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Biomass and integrated forage/biomass yields of switchgrass as affected by intercropped cool- and warm-season legumes

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Abstract: Switchgrass (*Panicum virgatum* L.) has potential as a biofuel feedstock for ethanol production on marginal soils not suitable for row crop production. Further, it is hypothesized that legumes may be interseeded into switchgrass to increase available soil nitrogen (N) and enhance switchgrass yields. Therefore the primary objective was to identify compatible legume species for intercropping with lowland switchgrass and determine if biomass yields and forage quality can be improved. Four cool- and two warm-season legume species were compared to application of 67 and 134 kg N ha^{-1} (59.8 and 119.6 lb N ac^{-1}) during 2009 and 2010 over a range of soils at three research and education centers in Tennessee. Cool-season legumes were alfalfa (*Medicago sativa* L.), red clover (*Trifolium pratense* L.), crimson clover (*Trifolium incarnatum* L.), and hairy vetch (*Vicia villosa* L.), and warm-season legumes included Illinois bundle flower (*Desmanthus illinoensis* L.) and partridge pea (*Chamaechrista fasciculata* L.). Legumes were evaluated for establishment (plant densities) and their effects on switchgrass yield and forage quality under a one-cut biomass (single, postdormancy biofuel) and an integrated two-cut (biomass/forage [preanthesis]) system. In the one-cut system, switchgrass yields (16.6 Mg ha^{-1} [6.7 tn ac^{-1}]) from the current recommended rate (67 kg N ha^{-1} [59.8 lb N ac^{-1}]) exceeded ($p < 0.05$) legume treatment yields (average 13.5 Mg ha^{-1} [5.5 tn ac^{-1}]). In the integrated harvest system, switchgrass yields from red (13.4 Mg ha^{-1} [5.4 tn ac^{-1}]) and crimson clover (12.8 Mg ha^{-1} [5.2 tn ac^{-1}]) intercrops were not different from 67 kg N ha^{-1} (14.5 Mg ha^{-1} [5.9 tn ac^{-1}]). Crude protein levels were greater ($p < 0.05$) for 134 kg N ha^{-1} (119.6 lb N ac^{-1}), compared to legume intercrops (except red clover). Partridge pea showed promise as a warm-season legume that can be grown compatibly with switchgrass for up to two years. Therefore, compatible legume-intercrop candidates, such as partridge pea and red clover, may enhance switchgrass yield and forage quality while displacing synthetic N in integrated biofuel/forage systems, but need to be further investigated in efforts to reduce nitrate (NO_3) leaching and emissions from fertilizing.

Key words: biomass—forage—legume intercropping—nitrogen—switchgrass

Switchgrass (*Panicum virgatum* L.) is a C4 perennial bunchgrass with excellent potential for producing biomass during warm, dry summer months in the southeastern United States (Cherney et al. 1991) and is currently being developed as a biofuel crop due to high yields with minimal inputs. Average annual biomass yields in the upper Southeast are 15.9 Mg ha^{-1} (6.1 tn ac^{-1}; Lemus et al. 2009), and yields may increase with nitrogen (N) fertilization, up to a point. As such, N fertilization is recommended in switchgrass production systems at a rate of 67 kg ha^{-1} (59.8 lb ac^{-1}; Garland et al. 2008), or approximately half the rate for corn (*Zea mays* L.). Nitrogen removal by switchgrass has shown to be twice as high in a two-cut compared to a one-cut harvest system (Fike et al. 2006; Lemus et al. 2009; Reynolds et al. 2000; Yang et al. 2009).

Nitrogen-fixing legumes may supply a portion of the N diet for switchgrass production. Experiments have shown that alfalfa (*Medicago sativa* L.) and birdsfoot trefoil (*Lotus corniculatus* L.) may transfer all N requirements directly or indirectly (through rhizodeposition) to companion grass stands (Brophy et al. 1987). Similarly, interseeded hairy vetch (*Vicia villosa* L.), red clover (*Trifolium pratense* L.), alfalfa, Persian clover (*Trifolium resupinatum* L), common vetch (*Vicia sativa*), arrowleaf clover (*Trifolium vesiculosum* L.), and crimson clover (*Trifolium incarnatum* L.) have all shown to supply substantial amounts of N to companion crops (Holderbaum et al. 1990; Tyler et al. 1987; Opitz von Boberfeld et al. 2005). Experiments with legume-switchgrass stands including white and yellow sweet clovers (*Melilotus alba* Medik and *Melilotus officinalis* L.), birdsfoot trefoil, red clover, and alfalfa resulted in yields that exceeded those from N-only stands, even at N levels of 240 kg ha^{-1} (214.3 lb ac^{-1}; George et al. 1995; Gettle et al. 1996).

Compatible legume intercrop establishment and persistence depends on growth habit, management, photosynthetic pathway, and rate of legume maturity (Blanchet et al. 1995; Posler et al. 1993). Alfalfa and hairy vetch persistence were both over 60% when seeded into the less robust upland switchgrass variety "Cave-In-Rock" (Blanchet et al. 1995). Companion species and main crops must be able to grow simultaneously, not eliminate one another from the stand, and take advantage of the co-crop growth pattern (Cherney et al. 1991). Legume presence also increases species diversity, which helps to maintain stable year-to-year production, break disease cycles, and increase beneficial arthropod communities (DeHaan et al. 2010; Tilman 2000).

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Grassland systems with greater species diversity have the potential to increase crude protein (CP) content (Barnett and Posler 1983; Posler et al. 1993), increase yields, and reduce weed pressures due to niche differentiation. The addition of legumes in cool-season pasture systems reportedly increased animal performance by 25% to 50% (Allen et al. 1992), as well as the overall forage quality. In vitro digestible dry matter (IVDDM) concentrations of switchgrass improved when interseeded with legumes; however, legume reseeding was necessary for continued IVDDM improvement after consecutive years of legume growth (Posler et al. 1993).

There are many unanswered questions regarding compatible cool- and warm-season legumes for switchgrass intercropping, especially for lowland ecotypes such as 'Alamo.' Consequently, objectives of this research were to (1) identify compatible legume intercrops in biomass and forage production systems by screening from a pool of six cool- and warm-season legume species; (2) determine whether switchgrass yields and forage quality are affected by a compatible, intercropped legume; and (3) assess legume density impacts from harvest timing and frequency.

Table 1

Average annual precipitation, and average annual ambient temperatures, and previous experimental site management at the Research and Education Centers at Knoxville (ETREC), Crossville (PREC), and Milan, Tennessee (RECM) during 2009 and 2010.

	Annual precipitation (cm)		Annual temperature (°C)		
Location	2009	2010	2009	2010	Previous experimental site management
ETREC	173	124	14.3	14.4	Orchardgrass *(Dactylis glomerata)* hay (4 years)
PREC	192	140	12.7	12.6	Tall fescue pasture
RECM	140	145	14.9	15.2	Row crops

Materials and Methods

Switchgrass and Legume Establishment. 'Alamo' switchgrass was planted at 9 kg ha^{-1} (8 lb ac^{-1}) pure live seed (PLS) in spring of 2007 at the University of Tennessee, East Tennessee Research and Education Center (ETREC), Knoxville (35°57′38″ N, 83°55′14″ W) and the Plateau Research and Education Center (PREC), Crossville (35°56′56″ N, 85°56′56″ W), as well as in 2004 at the Research and Education Center at Milan (RECM) Milan, Tennessee (35°55′11″ N, 88°45′32″ W). Soil series at ETREC was a Huntington silt loam (fine-silty, mixed, active, mesic Fluventic Hapludolls), a Lily silt loam (fine-loamy, siliceous, semiactive, mesic Typic Hapludults) at PREC, and a Collins silt loam (coarse-silty, mixed, active, acid, thermic Aquic Udifluvents) at RECM. Previous management practices, mean annual temperature, and precipitation at each location are presented in table 1. Weeds were controlled at ETREC by hand cultivation and by an application of nicosulfuron (2-[{(4, 6-dimethoxypyrimidin-2-yl) aminocarbonyl} aminosulfonyl]-N, N-dimethyl-3-pyridinecarboxamide) at a rate of 47 g ha^{-1} (0.7 oz ac^{-1}) in 2009. Weeds at PREC and RECM prior to legume plantings were controlled by 2, 4-dichlorophenoxyacetic acid at a rate of 0.9 L ha^{-1} (12.3 oz ac^{-1}).

Legume Treatments. This study evaluated the establishment (density) and subsequent impacts (yield and forage quality) of six legume species when interseeded into established 'Alamo' switchgrass, as well as three inorganic N rates. A randomized complete block design tested a single factor (legume, N, or some combination) independently for each harvest system in three blocks per location. Harvest regimes tested included (1) a single, end-of-season harvest in November (one-cut system), (2) an integrated forage/biofuel production paradigm in June and November (two-cut system) with each harvest treatment analyzed separately, and (3) the sum of the two-cut system under an integrated approach. These harvests were chosen to represent a biomass production and an integrated forage/biomass production scenario.

Cool-season legumes were alfalfa ('Evermore' [ALF]), red clover ('Cinnamon Plus' [RC]), hairy vetch ('variety not stated' [VNS; HV]), and crimson clover ('VNS' [CC]). The warm-season legumes were Illinois bundle flower (*Desmanthus illinoensis* L.; 'VNS' [IBF]), and partridge pea (*Chamaechrista fasciculata* L.; 'VNS' [PP]). Legumes were chosen based on growth habits and cycles that are potentially compatible with lowland switchgrass, and for their reported ability to fix high volumes of N_2. Legumes were no-till drilled into one-year old switchgrass ('Alamo') stubble at ETREC and PREC using a seven-row Hege plot drill (Colwich, Kansas), and into three-year-old switchgrass ('Alamo') at RECM with an eight-row ALMACO plot drill (Nevada, Iowa). Legumes were seeded at a depth ranging from 0.6 to 1.3 cm (0.25 to 0.50 in). Plot sizes at ETREC and PREC were 7.6 by 1.5 m (25 by 5 ft) and 7.6 by 1.8 m (25 by 4.25 ft), respectively, with 18 cm (7 in) row spacing. Plot size at RECM was 7.6 by 3.1 m (25 by 10 ft) with 25.4 cm (10 in) row spacing. Legume seeding rates were 14, 7, 7, 9, 14, and 9 kg ha^{-1} PLS (12, 6, 6, 8, 12, and 8 lb ac^{-1}) for ALF, CC, HV, IBF, PP, and RC, respectively. Seeding rates were adjusted from recommended pure stand rates for forage production to reduce competition with switchgrass early in the season. In order to establish adequate stand densities, legumes were seeded in 2008 and again in 2009 (table 2). Fall seeding occurred in late fall following switchgrass harvest. Seed of ALF, CC, and RC were inoculated with cow pea group inoculum (*Bradyrhizobium* spp.), whereas HV, IBF, and PP were not.

Legume stand densities were measured annually following green-up in the spring with a 1 m^2 (3.3 ft^2) frequency grid (Vogel and Masters 2001). Four density counts were taken on each experimental unit (plot). Switchgrass heights were also measured and averaged from four subsamples per plot.

Harvest Treatment: Biomass Only. The biomass-only experiment evaluated legume yield response with a single, postdormancy harvest and was conducted at ETREC, PREC, and RECM. This experiment included four cool-season legumes (ALF, CC, HV, and RC), two warm-season legumes (IBF and PP), two inorganic N rates (0 and 67 kg N ha^{-1} [0 and 147.7 lb ac^{-1}]), as well as ALF + 67 N and RC + 67 N for a total of 10 treatments. At the RECM location only, six legumes (ALF, CC, HV, IBF, PP, and RC) and those same legumes plus 67 kg N ha^{-1} (60 lb ac^{-1}) were included. The RECM location also included a 134 kg N ha^{-1} (120 lb ac^{-1}) rate. All inorganic N applications occurred when switchgrass broke dormancy in the spring and was approximately 30.5 cm (12 in) tall, which typically occurs late April. The synthetic N source, ammonium nitrate (NH_4NO_3), was broadcast via a mechanical spreader.

Harvest Treatment: Forage/Biomass. Dual-use (forage/biomass) harvests were

Table 2
Summary of legume seeding and switchgrass harvest dates at the Research and Education Centers at Knoxville (ETREC), Crossville (PREC), and Milan, Tennessee (RECM), for both single and two-cut harvest system experiments in 2009 and 2010.

	Seeding dates					
	ETREC		PREC		RECM	
Growing season	Cool*	Warm†	Cool	Warm	Cool	Warm
2009	Oct. 20, 2008 Mar. 24, 2009‡	Mar. 24, 2009	Nov. 4, 2008 Mar. 31, 2009‡	31 Mar. 2009	Apr. 9, 2009	
2010	Oct. 29, 2009		Oct. 22, 2009		Dec. 17, 2009	
	Harvest dates					
	ETREC		**PREC**		**RECM**	
Growing season	**Forage**	**Biomass**	**Forage**	**Biomass**	**Biomass**	
2009	June 10, 2009	Oct. 22, 2009	June 17, 2009	Oct. 21, 2009	Dec. 3, 2009	
2010	May 26, 2010	Nov. 8, 2010	June 9, 2010	Oct. 21, 2010	Nov. 23, 2010	

*Cool-season legume plantings included alfalfa, crimson clover, hairy vetch, and red clover.

†Warm-season legume plantings included Illinois bundle flower and partridge pea.

‡In 2009, cool-season legumes were replanted in March because of poor emergence and persistence in 2008 at ETREC and PREC due to a combination of wet and cold weather damage over winter months.

conducted at ETREC and PREC during boot stage (late May [ETREC] or mid-June [PREC]) with the second harvest occurring postdormancy (late October through early December [table 2]). This harvest system included four cool-season legumes (ALF, CC, HV, and RC), two warm-season legumes (IBF and PP), and three inorganic N treatments (0, 67, and 134 kg N ha^{-1} [0, 147.7, and 295.4 lb ac^{-1}]). The 134 kg N ha^{-1} treatment was applied as a split-application (spring green-up and after renewed green-up following the forage cut [approximately second week]). The control was represented by the 0 kg N ha^{-1} treatment.

Sample Collection. Switchgrass dry matter yields were measured from all plots at time of harvest (table 2) and analyzed for each location × year in 2009 and 2010. Plots were harvested at ETREC and PREC using a Carter forage harvester (Brookston, Indiana) with a 91 cm (36 in) cutting width at 20 cm (8 in) cutting height. A New Holland Crop Cruiser 850 forage chopper with a 2.1 m (82 in) cutting width at 20 cm (8 in) cutting height was used at RECM. Grab samples (1 to 2 kg [2 to 4.4 lb]) were collected from all plots at harvest, weighed, dried at 49°C (120.2°F) for 72 hours in a batch oven (Wisconsin Oven Corporation, East Troy, Wisconsin), and reweighed to determine moisture content. Samples were then ground to pass through a 2 mm (0.08 in) sieve on a Wiley mill (Thomas Scientific, Swedesboro, New Jersey). Forage nutritional components were only analyzed on the forage (first) cut of the two-cut harvest system. The analysis included moisture content at harvest, acid detergent fiber (ADF), neutral detergent fiber (NDF), CP, total digestible nutrients (TDN), and net energy lactation (NEL; Robertson and Van Soest 1981). Ground (2 mm) switchgrass tissue (separated from legumes) was analyzed with near-infrared spectroscopy (NIRS) using a LabSpec Pro Spectrometer (Analytical Spectral Devices, Boulder, Colorado). Five scans were taken per sample and the scan range was 1,003 to 2,500 nm. Samples were compiled across replications per legume seeding level such that only one sample was analyzed per species and N-level per harvest (n = 8 per site).

Data Analyses. Switchgrass yields, forage nutritional components, and legume densities were analyzed per harvest system using PROC MIXED with SAS v. 9.1.3 (SAS Institute, Cary, North Carolina). The ANOVA assumptions of normally distributed residuals (Shapiro-Wilk test) and homogeneity of variances (Levene's F-test) were confirmed. The fixed effect was legume and/or N treatment. Given that treatment assignments across locations were not balanced (RECM had 15 legume and/or N treatment combinations, whereas PREC and ETREC had 10), data were initially combined in a global model across years per treatment combination (i.e., PREC and ETREC analyzed together and RECM alone) with replication, location, and year being assigned as a fixed effects to test interactions. Given the interactions ($p < 0.05$) for year and location (where appropriate) for dependent variables (i.e., legume density, forage quality, and yield variables [per harvest regime]), data were therefore analyzed by year and location. The same model was used per harvest systems 1 through 4 (i.e., [1] biomass-only, [2] forage/biomass [integrated], [3] forage [as part of integrated system]; and [4] biomass [as part of integrated system]). Mean separation was performed with Tukey's at a Type-I error rate of 5%.

Results and Discussion

Biomass: Legume Establishment. Legume stand densities were influenced by date of seeding, weather after seeding, legume growing cycle, and switchgrass competition. Success in stand establishment, as measured by plant densities and legume heights, varied among sites and years ($p < 0.05$), but not across harvest systems. At ETREC and PREC, legume density (measured May of 2009) was greatest for CC, RC, and RC + 67 kg N ha^{-1} (RC + 59.8 lb N ac^{-1}; all exceeded 25 plants m^{-2} [2.3 plants ft^{-2}]) and was lowest (below 9 plants m^{-2} [0.83 plants ft^{-2}]) for IBF, and followed a similar pattern in 2010 (table 3; $p < 0.05$). At RECM for both years, legume density (measured May 19) was generally greatest for RC (≥26 plants m^{-2} [3.6 plants ft^{-2}]) and RC + 67 N (17 plants m^{-2} [2 plants ft^{-2}]) with densities of all other species being ≤5 plants m^{-2} (0.5 plants ft^{-2}). Although the number of plants for ALF (16 to 30 plants m^{-2} [2 to 3 plants ft^{-2}]) and IBF (5 to 12 plants m^{-2} [0.5 to 1.3 plants ft^{-2}]) in 2009 were reasonable, plants were small (3 to 5 cm [1 to 2 in]) and were out competed by switchgrass; consequently, the number of

Table 3
Average* legume (LG) plant density and height and switchgrass (SG) height in May of 2009 and 2010 at the Research and Education Centers at Knoxville (ETREC), Crossville (PREC), and Milan, Tennessee (RECM), sites in the one-cut biomass harvest experiments.

Year and treatment	ETREC			PREC			RECM		
	Plant density† (plants m⁻²)	Height LG (cm)	Height SG (cm)	Plant density (plants m⁻²)	Height LG (cm)	Height SG (cm)	Plant density (plants m⁻²)	Height LG (cm)	Height SG (cm)
2009									
Alfalfa	16	5	67	30	3	48	23	4	64
Alfalfa + 67 N‡	9	3	69	27	3	54	20	4	80
Crimson clover	31	10	69	32	9	54	23	7	63
Crimson clover + 67 N	—	—	—	—	—	—	22	9	86
Hairy vetch	12	22	66	15	10	51	17	30	68
Hairy vetch + 67 N	—	—	—	—	—	—	13	29	79
IL bundle flower	8	3	72	5	3	52	12	5	62
IL bundle flower + 67 N	—	—	—	—	—	—	9	5	83
Partridge pea	7	8	64	12	7	53	11	9	62
Partridge pea + 67 N	—	—	—	—	—	—	13	13	82
Red clover	25	6	69	36	10	54	34	9	65
Red clover + 67 N	25	9	65	37	10	53	24	8	84
0 N control	—	—	65	—	—	53	—	—	73
67 N‡	—	—	70	—	—	53	—	—	85
135 N	—	—	—	—	—	—	—	—	81
2010									
Alfalfa	0	—	111	0	—	103	0	—	79
Alfalfa + 67 N‡	0	—	115	0	—	105	0	—	81
Crimson clover	23	49	112	1	7	105	1	40	68
Crimson clover + 67 N	—	—	—	—	—	—	1	36	92
Hairy vetch	7	54	84	0	0	109	5	51	69
Hairy vetch + 67 N	—	—	—	—	—	—	4	45	86
IL bundle flower	0	—	117	0	—	110	2	4	75
IL bundle flower + 67 N	—	—	—	—	—	—	2	6	99
Partridge pea	2	14	106	4	16	100	3	16	71
Partridge pea + 67 N	—	—	—	—	—	—	4	18	96
Red clover	16	42	112	6	19	105	26	20	71
Red clover + 67 N	17	51	112	5	18	106	17	13	94
0 N‡ control	—	—	110	—	—	108	—	—	78
67 N‡	—	—	114	—	—	108	—	—	99
135 N	—	—	—	—	—	—	—	—	108

*Means across subsamples and replications.

†Plant density and plant height = average of four 1 m^{-2} subsamples per plot and three replications.

‡Nitrogen (N) applications are in kg ha^{-1}.

plants in 2010 was ≤3 plants m^{-2} (table 3). Switchgrass heights in 2009 were lower (64 to 72 cm [25 to 28 in]) than in 2010 (84 to 117 cm [33 to 46 in]; $p < 0.05$). The shorter heights of switchgrass when intercropped with HV was due to the competitive growth habit of HV (table 3). However, switchgrass did recover and yields were not negatively affected (table 4). None of the legumes survived at PREC in 2010 (table 3).

Precipitation at ETREC and PREC was greater in 2009 than in 2010, while RECM was approximately the same for both years (table 1), suggesting that precipitation was not the cause of observed height differences in these two years. Heavy rains and flooding at RECM during fall of 2009 and cold temperatures that occurred after fall seeding at all locations could have impacted legume establishment and persistence in 2010. Early season increases in switchgrass height shaded out ALF and IBF seedlings, which was also observed by Moore et al. (1991). Conversely, partridge pea (warm-season) was able to compete and grow compatibly with switchgrass. Theoretically, it may be possible to increase N supplied by increasing seeding rates above levels tested in this study; however, further work needs to be done to substantiate this.

Biomass Yields. Legumes had no detectable impacts on biomass yields at the three locations and for both years ($p > 0.05$). Switchgrass yields at ETREC averaged 14.0 and 13.2 Mg ha^{-1} (5.7 and 5.3 tn ac^{-1}), respectively, for 2009 and 2010, and did not

Table 4
Average dry matter yields (Mg ha^{-1}) of switchgrass from legume or legume + nitrogen (N) treatment for the one-cut biomass harvest at the Research and Education Centers at Knoxville (ETREC), Crossville (PREC), and Milan, Tennessee (RECM), sites from 2009 to 2010.

	2009			2010		
Treatment	ETREC	PREC	RECM	ETREC	PREC	RECM
Alfalfa	15.0a*	14.6ab	15.6bcd	10.2a	13.2a	15.9abcd
Alfalfa + 67 N†	13.9a	15.3ab	19.6ab	19.4a	15.1a	18.5ab
Crimson clover	11.4a	14.4ab	12.9d	12.7a	15.4a	10.5d
Crimson clover + 67 N	—	—	20.9a	—	—	20.4a
Hairy vetch	14.4a	16.9a	14.1cd	12.7a	13.8a	12.3cd
Hairy vetch + 67N	—	—	18.7abc	—	—	17.6abc
IL bundle flower	15.5a	15.9ab	12.6d	12.8a	16.4a	12.5bcd
IL bundle flower + 67 N	—	—	21.7a	—	—	21.5a
Partridge pea	13.4a	13.0b	12.8d	10.2a	14.4a	11.7cd
Partridge pea + 67N	—	—	20.8a	—	—	19.5a
Red clover	11.3a	14.1ab	12.9d	14.6a	14.0a	11.8cd
Red clover + 67 N	13.4a	15.0ab	20.5a	13.7a	15.9a	20.8a
0 N control	15.2a	14.3ab	13.4d	11.6a	13.7a	13.1bcd
67 N†	16.1a	15.5ab	19.0ab	14.2a	15.6a	19.3a
135 N	—	—	22.3a	—	—	21.8a

*Letters indicate statistical differences based on Tukey's at $p < 0.05$, which applies to columns across treatments within locations and year.
†Nitrogen applications are in kg ha^{-1}.

differ among treatments (table 4; $p > 0.05$). At PREC in 2009, switchgrass yields with HV (16.9 Mg ha^{-1} [6.8 tn ac^{-1}]) were greater ($p < 0.05$) than PP (13.0 Mg ha^{-1} [5.3 tn ac^{-1}]), with no others differing ($p > 0.05$), including 0, 67, and 134 kg N ha^{-1} rates. At RECM in 2009 and 2010, switchgrass yields from legume treatments without inorganic N were lower ($p < 0.05$) than legume treatments with additional 67 kg N ha^{-1} (20.4 Mg ha^{-1} [8.3 tn N ac^{-1}]), whereas yields from legume treatments without N were equivalent to that of the control. Thus, effects of legumes + N treatments (ALF, CC, HV, IBL, PP, and RC) on biomass yield were likely due to N applications and not legumes (table 4). Furthermore, biomass yields from the 134 kg N ha^{-1} rate were not greater than the 67 kg N ha^{-1} treatment ($p > 0.05$).

These results support the recommendation of 67 kg N ha^{-1} for lowland switchgrass production (Garland et al. 2008). We hypothesized that the N carryover effect of legumes on biomass yields would occur during the second year (2010), owing to modest N additions stimulating N_2 fixation (Zahran 1999). According to Mallarino et al. (1990), average N derived from legumes in tall fescue (*Schedonorus arundinaceus* Schreb) increased from 20% in the first year after seeding to 45% to 60% N the following year. However, this was not the case in this study.

Forage/Biomass System. Legume establishment in the two-cut harvest system was evaluated for two years at two locations. In 2009 at both locations, legume densities were high (except for IBF at PREC), ranging from 11 to 35 plants m^{-2} (1 to 4 plants ft^{-2}; table 5). However, in 2010, only CC, HV, and RC had reasonable plant stands at ETREC (15, 8, and 31 plants m^{-2}, respectively [2, 1, and 3 plants ft^{-2}]) and only RC at PREC (33 plants m^{-2} [3 plants ft^{-2}]). Only RC had consistently high densities across locations and years. Plant stands of other legumes were essentially nonexistent in the second year. Switchgrass heights during May in the forage/biomass experiment were higher for all treatments in 2010 compared to 2009 (table 5). As was the case in the one-cut biomass experiment, small plants of ALF and IBF were quickly shaded and overwhelmed by switchgrass. Townsend et al. (1975) also reported that IBF died after the first year when grown with warm-season grasses. On the other hand, small seedlings of PP were able to take advantage of the canopy removal during the forage cut, which allowed for accelerated vegetative growth, and subsequent reproduction by the end of the growing-season.

Maturation and ontogeny of legumes may be important in a two-cut harvest system. Earlier and late maturing legumes such as CC, RC, PP, and HV can take advantage of the open canopy following fall and spring harvests, respectively, by self-reseeding prior to canopy reclosure. To successfully reproduce, though, legumes must have reached their reproductive state before the forage harvest, or be able to mature in the presence of switchgrass regrowth.

Forage Yields. Overall, legumes had no consistent effect on forage yields at either location or year (table 6; $p > 0.05$). At ETREC in 2009, switchgrass yields of all legume treatments (5.5 Mg ha^{-1} [2.2 tn ac^{-1}]) with the exception of ALF (2.1 Mg ha^{-1} [0.9 tn ac^{-1}]), did not differ ($p > 0.05$) from the control (5.5 Mg ha^{-1} [2.2 tn ac^{-1}]; table 6). However, Heichel and Henjum (1991), reported high N_2 fixation capabilities of alfalfa (82 to 254 kg N ha^{-1}) when grown with a companion grass. Similarly, in 2010, switchgrass yields averaged 5.9 Mg ha^{-1} (2.4 tn ac^{-1}) across all treatments and were not different from the control (6.7 Mg ha^{-1} [2.7 tn ac^{-1}]). At PREC in 2009, forage yields were not different among legume treatments ($p > 0.05$) and averaged 5.1 Mg ha^{-1} (2.1 tn ac^{-1}; table 6). In 2010, switchgrass yield from the 134 kg N ha^{-1} treatment (5.7 Mg ha^{-1} [2.3 tn ac^{-1}]) was greater than that of ALF, HV, IBF, PP, and 0 N treatments, but was equivalent to CC, RC, and the 67 kg N ha^{-1} rate.

Forage Nutritional Components. Legumes can reportedly enhance switchgrass forage quality early in the season (George et al. 1995; Posler et al. 1993). However, differences among treatments and years were not

Table 5

Average* legume (LG) stand densities and heights and switchgrass (SG) heights at the Research and Education Centers at Knoxville (ETREC) and Crossville (PREC) sites in the two-cut (forage/biomass) harvest experiments in 2009 and 2010.

	ETREC						PREC					
	2009			2010			2009			2010		
	Plant	Height		Plant	Height		Plant	Height		Plant	Height	
Treatment	density† (plants m^{-2})	LG (cm)	SG (cm)	density (plants m^{-2})	LG (cm)	SG (cm)	density (plants m^{-2})	LG (cm)	SG (cm)	density (plants m^{-2})	LG (cm)	SG (cm)
Alfalfa	30	3	54	1	—	85	31	3	51	1	—	83
Crimson Clover	24	10	67	15	46	94	32	10	50	1	4	88
Hairy vetch	11	14	62	8	67	91	15	13	52	0	—	11
IL bundle Flower	17	3	62	1	2	88	3	6	53	0	—	82
Partridge pea	16	7	65	3	14	93	12	6	53	2	15	85
Red clover	30	9	72	31	54	93	35	10	52	33	47	89
0 N control	—	—	69	—	—	97	—	—	52	—	—	87
67 N‡	—	—	63	—	—	100	—	—	51	—	—	90
135 N	—	—	70	—	—	108	—	—	53	—	—	100

*Means across subsamples and replications.

†Plant density and plant heights = average of four subsamples per plot and three replications.

‡Nitrogen (N) applications are in kg ha^{-1}.

significant for ADF, NDF, NEL, and TDN ($p > 0.05$). ALF and IBF legume treatments were not included in the analysis due to inadequate plant densities in 2010. Averaged across locations and years, the 134 kg N ha^{-1} and RC treatment had the highest CP percentage (10.5% and 9.2% dry matter [DM], respectively; table 7). However, there were no differences ($p > 0.05$) in CP among legumes, the control, or 67 kg N ha^{-1} (table 7).

Biomass Yields Following a Forage Cut. Legumes evaluated had nominal effects on second-cut biomass yields at either location or year (table 6). Similarly, the second 67 kg N ha^{-1} application following the forage cut (134 kg N ha^{-1} total) was generally equivalent to the 67 kg ha^{-1} rate (table 6; $p > 0.05$). At PREC in 2009, switchgrass integrated biomass yields for the 134 kg N ha^{-1} treatment (7.3 Mg ha^{-1} [3.0 tn ac^{-1}]) was greater than ALF, HV, IBF, PP, and RC treatments (table 6). There were no differences among legume treatments, the control (4.8 Mg ha^{-1} [1.9 tn ac^{-1}]), 67 kg N ha^{-1} (5.9 Mg ha^{-1} [2.4 tn ac^{-1}]), nor any of the legumes and the 67 kg N ha^{-1} treatment (6.3 Mg ha^{-1} [2.6 tn ac^{-1}]) in either year or location ($p > 0.05$).

For both locations combined, yields in 2009 for 67 and 134 kg N ha^{-1} were greater ($p < 0.05$) than those from ALF, HV, IBF, and PP intercrops, but did not differ from yields of CC and RC intercrops, or the 0 N treatment (table 6). Yield from the CC treatment, 6.1 Mg ha^{-1} (2.5 tn ac^{-1}) was greater ($p < 0.05$) than those from all other legume treatments except RC (5.8 Mg ha^{-1} [2.3 tn ac^{-1}]). In 2010, yield from 134 kg N ha^{-1} (8.1 Mg ha^{-1} [3.3 tn ac^{-1}]) was greater ($p < 0.05$) than all legume treatments, but was not different from 67 kg N ha^{-1} (7.0 Mg ha^{-1} [2.8 tn ac^{-1}]).

Total Combined Yield. Combined forage and biomass yields were not consistently affected by legume intercrops. Generally, yields from legumes were not different from the control (no N) or the 67 kg N ha^{-1} rate (table 6). However, averaged across locations within a year, total combined forage and biomass yields from the split application of N (15.4 and 15.0 Mg ha^{-1} [6.2 and 6.1 tn ac^{-1}]; 2009 and 2010, respectively) were greater ($p < 0.05$) than the control (11.4 and 10.4 Mg ha^{-1} [4.6 and 4.2 tn ac^{-1}]; 2009 and 2010, respectively) and legume treatments, but not the 67 kg N ha^{-1} (table 6). At ETREC in 2009, switchgrass yields from the 134 kg N ha^{-1} treatment (16.5 Mg ha^{-1} [6.7 tn ac^{-1}]) were greater than ALF, HV, and IBF legume intercrops (table 6). Similar to integrated biomass yields, the 67 kg N ha^{-1} (15.4 Mg ha^{-1} [6.2 tn ac^{-1}]) and legume treatments (except ALF) did not differ.

At PREC in 2009, yields from the 134 kg N ha^{-1} rate were greater than (14.2 Mg ha^{-1} [5.7 tn ac^{-1}]) that of legume treatments (table 6). Yields from the 67 kg N ha^{-1} rate (11.7 Mg ha^{-1} [4.7 tn ac^{-1}]) did not differ from legumes, or 134 kg N ha^{-1}. Yields in 2010 for 134 kg N ha^{-1} (12.6 Mg ha^{-1} [5.1 tn ac^{-1}]) were greater than ALF, IBF, and PP legume intercrops.

Summary and Conclusions

Of the six legumes evaluated in this study, PP and RC were the most compatible for interseeding into established stands of 'Alamo' switchgrass in both dual-use (forage/biomass) and biomass-only systems. Although densities for ALF and IBF in 2009 were adequate, these species could not compete with switchgrass, and consequently are not suitable companion legumes for lowland switchgrass. Conversely, the density and size of HV at ETREC in both years provided heavy competition for the switchgrass early in the season. Harvest system did not affect legume densities considering both systems resulted in similar legume frequencies, as most variability arose from locations and years. Both annuals with reseeding capability, CC and HV, did not produce sufficient populations during the second year that would preclude reseeding; however, further determinations of legume persistence overtime and proper seeding recommendations are needed.

Legume intercrops had minimal-to-no yield effect on harvest systems tested herein when interseeded into switchgrass swards. Albeit, RC may prove to be a successful candidate, as in both harvest systems (biomass only, forage, or forage/biomass) yields were equivalent to the current recommended N rate (67 kg ha^{-1} [60 lb ac^{-1}]). Further, due to its in-season growing compatibility and reseeding success, PP also showed promise as a warm-season legume that can be intercropped with switchgrass. Consequently,

Table 6

Average dry matter yields (Mg ha⁻¹) of switchgrass from legume or legume + nitrogen (N) treatments of forage, biomass, and total forage/biomass (F + B) harvests at the Research and Education Centers at Knoxville (ETREC) and Crossville (PREC) for 2009 and 2010.

	ETREC			PREC			Two location average		
Treatment	Forage (F)	Biomass (B)	F + B	Forage	Biomass	F + B	Forage	Biomass	F + B
2009 (3-year-old 'Alamo' switchgrass stands)									
Alfalfa	2.1b*	5.7a	7.7c	4.5a	3.8b	8.3b	3.3c	4.7c	8.0c
Crimson clover	5.0ab	7.4a	12.4abc	4.7a	4.9ab	9.6b	4.9bc	6.1ab	11.0bc
Hairy vetch	3.8ab	6.2a	10.0bc	4.6a	4.0b	8.6b	4.2bc	5.1c	9.3c
IL bundle flower	4.0ab	5.4a	9.4bc	4.9a	4.7b	9.5b	4.5bc	5.0c	9.5c
Partridge pea	5.5a	5.9a	11.4abc	4.4a	4.4b	8.8b	5.0abc	5.1c	10.1bc
Red clover	5.4a	6.9a	12.3abc	5.4a	4.6b	10.0b	5.4ab	5.8bc	11.2bc
0 N control	5.5a	7.8a	13.3abc	4.6a	4.8ab	9.5b	5.1abc	6.3abc	11.4bc
67 N†	5.4a	10.0a	15.4ab	5.8a	5.9ab	11.7ab	5.6ab	8.0ab	13.6ab
134‡ N	6.8a	9.7a	16.5a	6.9a	7.3a	14.2a	6.7a	8.5ab	15.4a
2010 (4-year old 'Alamo' switchgrass stands)									
Alfalfa	4.3a	5.5ab	9.8b	3.0b	4.5b	7.5b	3.7b	5.0b	8.7c
Crimson clover	6.0a	5.6ab	11.6ab	3.9ab	5.7ab	9.6ab	5.0ab	5.7b	10.6bc
Hairy vetch	5.3a	5.4ab	10.7ab	3.5b	5.1ab	8.6ab	4.4b	5.3b	9.7bc
IL bundle flower	3.9a	5.0b	9.0b	3.0b	4.7ab	7.7b	3.5b	4.9b	8.4c
Partridge pea	5.4a	6.3ab	11.7ab	3.5b	5.0ab	8.5b	4.5b	5.6b	10.1bc
Red clover	6.7a	5.1b	11.8ab	4.3ab	5.3ab	9.6ab	5.5ab	5.2b	10.7bc
0 N control	6.7a	5.3ab	12.0ab	3.3b	5.4ab	8.7ab	5.0ab	5.3b	10.4bc
67 N†	6.7a	7.7ab	14.3ab	4.4ab	6.3ab	10.7ab	5.5ab	7.0ab	12.5ab
134‡ N	8.1a	9.4a	17.5a	5.7a	6.8a	12.6a	6.9a	8.1a	15.0a

*Letters indicate statistical differences based on Tukey's at $p < 0.05$, which applies to columns across treatments.

†N applications are in kg ha⁻¹.

‡Split application of 67 + 67 (at spring green-up and after forage harvest).

Table 7

Average crude protein* (% DM) in the forage cut of the two-cut forage/biomass harvest at the Research and Education Centers at Knoxville (ETREC) and Crossville (PREC) sites in 2009 and 2010.

	Crude protein						
Location	CC	HV	PP	RC	0 N‡	67 N	134 N
ETREC 2009	7.3a†	8.1a	7.3a	7.2a	6.9a	7.8a	7.7a
ETREC 2010	8.0b	7.7b	8.0b	8.6b	7.3b	7.0b	10.4a
PREC 2009	8.8b	9.2b	8.8b	8.7b	8.6b	9.5b	13.3a
PREC 2010	10.5a	10.2a	10.6a	12.3a	11.2a	11.7a	10.8a
Average	8.6b	8.8b	8.7b	9.2ab	8.5b	9.0b	10.5a

Notes: DM = dry matter. CC = crimson clover. HV = hairy vetch. PP = partridge pea. RC = red clover. N = nitrogen.

*The average acid detergent fiber, neutral detergent fiber, net energy lactation, and total digestible nutrients values did not differ among treatments or locations and years.

†Mean separations based on Tukey's at $p < 0.05$. Means followed by a common letter within a row (location year) are not significantly different.

‡N applications of 0, 67, and 134 kg N ha⁻¹.

further investigations are warranted with these two legume intercrops.

Results in this study further substantiate the current recommended rate of N for 'Alamo' switchgrass production (Garland et al. 2008; Mooney 2009). The double rate of N (134 kg ha^{-1} [120 lb ac^{-1}]) did not result in greater yields ($p < 0.05$) than the 67 kg ha^{-1} rate. However, the double rate did result in greater integrated (two-cut system) yields than the control and legumes (when averaged across locations and years). In addition, the split application of 67 kg N ha^{-1} following the forage harvest did not increase postdormancy biomass yields.

We hypothesized that cumulative N effects of legumes for biomass yields and forage quality would occur during the second year (2010); however, this was repudiated in our two-year study. Consequently, the null hypothesis was accepted, as presence of legumes did not consistently alter forage quality among legume treatments. The addition of 134 kg N ha^{-1} (120 lb ac^{-1}) increased CP content in dry matter, but did not affect other nutritional forage quality characteristics. If legumes such as RC or PP with longer persistence (>2 years Ashworth et al. 2015) can be successfully intercropped with switchgrass at appropriate densities, a portion of the inorganic N fertilizer requirement may be reduced, thereby minimizing inputs and energetic costs associated with N manufacturing. Based on these results, a follow-up study was initiated to evaluate required stand densities and persistence of successful legumes over time, as well as yields of switchgrass under a single, postdormancy biofuel and a forage/biomass harvest system (Ashworth et al. 2015).

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