Cultivar and Phosphorus Amendment Impacts on Organically Managed Forage Cowpea Yield and Composition

Samantha L. Hill, David A. Verbree, Gary E. Bates, and David M. Butler*

ABSTRACT

Cowpea (Vigna unguiculata Walp.) is a warm-season legume with many traits that make it an attractive forage or cover crop for organic systems. Eight cowpea cultivars were evaluated under organic management at two locations for stand establishment, forage yield and quality, and weed biomass. The experiment was arranged in a strip-plot design with two P fertilization rates, amended (45 kg P ha⁻¹) and unamended, to evaluate responsiveness to P fertilization in low native soil P status (Mehlich-1 $P < 10 \text{ mg P kg}^{-1}$). Cowpea was seeded at 209,000 seeds ha⁻¹. Stand density at 4 wk indicated the highest plant populations from cultivar Iron & Clay (166,000 plants ha⁻¹), intermediate populations from Speckled Purple Hull, IT82E-18, and IT85-867-5F (143,000 to 138,000 plants ha⁻¹), and lowest populations from IAR7/8-5-4-1, Coronet, KV×396, and IT97K-556-4 (128,000 to 118,000 plants ha⁻¹) primarily due to presence of seedling diseases caused by Fusarium spp. Speckled Purple Hull and Iron & Clay had the highest total yield (4922 and 4623 kg ha⁻¹, respectively). Yield was lowest from IT82E-18, Coronet and IAR7/8-5-4-1 (1958–2585 kg ha⁻¹), likely due to low plant populations (IAR7/8-5-4-1, Coronet) and higher weed biomass than cowpea biomass (IAR7/8-5-4-1, Coronet, IT82E-18). There was no statistical difference in cowpea biomass between unamended (3422 kg ha⁻¹) and P-amended plots $(3150 \text{ kg ha}^{-1})$, or differences in cowpea tissue P concentration. High forage quality values of top-performing cultivars suggest that they are well adapted to address low summer forage quality in applicable forage systems.

Core Ideas

- Cowpea is well-adapted for organic systems but cultivar differences are not well explored.
- Cultivars differed widely in biomass, stand density/seedling disease, and quality.
- Cowpea cultivars examined did not respond to P fertilizer in low soil P status soils.

Published in Agron. J. 109:1623–1631 (2017) doi:10.2134/agronj2016.11.0663

Copyright © 2017 by the American Society of Agronomy 5585 Guilford Road, Madison, WI 53711 USA All rights reserved

RGANIC CROPPING SYSTEMS in the southeastern United States can be limited by low soil N, weed pressure, insect and disease pressures, lack of commercially available, adapted varieties for organic systems, and the highly weathered, low-organic matter soils common in the region (Jordan, 2004; Lammerts van Bueren et al., 2011). In systems that integrate organic livestock production with crop production, there are additional issues, such as the difficulty in producing adequate quantity and quality of forage for grazing livestock during hot and potentially droughty summer months. During this period, air and soil temperatures often are elevated, and soil water potential often is reduced. These factors can increase plant stress and can reduce nutrient mineralization by soil biota. This period is a time during which cool-season perennial grasses decline in productivity and quality (Rao and Northup, 2009).

Integrating cowpea into existing organic crop rotations can help address many of these issues. As a warm-season legume native to sub-Saharan Africa, cowpea is drought and heat tolerant and associates with symbiotic N-fixing *Bradyrhizobium* spp. bacteria, making it a promising summer crop for organic production systems in the region (Ehlers and Hall, 1997). Cowpea requires few inputs, and it can enhance or maintain soil fertility through biological N₂ fixation and impart efficient uptake of poorly soluble soil P (Creamer and Baldwin, 2000; Khandaker, 1994; Sanginga et al., 2000).

A high degree of genetic diversity in cowpea cultivars exists, with a range of diverse cultivars targeted for fresh vegetable, dry grain, forage, or cover crop use. Cowpea cultivars differ widely in growth habit and phenotypic attributes, such as seed size, seed coat, pod and flower color, photosensitivity, determinacy, and nutritional value (Ehlers and Hall, 1997). Growth habit ranges from erect, semi-erect, semi-prostrate or prostrate, and determinate, bushy growth to indeterminate, vining growth. Although cowpea demonstrating an erect, determinate growth habit will likely be more suitable for mechanical harvest of dry grain, prostrate, indeterminate cowpea varieties may be more valuable as a forage or cover crop where maximum ground cover and biomass accumulation are essential functional traits (Harrison et al., 2006).

Photosensitivity plays an important role in regional cowpea adaptability in that many cultivars are short-day photosensitive.

Abbreviations: ADF, acid detergent fiber; CP, crude protein; aNDF, amylase-treated neutral detergent fiber; NIRS, near-infrared reflectance spectroscopy; TDN total digestible nutrients.

Dep. of Plant Sciences, Univ. of Tennessee, Knoxville, TN 37996. Received 17 Nov. 2016. Accepted 20 Mar. 2017. *Corresponding author (dbutler@utk.edu).

These cultivars are late maturing in the United States and often do not produce pods until very late in the growing season. Photoperiod-sensitive cultivars have the potential to produce much more biomass if planted during long day-lengths due to the extended duration of the vegetative stage preventing early transition into reproductive growth (Ehlers et al., 2002a; Hall et al., 2002). The photosensitive cultivar Iron & Clay produces a large amount of biomass throughout the season and has rapid regrowth ability given that nutrient allocation for seed production is delayed until daylight hours are short in the late summer and fall in the southeastern United States. Historically, Iron and Clay were separate cultivars and were not combined as a cultivar mixture with desirable traits until the early 20th century (Hayes and Garber, 1921). Iron was originally heralded for its root-knot nematode (*Meloidogyne* spp.), *Fusarium* spp. wilt and rust resistances, but it did not produce large seed yields or provide enough biomass to be utilized as forage (Nielsen, 1916). Clay is one of the oldest referenced U.S. cultivars of cowpea, dating back to the early to mid-19th century, and the name Clay was a group descriptor of similar cowpea cultivars with buff-colored seeds (Piper, 1912; Morse, 1920). Clay was considered a cultivar of "secondary value" to Iron due to it lacking natural resistances (Nielsen, 1916). However, Clay had a desirable growth habit for forage situations that Iron lacked; specifically, it was indeterminate and prostrate, produced high seed yields, and was also later maturing than Iron (Nielsen, 1916). Today, the Iron & Clay cultivar mixture is marketed widely as a cover crop and forage for being resistant to root-knot nematodes (Ehlers et al., 2002b; Hall et al., 2002), highly competitive with various weed species (Wang et al., 2004), and is often the standard cultivar used in cover crop research (e.g., Harrison et al., 2006; Butler et al., 2012). Iron & Clay serves as a control cultivar in this study as it is the only widely commercially available forage cowpea cultivar in the southeastern United States.

The objectives of this research were to (i) evaluate cowpea cultivar performance (establishment, biomass, regrowth, and weed competitiveness) as a forage crop under organic management in the southeastern United States, (ii) evaluate cowpea cultivar response to fertilizer P in low native P soils, and (iii) evaluate cowpea cultivar forage quality under organic management.

MATERIALS AND METHODS

In May 2014, a strip-plot experimental design was established at two locations, the Organic Crops Unit of the East Tennessee Agricultural Research and Education Center in Knoxville, TN (OCU) (35.88°N, 83.93°W), and the University of Tennessee Plateau Research and Education Center (PREC) in Crossville, TN (36.02°N, 85.13°W). Soil types were a Dewey loam (fine, kaolinitic, thermic Typic Paleudult) at the OCU and a Lily loam (fine-loamy, siliceous, semiactive, mesic Typic Hapludult) at PREC. The site at the OCU is USDA-certified organic. At each location, four blocks (replicates) were established with each containing two main plots (17.1 by 7.6 m) randomly assigned as either P amended or unamended. Within each block, eight subplots, 2.1-m wide, were assigned randomly to one of eight cowpea cultivars, creating a strip-plot design with 2.1 by 7.6 m plots as the experimental unit. Within each plot, four rows (38-cm spacing) were planted with a plot drill equipped with seed metering belt cones (OCU, ALMACO, Nevada, IA; PREC, Hege Maschinen, Waldenburg, Germany) at a seeding of 209,000 seeds ha^{-1} to the entire plot length (7.6-m). Cowpea cultivars included were: Iron & Clay, IT97K-556-4, KV×396, IT85F-867-5, IT82E-18, Speckled Purple Hull, IAR7/8-5-4-1, and Coronet (Table 1). Due to limited seed availability of several cultivars, cultivar germination was assessed by planting three replicates of 10 seeds in 10-cm pots filled with moist sand. All cultivars were confirmed to germinate at a rate of

| | | Days to | Days to | Seed | | | |
|-------------------------|------------------------------------------------------------------------------------|-----------|----------|---------|--------------------|----------------|---------------|
| Cultivar | Origin | flowering | maturity | weight† | Photo-sensitivity‡ | Growth habit | Determinacy |
| Iron & Clay | Heirloom, southeastern USA | 83 | 110 | 11.3 | Yes | Semi-prostrate | Indeterminate |
| Speckled Purple Hull | Heirloom, southeastern USA | 58 | 83 | 17.7 | No | Erect | Indeterminate |
| IT97K-556-4 | International Institute of Tropical Agriculture (IITA), Nigeria | 83 | 110 | 17.3 | Yes | Semi-prostrate | Indeterminate |
| KV×396 | Institut de l'Environnement et Recherches Agricoles (INERA), Burkina Faso | 52 | 87 | 13.8 | No | Erect | Determinate |
| IT85F-867-5 | International Institute of Tropical Agriculture (IITA), Nigeria | 37 | 64 | 13.8 | No | Erect | Indeterminate |
| IAR7/8-5-4-1 | Institute for Agricultural Research (IAR), Nigeria | 54 | 90 | 15.4 | No | Semi-erect | Determinate |
| IT82E-18 | International Institute of Tropical Agriculture (IITA), Nigeria | 40 | 64 | 16.9 | No | Erect | Determinate |
| Coronet | University of Georgia, USA | 37 | 83 | 17.1 | No | Semi-prostrate | Determinate |

Table I. Cowpea cultivar descriptors collected from visual field observation (Verbree et al.; unpublished data, 2013).

† g 100 seeds-1.

‡ Yes = short-day photosensitive.

80% or above, with no significant differences (P > 0.05) among cultivars. Cultivars were chosen based on their history of use as a cover crop and forage in the southeastern United States (Iron & Clay), observed indeterminate habit and high biomass in preliminary trials (Speckled Purple Hull and IT97K-556-4), and more determinate cultivars with potential for multipurpose use (Coronet, IAR7/8-5-4-1, and IT83E-18). All seeds were not treated with fungicide and were sourced from seed produced in preliminary trials at the University of Tennessee. Cowpea seeds were inoculated with N-Dure *Bradyrhizobium* spp. (*Vigna*) inoculum (INTX Microbials, Kentland, IN) immediately prior to seeding. Planting dates were 23 May 2014 at OCU and 4 June 2014 at PREC.

Soils at the OCU and PREC were sampled in the fall 2013 to confirm low native soil P status (Mehlich-1 $P < 10 \text{ mg P kg}^{-1}$). At the OCU, winter cover crops of triticale (× Triticosecale Wittm.) and crimson clover (Trifolium incarnatum L.) preceded cowpea in rotation. The cover crop was mowed with a flail mower and then incorporated with a disk. At PREC, a cover crop of winter wheat (Triticum aestivum L.) was mowed and incorporated with a disk. Steamed bone meal was applied in P-amended plots at both sites at 45 kg P ha⁻¹ and amended by hand broadcasting throughout main plots. Steamed bone meal is a moderately available form of P, especially in acidic soils, and is used as an organic fertilizer in some cropping systems (Klock and Taber, 1996). Data on rainfall and temperature averages were collected from weather stations at each site equipped with precipitation gauges and temperature sensors (OCU, Vantage Pro2, Davis Instruments Corp., Hayward, CA; PREC, CR3000 datalogger, Campbell Scientific, Logan, UT).

Stand counts were recorded on 20 June 2014 at the OCU and 25 June 2014 at PREC by counting every live cowpea in each plot. No weed control operations were performed during the course of the study other than mowing at harvest. Plots were harvested at the OCU on 15 Aug. 2014 and 2 Oct. 2014 and at PREC on 13 Aug. 2014 and 24 Sept. 2014. Cowpeas were at early bloom to early pod filling prior to the first harvest and regrew to pod filling to seeding stages at the second harvest. Subsamples of weed and cowpea biomass were taken prior to harvest. In the outer two rows, 1.8 linear m of cowpea rows were cut to 2 cm above the soil surface and collected for cowpea quality analyses. Weed biomass was sampled from three, 0.25-m² areas (2 cm above the soil surface) to assess total weed dry matter. A 5.8-m² (7.6 by 0.76 m) harvest area of the center two rows of each plot were then cut at a height of 15 to 20 cm using a forage harvester (OCU, ALMACO, Nevada, IA, or Swift Machine and Welding Ltd., Swift Current, SK, Canada; PREC, Carter Manufacturing Company Inc., Brookston, IN). Fresh weight of bulk-harvested biomass was determined in the field at harvest. Subsamples from the bulk biomass were collected and oven-dried (65°C for 72 h) and weighed to determine forage moisture content. Whole plant cowpea samples were oven-dried, weighed, and ground in a laboratory grinder (Thomas Model 4 Wiley Mill, Thomas Scientific, Swedesboro, NJ) to pass through a 1-mm sieve for forage quality analyses. Weed biomass samples also were oven-dried and weighed.

Forage samples were analyzed using near-infrared reflectance spectroscopy (NIRS) technology (FOSS 5000, FOSS NIRSystems, Inc., Laurel, MD; Win ISI II software, Infrasoft International LLC, State College, PA) to assess cowpea biomass quality, nutritive values, and P concentration. The legume equation developed by the NIRS Forage and Feed Consortium (NIRSC, Hillsboro, WI) was used to determine cowpea total protein, acid detergent fiber (ADF), amylase-treated neutral detergent fiber (aNDF), P concentration, lignin, and total digestible nutrients (TDN). Sample analyses were compared against the model and other warm-season legumes in the database and all analyses fit the equation at H < 3.0 accuracy (Murray and Cowe, 2004).

Three soil cores (1.75-cm internal diameter) were sampled at a depth of 0 to 15 cm from each plot on 25 June and 15 Oct. 2014 at OCU and on 26 June and 16 Oct. 2014 at PREC. Samples were taken several weeks after applying P amendments and at the end of the study after the second harvest date. Soils were air-dried and then gently crushed with a mortar and pestle and sieved (2 mm). The method described by Sims et al. (1995) and Sims (2006) was used to determine soil inorganic $N (NH_4 - N + NO_3 - N + NO_2 - N)$. Briefly, approximately 5 g of air-dried, sieved soil were placed into a tared centrifuge tube, and the exact soil weight was recorded. Soil was extracted with 40 mL of 1 M KCl on a reciprocating shaker for 60 min at 180 rpm, then centrifuged at 25,000 \times g for 5 min before filtering the supernatant (Whatman 42, Whatman Ltd., Kent, UK). Concentration of inorganic N constituents in filtrate was determined using a microplate reduction technique, and absorbance was measured at 550 nm (Sims et al., 1995; Powerwave XS, Biotek, Woonooski, VT). Extractable soil P was determined by adding Mehlich-1 extractant (0.0125 M $H_2SO_4 + 0.05$ M HCl; Mehlich 1953) at a ratio of 20 mL per 5-g soil and extracting by shaking for 5 min at 180 rpm. Samples were centrifuged for 5 min at $25,000 \times g$, and the supernatant was filtered prior to colorimetric analysis for P concentration. Filtrate was analyzed using the microplate method described by D'Angelo et al. (2001) where dissolved phosphates in soil extracts were reacted with ammonium molybdate tetrahydrate and then Malachite green carbinol hydrochloride in polyvinyl alcohol. Concentrations of inorganic P were determined by measuring absorbance at 630 nm. Final concentration of extracted N and P in soils was determined based on extract concentrations and exact weight of extracted soil.

Analysis of variance was performed using mixed models (PROC GLMMIX, SAS 9.4, Cary, NC) and least squares means computed and separated using Fisher's *F*-protected LSD at P =0.05. Total annual cowpea biomass, total annual weed biomass, and stand density were analyzed according to a strip-plot design. Cultivar and P amendment and their interaction were fixed factors in the model and site, block (nested within site), and the interaction of block with fixed effects (cultivar, P amendment, and their interaction) were random effects. For response variables associated with harvest dates, cultivar, P amendment, harvest and their interactions were fixed factors and site, block (nested within site), and the interaction of block with cultivar, block with P amendment, and block with cultivar × P amendment were random effects. The effect of site was not considered of interest and so was considered a random effect in statistical models to statistically address variation between the two site environments where the study was repeated.

RESULTS AND DISCUSSION

From planting (23 May 2014) to the first harvest at the OCU (15 Aug. 2014), plots at the OCU received 300 mm of total rainfall. In the 6 wk from the first harvest to the second harvest (2 Oct. 2014) plots received 100 mm of total rainfall. At PREC, total precipitation was higher at 480 mm, including 136 mm occurring in the month of June. Rainfall totaled 274 mm from planting (4 June 2014) to the first harvest (13 Aug. 2014) and 206 mm from the first harvest to the second harvest (23 Sept. 2014). Average temperatures were similar between the two locations; from planting to the first harvest the average temperature was 23°C at the OCU and 22°C at PREC. From the first harvest to the second harvest the average temperature was 22°C at both locations.

Mehlich I soil P was influenced by P amendment ($P \le 0.001$) and sampling time ($P \le 0.05$), but not the interaction. Increased soil P was observed in P-amended plots with 14.7 mg extractable P kg⁻¹ soil compared to 10.6 mg P kg⁻¹ soil in unamended plots averaged over sampling date. Soil P was higher at the June sampling dates (13.5 mg P kg⁻¹ soil) than the October sampling dates (11.8 mg P kg⁻¹ soil), averaged across amended and unamended plots. Inorganic soil N was affected by sampling time ($P \le 0.001$) but not by cultivar or the interaction. Extractable inorganic soil N was higher at the June samplings (21.5 mg N kg⁻¹ soil) than on the October samplings (7.5 mg N kg⁻¹ soil).

Cowpea Performance

Stand density 4 wk after planting indicated the highest plant populations from Iron & Clay (166,000 plants ha^{-1}), intermediate populations from Speckled Purple Hull, IT82E-18, and IT85F-867-5 (138,000–143,000 plants ha^{-1}), and lowest populations from IAR7/8-5-4-1, Coronet, KV×396, and

IT97K-556-4 (118,000–128,000 plants ha⁻¹) (Fig. 1). Diseased seedlings were collected from plots to verify causal pathogens, which included Fusarium proliferatum, F. oxysporum, and Macrophomina phaseolina (Shrestha et al., 2016a, 2016b; U. Shrestha, personal communication, 2016). Iron is known to have resistance to *M. phaseolina* and to at least some races of *F*. oxysporum and is used extensively by plant breeders as a source of resistance to both diseases (Singh et al., 1997). These results suggest that cultivars evaluated likely differ in resistance or tolerance to seedling pathogens and that resistance is an area that requires further study, especially for organic production. Given limited seed treatments available for organic production, planting at higher seed densities may be a management strategy for cultivars lacking sufficient resistance or tolerance to these diseases. This could potentially allow the crop to still produce an adequate plant density for crop productivity as demonstrated with Rhizoctonia seedling blight of field pea (Hwang et al., 2007).

Cowpea biomass at each harvest was influenced by cultivar only ($P \le 0.001$; Table 2). Speckled Purple Hull and Iron & Clay had the highest average biomass per harvest (2446 and 2330 kg ha⁻¹, respectively), and biomass was least from IAR7/8-5-4-1, IT82E-18, and Coronet (983–1302 kg ha⁻¹). The first harvest (1707 kg ha⁻¹) did not differ from the second (1585 kg ha⁻¹). Cowpea total biomass was influenced by cultivar ($P \le 0.001$), but not P amendment or the interaction (Table 2). Speckled Purple Hull and Iron & Clay had the highest annual cowpea biomass (4922 and 4623 kg ha⁻¹, respectively; Fig. 2). Both of these cultivars are indeterminate and produce tendrils (Table 1), allowing them to spread across rows and completely cover inter-row space effectively (Wang et al., 2006). Notably, photosensitive Iron & Clay was still in a vegetative growth stage when the first harvest occurred in August



Fig. 1. Stand density at 4-wk post planting as influenced by cultivar, averaged over location. Means followed by the same letter are not significantly different according to an *F*-protected LSD, P > 0.05.

Table 2. Mixed models analysis of variance for all response variables as affected by P amendment, cowpea cultivar, harvest (where applicable), and their interactions.

| | Stand | Total cowpea | Total weed | Сомраз | Wood | Protein | Protein | | |
|-------------------------------------|--------|--------------|------------|---------|---------|---------|---------------------|--|--|
| Fixed effects | count | biomass | biomass | biomass | biomass | % | kg ha ⁻¹ | | |
| | | Bualuo | | | | | | | |
| D | | r value | | | | | | | |
| P amendment | nsŢ | ns | ns | ns | ns | ns | ns | | |
| Cultivar | <0.001 | <0.001 | ns | <0.001 | ns | <0.001 | <0.001 | | |
| Cultivar × P amendment | ns | ns | ns | ns | ns | ns | ns | | |
| Harvest | na‡ | na | na | ns | <0.001 | <0.001 | <0.001 | | |
| P amendment × harvest | na | na | na | ns | ns | ns | ns | | |
| Cultivar × harvest | na | na | na | ns | ns | ns | ns | | |
| P amendment × cultivar × harvest | na | na | na | ns | ns | ns | ns | | |

 \dagger ns = not significant according to an *F*-protected LSD, *P* > 0.0.

‡ na = not applicable.

2014, allowing it to quickly re-establish its leafy biomass, which was maintained until final harvest in October 2014. Annual biomass was least from IAR7/8-5-4-1, Coronet, and IT82E-18 (2585 to 1958 kg ha⁻¹), likely due to low plant populations (IAR7/8-5-4-1, Coronet) and greater weed biomass than cowpea biomass (IAR7/8-5-4-1, Coronet, IT82E-18). Seed size and weight often is attributed to seedling vigor, vegetative growth, and reproductive behavior of cowpea due to larger seeds having greater energy reserves than smaller seeds (Ehoniyotan and Olorunmaiye, 2013). In this case, Iron & Clay is relatively small seeded and Speckled Purple Hull is relatively large seeded, but our data show no particular correlation of seed size and biomass (Table 1).

Yield per plant (harvested biomass divided by stand density) was influenced by cultivar ($P \le 0.001$), but not by P amendment (P = 0.11) or the interaction (P = 0.71). Speckled Purple Hull, IT97K-556-4 and Iron & Clay had the highest yields per plant (35, 31, and 30 g plant⁻¹, respectively), whereas

 $KV \times 396$, IT85F-865, and IAR7/8-5-4-1 were intermediate $(24-28 \text{ g plant}^{-1})$ and the lowest were Coronet and IT82E-18 $(18-20 \text{ g plant}^{-1})$. This result suggests that high total biomass of Iron & Clay and Speckled Purple Hull were due to a combination of high yield per plant and high stand density (Fig. 1). IT97K-556-4 had an equivalent yield per plant to Iron & Clay and Speckled Purple Hull, but total biomass of IT97K-556-4 was limited by a very low stand density. The worst-performing cultivars, Coronet and IT82E-18, were limited by low per plant biomass and low stand density.

The effect of P amendment on total cowpea biomass was not significant (P = 0.16), and if a Type II statistical error was made, higher annual cowpea biomass was observed in unamended plots compared to P-amended plots (3422 vs. 3150 kg ha⁻¹). This result emphasizes the non-responsiveness of the cowpea cultivars to P-amendments in the present study. Sanginga et al. (2000) evaluated cowpea breeding lines under P-amended and unamended environments for performance



Fig. 2. Total annual cowpea and weed biomass as influenced by cultivar, averaged over location. Means indicated by the same letter or no letters are not significantly different according to an F-protected LSD, P > 0.05.

indicators such as dry matter production, N_2 fixation, P-use efficiency, and arbuscular mycorrhizal fungi (AMF) symbioses. They concluded that P-use efficiency varies widely within cowpea germplasm with some cultivars not responding to P amendments even in low-P soils, or even displaying growth suppression in high P soils. The Sanginga et al. (2000) study evaluated 94 cowpea breeding lines, only one of which was included in the present forage study (IT82E-18). Our results suggest that at the low soil P ranges in the Ultisols evaluated in our study (Mehlich 1 P at 5–10 mg P kg⁻¹ soil), these cowpea cultivars are unlikely to respond to P fertilizer application, making cowpea a particularly useful forage crop for similar sites in the southeastern United States with low-P soils.

Weed biomass at each harvest was affected by harvest date $(P \le 0.001)$ (Table 2), but not by P amendment, cultivar, or any interactions (Table 2). The first harvest (1488 kg ha⁻¹) produced more weed biomass than the second harvest (893 kg ha⁻¹). Even though weed biomass differed with harvest date, the annual weed biomass was not affected by cultivar, P amendment, or the interaction. This response was interesting considering that determinate and indeterminate cultivars were evaluated. Weed biomass from P-amended plots (2675 kg ha⁻¹). did not differ from unamended plots (2398 kg ha⁻¹).

Growth habit, determinacy and photosensitivity all play a part in the phenotypic behavior of cowpea. Iron & Clay and IT97K-556-4 are both photosensitive and rely on short daylengths in the late summer and early fall to produce pods, thus they produce only vegetative biomass for the majority of the season and actively regrow that biomass after grazing or harvesting. Cultivars that tendril or display indeterminacy can produce rapidly growing biomass with good ground coverage. Determinate cultivars such as Coronet and IT82E-18 produced less biomass that would be problematic for an organically managed forage system with competitive weed populations. In comparison, Iron & Clay, IT97K-556-4, and Speckled Purple Hull are indeterminate cultivars and provide high biomass and good ground cover, suggesting that indeterminate cultivars might be preferred.

Forage Quality

Cultivar ($P \le 0.01$) affected forage quality for all quality components except lignin (Table 3). There was also a significant main effect of harvest for all quality components, and with the exception of protein, significant interaction effects between harvest and cultivar. There was no significant effect of P amendment nor any interactions of P amendment on forage quality components.

The percentage of protein content differed among cultivars ($P \le 0.001$) with IAR7/8-5-4-1 and KV×396 having the highest protein concentration in biomass at 210 g protein kg⁻¹ of biomass (i.e., 21.0%) and 206 g protein kg⁻¹, respectively (Fig. 3). Coronet had the lowest protein content at 166 g protein kg⁻¹. The first harvest had a greater average percentage of protein 206 g kg⁻¹ than the second harvest 177 g protein kg⁻¹ ($P \le 0.001$). Iron & Clay, Speckled Purple Hull, and IT97K-556-4 had the highest total forage protein production (biomass × protein concentration) at 939, 985, and 729 kg protein ha⁻¹, respectively (Fig. 3). Coronet and IT82E-18 had the lowest protein production at 348 and 444 kg protein ha⁻¹, respectively. The first harvest

| Table 3. Cowpea cultivar and harvest interaction | effects on biomass, tiss | sue P, and forage quality | parameters crude prote | ein, acid detergent |
|---------------------------------------------------|----------------------------|---------------------------|------------------------|---------------------|
| fiber (ADF), neutral detergent fiber (aNDF), lign | in, and total digestible r | nutrients (TDN). | | - |

| Cultivar | Biomass | Pro | tein | ADF | aNDF | Lignin | TDN | | Р |
|----------------------|----------|------------------|--------|--------|--------------------|--------|--------|--------|--------|
| | ——— kg h | ha ⁻¹ | | | g kg ⁻¹ | | | | |
| First harvest | | | | | | | | | |
| Iron and Clay | 2431 | 524 | 200 | 284 | 361 | 30.6 | 702 | 3.15 | 7.8 |
| Speckled Purple Hull | 2424 | 530 | 212 | 263 | 336 | 28.5 | 726 | 3.23 | 7.9 |
| IT97K-556-4 | 1826 | 407 | 207 | 286 | 373 | 30.5 | 700 | 3.23 | 6.0 |
| KV×396 | 1503 | 339 | 215 | 238 | 307 | 27.0 | 754 | 3.20 | 5.0 |
| IT85F-867-5 | 1761 | 374 | 205 | 249 | 315 | 24.7 | 742 | 3.09 | 5.6 |
| IAR7/8-5-4-1 | 1335 | 324 | 216 | 232 | 294 | 27.8 | 761 | 3.25 | 4.6 |
| IT82E-18 | 1170 | 265 | 216 | 249 | 324 | 26.0 | 741 | 3.35 | 4.0 |
| Coronet | 1210 | 227 | 171 | 254 | 321 | 34.8 | 735 | 2.88 | 3.6 |
| Second harvest | | | | | | | | | |
| Iron and Clay | 2229 | 415 | 176 | 258 | 334 | 36.3 | 731 | 2.82 | 6.3 |
| Speckled Purple Hull | 2469 | 454 | 179 | 267 | 344 | 38.2 | 721 | 2.87 | 7.1 |
| IT97K-556-4 | 1730 | 323 | 180 | 270 | 358 | 38.6 | 718 | 2.88 | 5.0 |
| KV×396 | 1802 | 361 | 192 | 256 | 326 | 35.9 | 733 | 3.03 | 5.5 |
| IT85F-867-5 | 1425 | 249 | 165 | 279 | 359 | 38.6 | 707 | 2.74 | 3.9 |
| IAR7/8-5-4-1 | 1269 | 254 | 194 | 251 | 316 | 34.0 | 739 | 3.06 | 3.9 |
| IT82E-18 | 998 | 179 | 180 | 293 | 364 | 39.5 | 691 | 3.03 | 3.0 |
| Coronet | 757 | 120 | 151 | 271 | 340 | 37.5 | 716 | 2.79 | 2.1 |
| $LSD\alpha$ = 0.05 | 596 | 127 | 16 | 21 | 25 | 4.4 | 24 | 0.12 | 1.9 |
| | | P values | | | | | | | |
| Cultivar | <0.05 | <0.001 | <0.001 | <0.01 | <0.001 | ns† | <0.01 | <0.001 | <0.001 |
| Harvest | ns | <0.001 | < 0.00 | <0.01 | <0.01 | <0.001 | <0.01 | <0.001 | <0.01 |
| Cultivar × harvest | ns | ns | ns | <0.001 | <0.01 | <0.01 | <0.001 | <0.01 | ns |

† ns = not significant, P > 0.05. No significant main effects or interaction effects were observed for P amendment, which is omitted from the table.

 $(374 \text{ kg protein ha}^{-1})$ produced 80 kg ha⁻¹ more protein than the average of the second harvest (294 kg protein ha⁻¹).

Acid-detergent fiber and aNDF are used to estimate the digestibility and intake by animals consuming the forage. All cell wall material is represented by aNDF, whereas ADF represents only the lignified or indigestible portions (Amiri and Shariff, 2012; Ball et al., 2007). High ADF and aNDF values are associated negatively with digestibility and voluntary forage intake by the animal, respectively. Cultivar, harvest date, and the interaction between cultivar and harvest date affected ADF and aNDF ($P \le 0.01$; Table 3). IT97K-556-4, IT82E-18, and Iron & Clay had the highest ADF at 278, 271, and 271 g ADF kg⁻¹, respectively, indicating more fiber content and less animal digestibility. Alternately, KV×396 and IAR7/8-5-4-1 had the lowest ADF at 247 and 241 g ADF kg⁻¹, respectively, indicating less fiber content and greater digestibility (Amiri and Shariff, 2012; Ball et al., 2007). The second harvest had greater ADF (268 g ADF kg⁻¹) compared to the first (257 g ADF kg⁻¹). Similarly, IT97K-556-4, Iron & Clay, and IT82E-18 had the highest aNDF at 365, 347, and 344 g aNDF kg⁻¹, respectively ($P \le 0.001$), indicating likelihood of less intake by grazing livestock. In comparison, IAR/8-5-4-1 and KV×396 had the lowest aNDF at 305 and 316 g aNDF kg⁻¹, respectively, indicating likelihood of higher voluntary intake by the grazing animal (Ball et al., 2007; Table 3). As expected, digestibility was highest in earlier maturity stages (Buxton, 1996).

Lignin content was influenced by the interaction between harvest and cultivar ($P \le 0.001$), but not P amendment (Table 3). Lignin content was highest after the second harvest (37.8 vs. 28.7 g lignin kg⁻¹) in all cultivars, a result that is expected, as lignin is more prevalent with increasing plant maturity (Ball et al., 2007; Buxton, 1996; Muir et al., 2008). Muir et al. (2008) in a study with nine warm-season legumes (including Iron & Clay cowpea), reported that the crude protein values decreased from early season to late season in all species. Similarly, Chemey and Chemey (2002) and Ball et al. (2007) state that plant maturity is the primary cause for legume forage quality decline. The cultivar by harvest interaction indicates relative differences of nutritional quality among cultivars and changes in chemical composition of cultivars as related to maturity (Schut et al., 2010). In general, earlier growth stages of these cultivars will provide higher quality forages with less lignin and indigestible fibers.

Total digestible nutrients (TDN) were influenced by the interaction between harvest and cultivar ($P \le 0.001$), but not P amendment (P > 0.05; Table 3). The highest TDN was observed in IAR7/8-5-4-1, KV×396, IT85F-867-5, and IT82E-18 at the first harvest (741–761 g TDN kg⁻¹; Table 3). IT97K-556-4 and Iron & Clay at the first harvest, and IT82E-18 and IT85F-867-5 at the second harvest had the lowest TDN (691–707 g TDN kg⁻¹). Total digestible nutrients are the sum of the digestible fiber, protein, lipid and carbohydrate components of a diet and are calculated from ADF; thus they are directly related to digestible energy making it a useful measurement for forage quality (Rasby and Martin, 2014).

Cowpea tissue P concentration was significantly affected by the interaction of cultivar and harvest ($P \le 0.01$), but not P amendment (P > 0.05; Table 3). The highest tissue P concentration was with IT82E-18 (3.4 g P kg^{-1}), IAR7/8-5-4-1 (3.3 g P kg^{-1}), IT97K-556-4 (3.2 g P kg^{-1}), and Speckled Purple Hull (3.2 g P kg^{-1}), all at the first harvest (Table 3).The lowest tissue P was observed in IT85F-867-5 (2.7 g P kg^{-1}), Coronet (2.8 g P kg^{-1}), and Iron & Clay (2.8 g P kg^{-1}), all at the second harvest. These values are all within or slightly above the reported P sufficiency range for cowpea of 2.3 to 3.0 g P kg^{-1} at early flowering (Godfrey et al., 1959; Sanchez, 2007), suggesting again that P deficiency would be unlikely for any of these cultivars in the environmental conditions of this study. Relatively minor differences in P concentration among cultivars may be related to



Fig. 3. Total protein production and the protein content per variety as influenced by cultivar and averaged over location. Means followed by the same letter or no letters are not statistically different according to an *F*-protected LSD, P > 0.05.

slight differences in cultivar growth stage at harvest given that P concentration in cowpea forage tissue declines with progression to reproductive growth stages (Sanchez, 2007; Godfrey et al., 1959), or physiological differences in P use efficiency, uptake, and accumulation (Sanginga et al., 2000). All cultivars had P content that was similar or higher at the first harvest as compared to the second harvest, which is similar to results reported by Godfrey et al. (1959). On average, the first harvest contained 3.1 g P kg⁻¹ and the second harvest contained 2.9 g P kg⁻¹. There was also no effect of P amendment on total P in cowpea biomass, with 5.7 to 15.0 kg P ha⁻¹ harvested in cowpea biomass over both of the harvests (Table 3).

CONCLUSIONS

Among the eight cultivars tested in the southeastern United States, cultivars Iron & Clay and Speckled Purple Hull produced the greatest biomass over two test sites, suggesting that they offer the greatest potential for forage or cover crop use in regional, organic, and other low-input systems. The two highest yielding cultivars displayed indeterminate growth and were relatively high in protein production (939–985 kg protein ha⁻¹ season⁻¹). Relatively high stand densities were observed from both cultivars, suggesting that they are potentially more resistant to endemic seedling diseases. The impact of seedling diseases, especially by Fusarium spp., on stand density in this study suggests that these diseases may be a limiting factor for organic cowpea production in the region. Cultivars screened in this trial did not respond to P fertilization with steamed bone meal and yet contained sufficient tissue P concentrations, suggesting that cowpea production may be a sustainable option on similar soils with low P availability. High forage quality values of screened cowpea cultivars also suggest that cowpea is well adapted to fill niches in forage crop systems where low summer forage quality limits animal productivity.

ACKNOWLEDGMENTS

Partial funding provided by Tennessee Agricultural Experiment Station supported in part by funds provided by the Hatch Act (USDA-NIFA Hatch project TEN-00412), and University of Tennessee Extension. Technical support provided by Sarah Eichler Inwood, Heather Toler, and staff at the University of Tennessee Organic Crops Unit and Plateau Research and Education Center is gratefully acknowledged.

REFERENCES

- Amiri, F., and A.R.B.M. Shariff. 2012. Comparison of nutritive values of grasses and legume species using forage quality index. Songklanakarin J. Sci. Technol. 34:577–586.
- Ball, D.M., C.S. Hoveland, and G.D. Lacefield. 2007. Southern forages. 4th ed. Potash & Phosphorus Inst. and the Foundation for Agronomic Research. Norcross, GA.
- Butler, D.M., E.N. Rosskopf, N. Kokalis-Burelle, J.P. Albano, J. Muramoto, and C. Shennan. 2012. Exploring warm-season cover crops as carbon sources for anaerobic soil disinfestation (ASD). Plant Soil 355:149–165. doi:10.1007/s11104-011-1088-0
- Buxton, D.R. 1996. Quality-related characteristics of forages as influenced by plant environment and agronomic factors. Anim. Feed Sci. Technol. 59:37–49. doi:10.1016/0377-8401(95)00885-3
- Chemey, J.H., and D.J.R. Chemey. 2002. Legume forage quality. J. Crop Prod. 5(1-2):261–284. doi:10.1300/J144v05n01_11

- Creamer, N.G., and K.R. Baldwin. 2000. An evaluation of summer cover crops for use in vegetable production systems in North Carolina. HortScience 35:600–603.
- D'Angelo, E., J. Crutchfield, and M. Vandiviere. 2001. Rapid, sensitive, microscale determination of phosphate in water and soil. J. Environ. Qual. 30:2206–2209. doi:10.2134/jeq2001.2206
- Ehlers, J.D., R.L. Fery, and A.E. Hall. 2002a. Cowpea breeding in the USA: New cultivars and improved germplasm. In: C.A. Fatokun et al., editors, Challenges and opportunities for enhancing sustainable cowpea production. Int. Inst. of Tropical Agric., Ibadan, Nigeria. p. 62–77.
- Ehlers, J., and A.E. Hall. 1997. Cowpea (*Vigna unguiculata* L. Walp.). Field Crops Res. 53:187–204. doi:10.1016/ S0378-4290(97)00031-2
- Ehlers, J.D., W.C. Matthews, A.E. Hall, and P.A. Roberts. 2002b. Breeding and evaluation of cowpeas with high levels of broadbased resistance to root-knot nematodes. In: C.A. Fatokun et al., editors, Challenges and opportunities for enhancing sustainable cowpea production. Int. Inst. of Tropical Agric., Ibadan, Nigeria. p. 41–51.
- Ehoniyotan, O.I., and K.S. Olorunmaiye. 2013. Seed size influence on germination and seedling development of cowpea (*Vigna* unguiculata (L) Walp). Albanian J. Agric. Sci. 12:389–395.
- Godfrey, C.L., F.L. Fisher, and M.J. Norris. 1959. A comparison of ammonium metaphosphate and ammonium orthophosphate with superphosphate on the yield and chemical composition of crops grown under field conditions. Soil Sci. Soc. Proc. 23:43–46. doi:10.2136/sssaj1959.03615995002300010020x
- Hall, A.E., A.M. Ismail, J.D. Ehlers, K.O. Marfo, N. Cisse, S. Thiaw, and T.J. Close. 2002. Breeding cowpea for tolerance to temperature extremes and adaptation to drought. In: C.A. Fatokun et al., editors, Challenges and opportunities for enhancing sustainable cowpea production. Int. Inst. of Tropical Agric., Ibadan, Nigeria. p. 14–21.
- Harrison, H.F., J.A. Thies, R.L. Fery, and J.P. Smith. 2006. Evaluation of cowpea genotypes for use as a cover crop. HortScience 48:1145–1148.
- Hayes, H.K., and R.J. Garber. 1921. Breeding crop plants. McGraw-Hill, New York. p. 145.
- Hwang, S.F., B.D. Gossen, R.L. Conner, K.F. Chang, G.D. Turnbull, K. Lopetinsky, and R.J. Howard. 2007. Management strategies to reduce losses caused by Rhizoctonia seedling blight of field pea. Can. J. Plant Sci. 87:145–155. doi:10.4141/P04-172
- Jordan, C.F. 2004. Organic farming and agroforestry: Alleycropping for mulch production for organic farms of southeastern United States. Agrofor. Syst. 61:79–90.
- Khandaker, Z.H. 1994. Effect of mixed cropping of maize (*Zea mays*) and cowpea (*Vigna unguiculata*) forage on fodder yield, chemical composition and its in-vitro digestibility. Indian J. Anim. Nutr. 11:55–57.
- Klock, K.A., and H.G. Taber. 1996. Comparison of bone products for phosphorus availability. Horttechnology 6:257–260.
- Lammerts van Bueren, E.T., S.S. Jones, L. Tamm, K.M. Murphy, J.R. Myers, C. Leifert, and M.M. Messmer. 2011. The need to breed crop varieties suitable for organic farming, using wheat, tomato and broccoli as examples: A review. NJAS Wagening. J. Life Sci. 58:193–205. doi:10.1016/j.njas.2010.04.001
- Mehlich, A. 1953. Determination of P, Ca, Mg, K, Na, and NH₄. North Carolina Soil Testing Div., North Carolina State Univ., Raleigh.
- Morse, W.J. 1920. Cowpeas: Culture and varieties. Farmers' Bull. 1148. USDA Bureau of Plant Industry, Washington, DC.
- Muir, J.P., T.J. Butler, R.M. Wolfe, and J.R. Bow. 2008. Harvest techniques change annual warm-season legume forage yield and nutritive value. Agron. J. 100:765–770. doi:10.2134/ agronj2007.0042

Murray, I., and I. Cowe. 2004. Sample preparation. In: C.A. Roberts et al, editors, Near-Infrared spectroscopy in agriculture. ASA, CSSA, and SSSA, Madison, WI. p. 75–112.

Nielsen, H.T. 1916. Cowpeas. Farmers' Bull. 318. USDA Bureau of Plant Industry, Washington, DC.

Piper, C.V. 1912. Agricultural varieties of the cowpea and immediately related species. Bull. 229. USDA Bureau of Plant Industry, Washington, DC.

Rao, S., and B. Northup. 2009. Capabilities of four novel warmseason legumes in the southern Great Plains: Biomass and forage quality. Crop Sci. 49:1096–1102. doi:10.2135/ cropsci2008.08.0499

Rasby, R., and J. Martin. 2014. Understanding feed analysis. Univ. of Nebraska Lincoln Ext., Univ. of Nebraska, Lincoln. https://beef. unl.edu/learning/feedanalysis.html (accessed 30 Apr. 2015).

Sanchez, C.A. 2007. Phosphorus. In: A.V. Barker and D.J. Pilbeam, editors, Handbook of plant nutrition. Taylor & Francis, Boca Raton, FL. p. 51–90.

Sanginga, N., O. Lyasse, and B.B. Singh. 2000. Phosphorus use efficiency and nitrogen balance of cowpea breeding lines in a low P soil of the derived savanna zone in West Africa. Plant Soil 220:119–128. doi:10.1023/A:1004785720047

Schut, A., S. Gherardi, and D. Wood. 2010. Empirical models to quantify the nutritive characteristics of annual pastures in south-west Western Australia. Crop Pasture Sci. 61:32–43. doi:10.1071/CP08438 Shrestha, U., B.H. Ownley, and D.M. Butler. 2016a. First report of dry root and stem rot of cowpea caused by *Fusarium proliferatum* in the United States. Plant Dis. 100:860. doi:10.1094/ PDIS-09-15-1103-PDN

Shrestha, U., B.H. Ownley, and D.M. Butler. 2016b. First report of stem and root rot of cowpea caused by *Fusarium oxysporum* in Tennessee. Plant Dis. 100:649. doi:10.1094/ PDIS-08-15-0934-PDN

Sims, G.K. 2006. Letter to the Editor on "Using the Berthelot Method for Nitrite and Nitrate Analysis.". Soil Sci. Soc. Am. J. 70:1038. doi:10.2136/sssaj2005.0408l

Sims, G.K., T.R. Ellsworth, and R.L. Mulvaney. 1995. Microscale determination of inorganic nitrogen in water and soil extracts. Commun. Soil Sci. Plant Anal. 26:303–316. doi:10.1080/00103629509369298

Singh, B.B., O.L. Chambliss, and B. Sharma. 1997. Recent advances in cowpea breeding. In: B.B. Singh et al., editors, Advances in cowpea research. IITA, Ibadan, Nigeria. p. 30–49.

Wang, G., J.D. Ehlers, E.J. Ogbuchiekwe, S. Yang, and M.E. McGiffen. 2004. Competitiveness of erect, semierect, and prostrate cowpea genotypes with sunflower (*Helianthus annuus*) and purslane (*Portulaca oleracea*). Weed Sci. 52:815–820. doi:10.1614/WS-03-148R

Wang, G., M.E. McGiffen, J.D. Ehlers, and E.C.S. Marchi. 2006. Competitive ability of cowpea genotypes with different growth habit. Weed Sci. 54:775–782. doi:10.1614/WS-06-011R.1