatively, animals could graze IBF and be removed before seed set. However, more research is needed to identify how late in the growing season IBF can be harvested without reducing persistence and how its yield and persistence would be affected by mixing it with perennial warm-season grasses in the upper midwestern USA.

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