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Cowpea Adaptability to Southeastern Organic Farming Systems: Forage Productivity and Charcoal Rot Susceptibility

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To the Graduate Council:

I am submitting herewith a thesis written by Samantha Lindsey Hill entitled "Cowpea Adaptability to Southeastern Organic Farming Systems: Forage Productivity and Charcoal Rot Susceptibility." I have examined the final electronic copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Plant Sciences.

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Cowpea Adaptability to Southeastern Organic Farming Systems:

Forage Productivity and Charcoal Rot Susceptibility

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Abstract

Cowpea (*Vigna unguiculata* (L.) Walp.) is a warm-season, multi-purpose legume that is well-adapted to the southeastern USA and has many traits that make it an attractive forage or cover crop for integration into organic production systems, including high rates of nitrogen (N) fixation, phosphorus (P) use efficiency, regrowth ability, and high digestibility. Eight cowpea cultivars were evaluated under organic management at two locations in summer 2014 for stand establishment, forage yield and quality, and weed biomass.

Charcoal rot [*Macrophomina phaseolina* (Tassi) Goid.] is a fungal disease that is economically important to many host plant species. High temperatures and drought conditions favor disease development making it difficult to predict when disease outbreak will occur. Cowpea (*Vigna unguiculata* L. Walp.) is an important crop for many regions of the globe and is a host species for *M. phaseolina*. Efforts have been made to breed genetic lines that are resistant to *M. phaseolina* but little research has been done to screen many popular cowpea cultivars for resistance. This study includes an inoculated field trial and greenhouse seedling screening of twenty-six cowpea lines to identify resistance to charcoal rot.

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Introduction

Cowpea (*Vigna unguiculata* (L.) Walp.) is a warm-season, multi-purpose annual legume that is well-adapted to the southeastern USA and has many traits that make it an attractive forage, grain or cover crop for integration into organic production systems, including high rates of nitrogen (N) fixation, phosphorus (P) use efficiency, regrowth ability, and high digestibility (Singh *et al*., 1997). Cowpea is native to sub-Saharan Africa where it is commonly grown as a dual-purpose crop for both grain and forage production (Boe et al. 1991). Cowpea has the ability to improve soil structure with its deep roots and to decrease erosion based on rapid growth and soil coverage (Clark, 2007). When properly inoculated cowpea produces large rhizobial root nodules fixing between 145 and 224 kg N ha^{-1} that can potentially be used by intercrops and subsequent cool-season forages (Clark, 2007; Creamer et al. 2000; Khandaker, 1994). Cowpea is also a rapidly maturing crop with the earliest flowering at 48 days and maturing by 60 days (Clark, 2007). This makes it ideal for organic systems that may need quick forage production, a quick legume grain crop, or soil improvement between major crops.

Organic (and conventional) agricultural production in the southeastern US is often hindered during the summer months by anomalous weather patterns including extreme heat, high humidity, and periods of drought that can be difficult to predict (Li *et al*, 2011; Wang *et al*, 2010). In addition to increased plant stress and evapotranspiration during these periods, soil temperatures rise and water availability becomes limited which reduces nutrient mineralization by soil biota (Collins *et al*., 1990); as such, low-input organic crops can suffer from nutrient deficiencies that affect growth and reproduction (Van Bueren et al., 2011). This is also the period during which perennial forages and grasses decline in productivity, which can be problematic considering that the USDA-organic certification framework requires that for ruminant livestock production that the livestock to graze for the entire grazing season, no less than 120 days, and that livestock obtain at least 30% of their dry matter intake from grazing (USDA-AMS, 2015).

Literature Review

Cowpea is traditionally cultivated in semiarid West Africa where it is at home in dry, sandy soils with infrequent rainfall. Considered one of the most important food crops in this region, it provides local people with both food and forage for livestock (Quin, 1997). The grain is valued for its nutritive quality and serves as a major source of protein in rural areas; the leaves and green pods are also consumed as a leafy vegetable (Singh *et al*., 1997). It is commonly intercropped with warm-season grains such as sorghum (*Sorghum bicolor*) and pearl millet *(Pennisetum glaucum)* where is sustains agricultural production through the hot, dry growing season (Singh and Tarawali, 1997). It is particularly useful as a N-fixing legume in this area as the soils are generally nutrient poor and need inexpensive carbon and nutrient inputs (Singh *et al* 1997).

Cowpea fixes N_2 through symbiotic rhizobial bacteria, which improves soil fertility, and could potentially make N available for succeeding crops that may be used in an organic cropping system. Dwivedi *et al* (2002) studied the relationship of soil N uptake between cowpea in rotation with rice (*Oryza sativa*) and wheat (*Triticum spp.*) in India. They hypothesized that cowpea may benefit wheat crops by increasing available organic carbon through root decomposition and cowpea root decomposition may favor wheat root growth by allowing wheat roots to penetrate into deeper soil layers allowing them access available soil N. In addition cowpea was also effective in minimizing nutrient leaching when combine with cereal crops in rotation and appropriate fertilizer applications (Dwivedi *et al*., 2002).

Organic cropping systems are often limited by N availability, but in highly weathered soils, P-availability can also be an issue due to its low solubility (Krasilnikoff *et al*, 2003). Cowpea has the ability to make inorganic soil P available to the organic pool due to various mechanisms in the soil-root interface (Krasilnikoff *et al*, 2003). Root-hair exudates secrete organic acids and phosphatase in to the rhizoshpere allowing for increased crop nutrient uptake due to beneficial mycorrhizal colonization (Krasilnikoff *et al*., 2003; Sanginga *et al*., 1999). However, cowpea genotypes are quite different in their ability to access soil P pools due to differences in root growth habit and genotypic differences in soil P uptake ability (Krasilnikoff *et al*., 2003; Sangina *et al*., 1999).

Weed suppression

Because cowpea cultivars have many phenotypic characteristics, they can fit an array of ecological niches. Cowpea is commonly intercropped with cereals such as sorghum and pearl millet (Olufajo et al. 2002). Several studies show that cowpea not only performs well in intercropping systems, but may also perform better when intercropped with sorghum-sudangrass when compared to monoculture (Creamer et al, 2000; Olufajo et al, 2002). Sorghum-sudangrass (*Sorghum bicolor x*) often has low seed costs and is also effective at suppressing weeds. Creamer et al. (2000) found that intercropped sorghum-sudan grass and cowpea were especially efficient at weed suppression due to the combined crops' high biomass and high biomass N. Nelson & Robichaux (1997) observed that shorter, bushier cultivars, such as 'California Blackeye 46', may not be suitable for intercropping due to being shaded by sorghum's height. Thus a legume-grass mixture between cowpea and sorghum-sudangrass may provide sufficient weed suppression, biomass and nutritional content to provide low-cost, low-input organic forage, but cultivar selection is crucial when considering production objectives.

Alder & Chase (2007) expanded the view of cowpea's weed suppression abilities by evaluating its allelopathic potential. Aqueous foliar extracts of cowpea at differing levels (5% and 10%) consistently reduced seed germination in both goosegrass (*Eleusine indica*) and livid amaranth (*Amaranthus lividus*). This suggests that cowpea is an aggressive competitor both physically and chemically and can effectively contribute to minimizing herbicide input for organic agriculture. Due to its wide phenotype diversity, cowpea cultivars require selection based on specific production system traits and objectives.

Insect resistance

Some common insects that affect global cowpea production in the field are Mexican bean beetles (*Epilachna varivestis*), bean leaf beetles (*Cerotoma trifucata*), cowpea curculios (*Chalcodermus aeneus*), grasshoppers, aphids, green stink bugs, lesser cornstalk borers, and weevils (during seed storage) (Sheahan, 2012). Maruca pod borer and pod bugs infect cowpea pods and cause significant damage and yield reduction (Singh, 1997). Cowpea is most susceptible to insect infestation during seedling stages.

Efforts have been made to cross-breed *Vigna* wild-type species with commercial cowpea but species compatibility was low and no viable progeny were produced (Fatokun, 2002). Developing insect resistant cultivars of cowpea has proven challenging due to the variety of insects present in different regions. It is important to choose insect resistant lines that are regionally specific especially for low-input systems that cannot rely on pesticide application during infestation.

Disease resistance

Fusarium spp. is of particular interest to cowpea breeders because genetic resistance to the fungus is a simple inheritance of one or two gene pairs and is relatively simple to incorporate into conventional breeding programs (Singh *et al.,* 1997). The parent breeding line 'Iron,' of Iron & Clay, is a known carrier for the gene that is resistant to both *Fusarium spp.* and *Macrophomina phaseolina* and is used extensively by breeders as a source of resistance to both (Singh *et al.,* 1997).

Macrophomina phaseolina (Tassi) Goid. is the causal fungal agent of charcoal rot—a soil- borne pathogen that causes economically important yield losses of over 500 different host plant species globally (Afouda *et al.*, 2008; Pearson *et al.,* 1984; Su *et al.,* 2000; You *et al.,* 2011). It is common in subtropical and tropical countries with a semiarid climate and is severe in arid regions that often have sustained drought periods (You *et al.*, 2011). Drought stress causes negative effects to host plant physiology, weakening plant tissues and predisposing crops to infectious facultative parasites such as *M. phaseolina* (Mayek-Perez *et al.*, 2002). This fungus survives in the soil as sclerotia embedded in organic debris or free in soil and can persist due to the high number of species in its host range (Abawi and Pastor-Corrales, 1988; Songa *et al.*, 1997). There is a strong association between the occurrence of drought and susceptibility to *M. phaseolina* (You *et al*., 2011). Host crops show a variety of disease symptoms that can coincide with any stage of development. In seedlings, *M. phaseolina* can cause pre- or post-emergent damping off, black cotyledonary lesions at varying degrees of severity or it can persist in a crop showing little to no disease symptoms. In soybean (*Glycine max* (L.) Merr.), aboveground symptoms are typically not apparent until after flowering and reproductive growth has occurred

(R5-R7) (Fehr *et al.,* 1971; Mengistu *et al.,* 2007); while in common bean (*Phaseolus vulgaris* L.) damage is mainly significant in the early stages of development (Mayek-Perez *et al.,* 2002). Afouda *et al*. (2008) states that many stress factors are involved in the development of *M. phaseolina* including plant age, high temperatures, and drought stress. Collins *et al*. (1990) showed that water hindered microsclerotial growth and development of *M. phaseolina* by limiting the exchange of O_2 and CO_2 where microbiological activity was occurring. One genetic mechanism that could be involved in charcoal rot development is the ability of the cultivar to maintain internal water turgor pressure during water stress (Mayek-Perez *et al*., 2002). Drought stress causes plant tissues to weaken and allows space for microsclerotia to infect the internal plant structure blocking xylem vessels and causing plants to wilt (You *et al*., 2011; Mayek-Perez *et al*., 2002). Mayek-Perez *et al*. (2002) studied the mechanisms involved in common bean resistance to *M. phaseolina* and concluded those cultivars that showed higher water and turgor potentials were more resistant to *M. phaseolina* than susceptible cultivars; thus, cultivars that are resistant to drought stress may also be resistant to root rot pathogens and vice versa.

Drought Tolerance

The impacts of increased climate variability through climate change portend additional challenges for forage crop production. It is likely that plant production in dry regions will experience increased losses even beyond those that are currently estimated (Wang *et al*., 2010). Plant characteristics such as high water use efficiency, caused by stomatal closure and greater root densities under elevated CO_2, may alleviate some drought pressures (Tubiello et al, 2007). Thus, species that are capable of producing forage under adverse conditions warrant consideration (Boe et al, 1991). Cowpea cultivars have a vast array of phenotypes, some which are photoperiod sensitive and if planted during long days would continue to produce new leaves and flowers after drought episodes had past (Anyia & Herzog, 2004; Foster et al, 2009). This is an important characteristic for forage situations in particular; cultivars that cannot recover from grazing or haying practices will not suitable for this system.

Mai-Kodomi et al. (1999) distinguishes two types of drought tolerant cowpea. Type 1 drought tolerant lines discontinued growth after the onset of drought stress and displayed declining turgidity in all tissues. The unifoliates, emerging trifoliates and epicotyl gradually dried at the same time. Type 2 cultivars remained green for longer and continued trifoliate growth even after the onset of drought stress. In a more recent study, Verbree et al (2014) showed that these Type 2 drought-tolerant lines that maintain trifoliate growth are a better indicator of tolerance during stress. Forages that are chosen for drought periods must remain subsistent in order to maintain quality forage and nutrition throughout the season.

Nutrition

Cowpea grain and forage biomass contain a dense nutritional profile that is beneficial for livestock. The grain contains between 22% and 32% protein on a dry weight basis (Fatokun, 2002; Panella et al, 1993). Fodder haulms are often fed to forage cattle in rural parts of the world as a nutritious supplement. Legumes supply ruminants with fermentable nitrogen, other nutrients for the rumen microbes, readily fermentable carbohydrates and bypass protein (Khandakar 1994). It is a major bioavailable source of micronutrients such as zinc and iron (Ojwang et al, 2012). A varietal nutrient test conducted by Singh (1999) showed that on a fresh weight basis (about 10% moisture), the protein content ranged from 20 to 26%, fat content from 0.36% to 3.34%, iron content from 56 ppm to 95.8 ppm, and manganese content from 5 ppm to 18 ppm

(Singh *et al*., 2002). The grain also contains flavonoids, which are important for their antioxidant and anti-inflammatory properties. Ojwang et al (2012) showed that seed coat color had a major influence on flavonol composition. The average mean for flavonol content was highest in the red phenotype (970 µg/g) and lowest in white phenotypes (270 µg/g). Thus, defining characteristics such as seed coat color may be a useful indicator of greater nutritional value in cowpeas.

Forage quality is defined as the capacity of forage to provide the required nutrients to livestock (Amiri *et al.,* 2012). Near infrared reflectance spectroscopy (NIRS) is used to determine forage nutritive value quickly and accurately (Norris *et al.,* 1976). The data produced by NIRS is a list of forage parameters and measurements. Crude protein (CP) is considered one of the most important qualities of forages. Pinkerton and Cross (1991) describe crude protein as a good indicator for high forage quality as high protein diets are essential for beef and dairy cattle to gain weight and produce milk. However, crude protein cannot be the sole predictor for high quality forages because of the limiting nutrient concept. Put simply, any excess of protein will not increase animal performance if there is another energy nutrient that is deficient in the diet. Thus, other energy and digestibility measurements are also analyzed to gain a complete profile of the forage at hand. As a negative performance indicator, fiber measures of ADF and aNDF were used to determine the digestibility of the cultivars. Neutral detergent fiber (aNDF) represents all cell wall material, while acid-detergent fiber (ADF) represents only the lignified or indigestible portions (Amiri *et al.,* 2012, Ball *et al.,* 2007). High ADF and NDF values are negatively correlated with digestibility and voluntary forage intake by the animal, respectively (Ball *et al.,* 2007). Dry matter digestibility (DMD) is a percentage measure representing the digestible portion of the sample. It is also measured from the level of ADF present in the sample; as a consequence, DMD decreases with increasing lignin (Amiri *et al.,* 2012, Ball *et al.,* 2007). Plant cell walls become lignified at later maturity stages thus reducing the overall forage quality from the beginning to the end of the growing season (Pinkerton and Cross, 1991).

Energy is the other major indicator for forage quality. Total digestible nutrients (TDN) are the sum of the digestible fiber, protein, lipid and carbohydrate components of a diet. TDN is calculated from ADF and is thus directly related to digestible energy making it a useful measurement for forage rations (Rasby, 2014). The net energy system can be broken down into several measurements: The net energy for lactation (NEL) is a measure of the amount of feed energy available for maintenance and milk production after digestive and metabolic losses. It is inversely related to ADF. The net energy for maintenance (NEM) is the energy needed for breathing, walking, and performing everyday functions. The net energy for growth or gain (NEG) is the amount of feed energy needed for muscle and bone production. (Belyea *et al.,* 1999; Encinias, 2000; Rasby, 2014). The estimated net energy (ENE) accounts not only for the amount of digestible nutrients in a feed or forage but also for the amount of energy which is wasted by the livestock and not used for productive purposes, i.e. heat loss (West, 2003).

Other forage quality parameters can be used to get a more vivid profile of each cultivar. Sugars in the form of water-soluble carbohydrates (WSC) include glucose, fructose, sucrose and fructans (Suzuki, 1993). These sugars are accumulated and stored in the stem to be later used for grain filling (Ritchie *et al.,* 2003). Greater carb storage in stems means improved grain filling and increased grain yields, and is often an indicator of the plant transitioning nutrient allocations from vegetative to reproductive growth (Huijser and Schmid, 2011). Minerals and vitamins play specific roles in forage animals. Calcium (Ca), Phosphorus (P), Potassium (K) and Magnesium (Mg) are minerals used in skeletal development and maintenance, nervous system function, lactation and also aide in biological energy production (Rasby *et al.,* 2011).

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Chapter 1

Cowpea biomass productivity under organic management in the southeastern USA as influenced by cultivar and phosphorus amendment

Cowpea biomass productivity under organic management in the southeastern USA as influenced by cultivar and phosphorus amendment

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Abstract

Cowpea (*Vigna unguiculata* (L.) Walp.) is a warm-season, multi-purpose legume that is well-adapted to the southeastern USA, having many traits that make it an attractive forage or cover crop for integration into organic production systems, including high rates of nitrogen (N) fixation, phosphorus (P) use efficiency, regrowth ability, and high digestibility. Eight cowpea cultivars were evaluated under organic management at two locations in summer 2014 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 cultivar responsiveness to P fertilization in soils of low native soil P status (Mehlich-1 P < 10 mg P kg^{-1}). Cowpea was seeded at 209,000 seeds ha^{-1}, managed organically, and biomass harvested twice during the growing season. Stand density four weeks after planting indicated the highest plant populations from 'Iron & Clay' (166,000 plants/ha), intermediate populations from 'Speckled Purplehull', 'IT82E-18' and 'IT85-867-5F' (143,000 to 138,000 plant/ha) and lowest populations from 'IAR7/8-5-4-1', 'Coronet', 'KVx396', and 'IT97K-556-4' (128,000 to 118,000 plants/ha) likely due to presence of seedling diseases caused by *Fusarium* spp. Speckled Purplehull and Iron & Clay had the highest total yield over both seasons (4922 and 4623 kg ha^{-1}, respectively). Annual biomass was least from IT82E-18, Coronet and IAR7/8-5-4-1 (1958 to 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 ($p = 0.16$) between plots unamended with soil P and P-amended plots (3422 vs. 3150 kg ha^{-1}), or differences in cowpea P-uptake. Annual weed biomass likewise did not differ ($p = 0.26$) between plots unamended with soil P (2398 kg ha^{-1}) and P-amended plots (2398 kg ha^{-1} vs. 2675 kg ha^{-1}). In general, harvest date, cultivar and the interaction between harvest date and cultivar significantly affected forage quality ($p < 0.05$). Speckled Purplehull was the only cultivar that was similar to Iron & Clay in both biomass production and indicators of forage quality. Results suggest that cultivar choice is an important consideration given wide variability in cultivar biomass production, forage nutritive quality and likely differences in seedling disease susceptibility.

Abbreviations: NIRS, near infrared reflectance spectroscopy; CP, crude protein; ADF, acid detergent fiber; NDF, neutral detergent fiber; TDN total digestible nutrients, DMD, dry matter digestibility; WSC, water soluble carbohydrate; ENE, estimated net energy; NEL, net energy for lactation; NEM, net energy for maintenance; NEG, net energy for gain

Introduction

Organic cropping systems in the southeastern US can be limited by low soil N, weed pressure, insect and disease pressure, lack of commercially-available adapted cultivars for organic systems, and the highly weathered, low organic matter soils common in the region. In systems that integrate organic livestock production at the farm scale 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 are often elevated and soil water potential is often reduced which increases plant stress and can reduce nutrient mineralization by soil biota. This is also the period during which cool-season perennial grasses decline in productivity and quality (Rao and Northup, 2009).

Integrating cowpea (*Vigna unguiculata* (L.) Walp.) into existing organic crop rotations can help address many of these issues. As a warm-season legume native to sub-Saharan Africa, cowpeas are drought and heat tolerant and fix N, making them a promising summer crop for organic production systems in the region (Ehlers and Hall, 1997). They require few inputs and can enhance or maintain soil fertility through N fixation and efficient uptake of poorly soluble soil P (Sangina *et al.,* 2000). In association with *Bradyrhizobium* spp., they produce large rhizobial root nodules fixing between 145 kg and 224 kg N ha^{-1} that can be used by intercrops and subsequent cool-season forages (Clark, 2008; Creamer et al., 2000; Khandaker, 1994).

Cowpea is known by many common names including southern field pea, crowder pea, cream pea, zipper pea, purple hulls, pink eyes, and black-eyed pea. These common names refer to different market classes of the same species and the lack of consistent common name recognition has impeded the development and adoption of new cultivars. The high degree of genetic diversity in cowpea has caused further confusion. Great varietal diversity exists with cultivars targeted for fresh vegetable, dry grain, forage, and/or cover crop use, but there is little research or guidance for growers on varietal selection or how to integrate cowpeas into organic forage production systems. Many of the cultivars tested in this study historically served dual purposes as grain or forage producers. For example, the widely grown ‘pink-eye’ cultivar Coronet is often grown for grain production, but is an erect cultivar that produces tendrils (Hall *et al.,* 2003). Cultivars differ in growth habit and phenotypic attributes such as seed size, seed coat color, pod color and flower color, photosensitivity, determinacy, and nutritional value (Ehlers *et al.,* 1997). Growth habits range from erect, semi-erect, semi-prostrate or prostrate and determinate, bushy growth to indeterminate, tendriling growth. Whereas cowpea demonstrating an erect, determinate growth habit will likely be more suitable for mechanical harvest of dry grain; prostrate, indeterminate cowpea cultivars could be more valuable as forages or cover crops where maximum ground cover and biomass accumulation are essential functional traits (Harrison *et al.,* 2006).

Photosensitivity plays a part in regional cowpea adaptability in that many cultivars are short-day photosensitive. These cultivars are late maturing in the United States and often don't produce pods until very late in the growing season. Photoperiod sensitive cultivars have the potential to produce much more biomass if planted during longer day-lengths due to the extended duration of the vegetative stage preventing early transition into reproductive growth (Ehlers *et al.,* 2002a, Hall *et al.,* 2003). The photosensitive cultivar Iron & Clay produces a large amount of biomass throughout the season and has rapid regrowth ability given that nutrient allocation for grain production is delayed until daylight hours are significantly shorter in the fall. Iron & Clay is widely marketed as a cover crop and forage for being resistant to root-knot nematodes (*Meloidogyne* spp.; Ehlers *et al.,* 2002b, Hall *et al.,* 2003), highly competitive with various weed species (Wang *et al.,* 2004), and is often the standard cultivar used extensively in cover crop research (Harrison *et al.,* 2006). Iron & Clay serves as a control cultivar in this study as it is perhaps the only widely-available forage cowpea cultivar in the southeastern USA.

Pinkerton and Cross (1991) describe crude protein as a good indicator for high forage quality as high protein diets are essential for beef and dairy cattle to gain weight and produce milk. However, crude protein cannot be the sole predictor for high quality forages because adequate protein will not increase animal performance if other nutrients are limiting. As a negative performance indicator, fiber measures of acid-detergent fiber (ADF) and neutral detergent fiber (NDF) are used to estimate the digestibility and intake of animals consuming the forage. All cell wall material is represented by NDF, while ADF represents only the lignified or indigestible portions (Amiri *et al.,* 2012, Ball *et al.,* 2007). High ADF and NDF values are negatively associated with digestibility and voluntary forage intake by the animal, respectively (Ball *et al.,* 2007). Plant cell walls typically become more lignified at later maturity stages, thus reducing the overall forage quality from the beginning to the end of the growing season (Pinkerton and Cross, 1991).

Total digestible nutrients (TDN) is the sum of the digestible fiber, protein, lipid and carbohydrate components of a diet. Total digestible nutrients are calculated from ADF and is thus directly related to digestible energy making it a useful measurement for forage rations (Rasby, 2014). The net energy system can be broken down into several measurements: The net energy for lactation (NEL) is a measure of the amount of feed energy available for maintenance and milk production after digestive and metabolic losses. It is inversely related to ADF. The net energy for maintenance (NEM) is the energy needed for breathing, walking, and performing everyday functions. The net energy for growth or gain (NEG) is the amount of feed energy needed for muscle and bone production (Belyea *et al.,* 1999; Encinias, 2000; Rasby, 2014). The estimated net energy (ENE) accounts not only for the amount of digestible nutrients in a feed or forage but also for the amount of energy that is wasted by the livestock (i.e. heat loss) and not used for productive purposes (West, 2003).

Sugars in the form of water-soluble carbohydrates (WSC) include glucose, fructose, sucrose and fructans (Suzuki, 1993). These sugars are accumulated and stored in the stem to be later used for grain filling (Ritchie *et al.,* 2003). Greater carbohydrate storage in stems may improve grain filling and increase grain yields, and is often an indicator of the plant transitioning nutrient allocations from vegetative to reproductive growth (Huijser and Schmid, 2011). Minerals and vitamins play specific roles in forage animals. Ca, P, K and Mg are minerals used in skeletal development and maintenance, nervous system function, lactation and also aide in biological energy production (Rasby *et al.,* 2011).

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

Materials and Methods

In May 2014 a randomized complete block design with a strip plot was established in two locations at the Organic Crops Unit of the East Tennessee Agricultural Research and Education Center in Knoxville, TN, USA (OCU) and the University of Tennessee Plateau Research and Education Center (PREC) in Crossville, TN. 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 were established each containing two main plots (17.1 by 7.6-m) randomly assigned as either amended or unamended with P. Within each block, eight subplots 2.1-m wide were randomly assigned to one of eight cowpea cultivars, creating a strip-plot design with 2.1-m 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, USA;

PREC, Hege Maschinen, Waldenburg, Germany) at a seeding rate of 209,000 seeds ha^{-1} to the entire plot length (7.6-m). Cowpea cultivars included were: Iron & Clay, IT97K-556-4, KVx396, IT85F-867-5, IT82E-18, Speckled Purple Hull, IAR7/8-5-4-1, and Coronet (Table 2). Due to limited seed availability, cultivar germination was assessed by planting 3 replicates of 10 seeds in 10 cm pots filled with moist sand. All cultivars were confirmed to germinate at a rate of 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 US (Iron & Clay), observed indeterminate habit and high biomass in preliminary trials (Speckled Purplehull 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 untreated and were sourced from seed produced in preliminary trials at the University of Tennessee. Cowpea seed was inoculated with N-Dure *Bradyrhizobium* sp. (Vigna) inoculum (INTX Microbials, Kentland, IN, USA) immediately prior to seeding. Planting dates were May 23, 2014 at OCU and June 4, 2014 at PREC.

Soils at the OCU and PREC were both sampled in the fall of 2013 to confirm low native soil P status (Mehlich-1 P < 10 mg P kg^{-1}). At the OCU, winter cover crops of triticale (*xTriticosecale* 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, winter wheat (*Triticum aestivum*) was mowed and incorporated with a disk. Bone meal was applied in P-amended plots at both sites at a rate of 44.8 kg P ha^{-1} and amended by hand broadcasting throughout main plots. At the PREC location, previous season soil tests indicated low soil K and the entire site was amended at a rate of 74 kg K ha^{-1}(KCl). 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, USA; PREC, CR3000 datalogger, Campbell Scientific, Logan, UT, USA).

Stand counts were recorded on June 20, 2014 at the OCU and June 25 at PREC by counting every germinated, 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 August 15, 2014 and again on October 2, 2015 and August 13, 2014 and September 24, 2014 at PREC. Cowpeas were at early bloom (R1) to early pod filling (R3) prior to the first harvest and regrew to seeding stages (R5 to R6) 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 were cut to 2 cm above the soil surface and collected for cowpea quality analyses. Weed biomass was sampled from three, 0.25-m^2 areas (2-cm above the soil surface) to assess total weed dry matter. A 5.8-m^2 (7.6-m x 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 flail-type forage harvester (OCU, ALMACO, Nevada, IA, USA or Swift Machine and Welding Ltd., Swift Current, SK, Canada; PREC, Carter Manufacturing Company Inc., Brookston, IN, USA). 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 hours) and weighed to determine bulk forage moisture content. Samples of cowpea for quality analyses and weed biomass samples were similarly oven-dried and then weighed. Cowpea samples for forage quality were ground in a lab grinder (Thomas Model 4 Wiley Mill, Thomas Scientific, Swedesboro, NJ, USA) through a 1-mm sieve. Cowpeas were ground as the entire plant including stems, leaves and pods, if present. The grinder was thoroughly cleaned between samples to avoid sample cross-contamination.

Forage samples were analyzed using near-infrared spectroscopy (NIRS) using a "mixed legume" calibration equation typically used for forage soybeans (Foss 6500, Eden Prairie, MN, USA). Parameters analyzed included: total protein, acid detergent fiber (ADF), neutral detergent fiber (NDF), minerals (Ca, Mg, P, K), lignin, water soluble carbohydrates (WSC), total digestible nutrients (TDN), estimated net energy (ENE), net energy for lactation (NEL), net energy for maintenance (NEM) and net energy for gain (NEG).

Three soil cores (1.75-cm internal diameter) were sampled a depth of 0 to 15-cm from each plot on June 25 and October 15, 2014 at OCU and on June 26 and October 16, 2014 at PREC. Samples were taken several weeks after applying P amendments and at the end of the study just 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 was placed into a tared centrifuge tube and exact soil weight recorded. Soil was extracted with 40 mL of 1-M KCl on a reciprocating shaker for 60 min at 180 rpm, then centrifuged at 3500 rpm for 5 min before filtering the supernatant (Whatman 42, Whatman Ltd., Kent, United Kingdom). Concentration of inorganic N constituents in filtrate was determined using a microplate reduction technique and absorbance measured at 550 nm (Powerwave XS, Biotek, Woonooski, VT, USA). 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 20mL per 5-g soil and extracting by shaking for 5 min at 180 rpm. Samples were centrifuged for 5 min at 3500 rpm and supernatant 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 (Powerwave XS). 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, USA) and least squares means computed and separated with LSD. Differences between means were considered significant at $p \leq 0.05$. Total annual cowpea biomass, total annual weed biomass and stand density were analyzed using a randomized complete block design with a split plot. Cultivar and applied soil P and their interaction were considered fixed factors in the model, and site, block (nested within site), and the interaction of block with fixed effects (cultivar, soil P and their interaction). For response variables associated with harvest dates, cultivar, soil P, harvest and their interactions were considered as fixed factors and site, block (nested within site), and the interaction of block with cultivar, block with soil P and block with cultivar x soil P considered as random effects.

Results and Discussion

Precipitation from May through October at the OCU totaled 400 mm and rainfall was variable throughout the season (Figure 1a). From planting (May 23, 2014) to the first harvest at the OCU (August 15, 2014), plots received 300 mm of total rainfall. In the six weeks from the first harvest to the second harvest (October 2, 2014) plots received 100 mm of total rainfall with 70% of that occurring on just four days. At PREC, total precipitation was higher at 480 mm, including 136 mm occurring in the month of June (Figure 1b). Rainfall totaled 274 mm from planting (June 4, 2014) to the first harvest (August 13, 2014) and 206 mm from the first harvest to the second harvest (September 23, 2014). Average temperatures were similar between the two locations (Figures 1a and 1b). 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 ($p < 0.001$) and sampling time ($p < 0.05$), but not the interaction. Increased soil P was observed in P-amended plots with 14.7 mg P kg^{-1} soil as 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 ($p < 0.001$), but not cultivar or the interaction. 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 four weeks after planting indicated the highest plant populations from Iron & Clay (166,000 plants ha^{-1}), intermediate populations from Speckled Purplehull, IT82E-18 and IT85F-867-5 (143,000 to 138,000 plants ha^{-1}) and lowest populations from (IAR7/8-5-4-1, Coronet, KVx396, and IT97K-556-4; 128,000 to 118,000 plants ha^{-1}) (Figure 2). Diseased seedlings were collected from plots to verify causal pathogens, and both *Fusarium spp.* and *Macrophomina phaseolina* were identified (Shrestha *et al*., unpublished data). *Fusarium spp.* is of particular interest to cowpea breeders because genetic resistance to the fungus is a simple inheritance of one or two gene pairs and is relatively simple to incorporate into conventional breeding programs (Singh *et al.,* 1997). The parent breeding line 'Iron,' of Iron & Clay, is a known carrier for the gene that is resistant to both *Fusarium spp.* and *Macrophomina phaseolina* and is used extensively by breeders as a source of resistance to both (Singh *et al.,* 1997). These results suggest that cultivars evaluated likely differ widely in resistance or tolerance to seedling pathogens, and is an area that requires further study, especially for organic production. Given limited seed treatments available for organic production, planting at a higher seed densities may be necessary for cultivars that are less resistant to these diseases to still produce an adequate plant density for crop productivity (Hwang *et al.,* 2007).

Cowpea biomass at each harvest was significantly influenced by cultivar only ($p < 0.001$; (Table 1). Speckled Purplehull 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 (1302 to 983 kg ha^{-1}). The first harvest average biomass (1707 kg ha^{-1}) did not differ from the second (1585 kg ha^{-1}). Although not significant ($p > 0.05$), Iron & Clay and Speckled Purplehull biomass was over 500 kg ha^{-1} higher than other cultivars on both harvest dates (Table 3a). Both of these cultivars are indeterminate and produce tendrils (Table 2) allowing them to spread across rows and completely cover inter-row space effectively shading out all but the taller and more competitive weeds (Wang *et al.,* 2006). Notably, photosensitive Iron & Clay was still in a vegetative growth stage when the first harvest occurred in August 2014 allowing it to quickly re-establish its leafy biomass, which was maintained until final harvest in October 2014.

Annual cowpea biomass was significantly influenced by cultivar ($p < 0.001$), but not soil P ($p = 0.16$) or the interaction ($p = 0.77$; Table 1. Speckled Purplehull and Iron & Clay had the highest annual cowpea biomass (4922 and 4623 kg ha^{-1}, respectively; Figure 3). 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 (IAR7/8-5-4-

1, Coronet, IT82E-18). Interesting, although not statistically significant ($p = 0.16$), there was a trend of higher annual cowpea biomass in unamended soil P plots compared to amended plots (3422 kg ha^{-1} vs. 3150 kg ha^{-1}). Sanginga *et al.* (2000) evaluated cowpea breeding lines under P-amended and unamended environments for performance indicators such as dry matter production, N-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. This study evaluated 94 cowpea breeding lines, only one of which was included in our 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 to 10 mg P kg^{-1} soil), these cowpea cultivars are unlikely to respond to P fertilizer application. Cowpea may be a particularly useful forage crop for sites in the southeastern USA with low soil P values.

Weed biomass at each harvest was significantly affected by harvest date ($p < 0.001$) (Table 1), but not by soil P ($p = 0.19$), cultivar ($p = 0.45$), or any interactions ($p > 0.05$; Table 1). The first harvest (1488 kg ha^{-1}) produced significantly more weed biomass than the second harvest (893 kg ha^{-1}). Annual weed biomass was not significantly affected by cultivar ($p = 0.14$), soil P ($p = 0.26$) or the interaction ($p = 0.21$). Interestingly, the trends indicated higher annual weed biomass from P-amended plots compared to unamended plots (2675 kg ha^{-1}vs 2398 kg ha^{-1}) indicating that that the addition of P may give grass weed populations a slight competitive advantage due to increased P availability and the cowpea cultivars' neutral response to added P (Sanginga *et al.,* 2000). Wang et al. (2006) looked at three cowpea cultivars (Iron & Clay, IT89KD-288 and UCR 277) of differing phenotypic growth habits (erect, semi-erect and prostrate, respectively) against four densities of two weed species with differing statures, common purslane (*Portulaca oleracea*) and common sunflower (*Helianthus annuus*). They concluded that cowpea biomass declined as weed density increased but the pattern of reduction varied with weed species' stature. At the OCU, while there was still vigorous cowpea growth from some indeterminate cultivars, the determinate, bushy cultivars such as Coronet and IT82E-18 seemed poor competitors with the taller grasses (i.e., fall panicum). High biomass cowpea at PREC in competition with much shorter sedge, canopied more completely and were able to more effectively compete for light within the plots. These cultivars (Iron & Clay, Speckled Purplehull, IT97K-556-4) are either photosensitive or displayed indeterminacy and tendriling to achieve maximum ground coverage and weed suppression for the length of the growing season (Table 2).

If growing cowpeas as cover crop for weed competition, it is recommended that a producer choose a cultivar that has a competitive ability over endemic weed species. 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 day lengths 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 will produce less biomass for an organically managed forage system with vigorous weed populations. Iron & Clay, IT97K-556-4 and Speckled Purplehull are indeterminate cultivars and provide good coverage throughout the plot, suggesting that determinacy is a more effective indicator of weed suppressive ability than growth habit.

Forage quality

Cultivar significantly ($p < 0.01$) affected forage quality for all quality components except lignin in which the interaction of cultivar and harvest was significant ($p < 0.001$; Table 3). Harvest and the interaction between harvest and cultivar were also significant for all quality components ($p < 0.05$). There were no significant three-way treatment interactions ($p > 0.05$; Table 1).

Speckled Purplehull, IT85F-867-5 and Iron & Clay had the highest total forage protein content based on biomass and protein percentage at 1164, 664, and 633 kg protein ha^{-1}, respectively. Coronet and IAR7/8-5-4-1 had the lowest protein production at 277 and 412 kg protein ha^{-1}, respectively (Figure 4). The first harvest (654 kg protein ha^{-1}) produced almost 100 kg ha^{-1} more protein than the average of the second harvest (551 kg protein ha^{-1}). Buxton *et al.* (1996) stated that as a forage legume matures, voluntary intake declines and this quality decline is more closely related to plant maturity rather than plant age. Cowpeas were harvested at early bloom (R1) to beginning pod development (R3) after the first harvest and then matured to beginning seed (R5) to full seed after the second harvest (R6). Forages, especially warm season legumes, mature more rapidly in warm environmental conditions than in cooler conditions (Buxton *et al.,* 1996). Typically, the southeastern US does not experience consistent cooler temperatures until mid to late October; thus, these cowpeas were exposed to high temperatures and inconsistent precipitation throughout the growing season allowing them to mature rapidly after the first harvest (Figure 1).

The percentage of protein content also differed among cultivars ($p < 0.001$) with IAR7/8-5-4-1and KVx396 having the highest protein proportion in biomass at 205 g protein kg^{-1} of biomass (20.5%) and 204 g protein kg^{-1}, respectively (Figure 4). Coronet and IT85F-867-5 had the lowest protein content 161 g protein kg^{-1} and 185g protein kg^{-1}, respectively. The first harvest had a greater percentage of protein 206 g kg^{-1} than the second harvest 177 g protein kg^{-1} ($p < 0.001$) but the interaction between cultivar and harvest was not significant ($p > 0.05$) (Table 1).

Cultivar, harvest date and the interaction between cultivar and harvest date significantly affected both ADF and NDF ($p < 0.01$; 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, KVx396 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 *et al.,* 2012; Ball *et al.,* 2007). Harvest significantly ($p = 0.001$) affected ADF with the second harvest having 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 NDF at 365, 347, and 344 g NDF kg^{-1}, respectively ($p < 0.001$), indicating likelihood of less intake by grazing livestock. IAR/8-5-4-1 and KVx396 had the lowest NDF at 305 and 316 g NDF 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 harvest ($p < 0.0001$) and the interaction between harvest and cultivar ($p < 0.001$), but not cultivar ($p = 0.12$) or soil P ($p > 0.05$; Table 3). Lignin content was highest after the second harvest (37.8 g lignin kg^{-1} vs. 28.7 g lignin kg^{-1}) in all cultivars, which is expected, as lignin is more prevalent in plants that are more mature (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, Cherney and Cherney (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 they transition from vegetative to reproductive growth stages (Schut *et al.,* 2010). In this study, the harvest by cultivar interaction was significant for most parameters indicating that the relationship of quality amongst cultivars differed over the two harvest dates. Thus, earlier growth stages of these cultivars will provide higher quality forages with less lignin and indigestible fibers.

Minerals Ca, Mg, P, and K all were significantly influenced by cultivar ($p < 0.01$), harvest ($p < 0.001$), and the interaction ($p < 0.01$) but not soil P ($p > 0.05$; Table 4). All cultivars contained between 8.7 and 9.1 g Ca kg^{-1} except Coronet, which was lower at 8.2 g Ca kg^{-1} (p = 0.009). The first harvest contained 9.2 g Ca kg^{-1}compared to 8.3 g Ca kg^{-1} at the second harvest. The highest biomass P content was observed from IT82E-18 (3.2 g P kg^{-1}), IAR7/8-5-4-1 (3.2 g P kg^{-1}) and KVx396 (3.1 g P kg^{-1}), and the lowest in Coronet (2.8 g P kg^{-1}) and IT85F-867-5 (2.9 g P kg^{-1}). The first harvest contained 3.1 g P kg^{-1} and the second harvest contained 2.9 g P kg^{-1}. All cultivars had a higher P content after the first harvest than the second harvest. The highest Mg content occurred in IT82E-18 (4.7 g Mg kg^{-1}), IT97K-556-4 (4.4 g Mg kg^{-1}) and IT85F-867-5 (4.2 g Mg kg^{-1}) and the lowest content in Coronet (3.8 g Mg kg^{-1}) and KVx396 (4.1 g Mg kg^{-1}). The first harvest contained 4.4 g Mg kg^{-1}while the second harvest had 4.0 g Mg kg^{-1}. All cultivars had higher Mg content after the first harvest than the second harvest, except for Coronet, which had a lower Mg content after the first harvest (3.7 g Mg kg^{-1}) than the second (3.9 g Mg kg^{-1}; Table 3b). KVx396 and IAR7/8-5-4-1 both contained the highest K content at 23 g K kg^{-1} with Speckled Purplehull and IT97K-556-4 both containing 22 g K kg^{-1}. Coronet was the lowest in K content at 20.6 g K kg^{-1}. K content was higher after the first harvest (23 g K kg^{-1}) and lower after the second harvest (21 g K kg^{-1}). Overall, minerals declined from the first harvest to the second, further supporting that nutrient forage quality and content quantity decreases with plant maturity (Buxton, 1996).

WSC were influenced by cultivar ($p < 0.001$), harvest ($p < 0.01$) and the interaction between harvest and cultivar ($p < 0.05$; Table 5). Coronet, IT85F-867-5 and IAR7/8-5-4-1 are more typical of grain-type cultivars and contained the most WSC at 183, 166 and 158 g WSC kg^{-1}, respectively. IT97K-556-4 contained just 139 g WSC kg^{-1}, which is likely due to the photosensitivity of this cultivar (Table 2). Photosensitivity can cause plants to transition to reproductive growth much later in the growing season when the day length shortens if they are planted during longer day lengths. Lower WSC in photosensitive cultivars suggests that the plant has not fully transitioned to reproductive grain filling (Ritchie *et al.,* 2003; Huijser and Schmid, 2011). Indeed, although IT97K-556-4 produces substantial biomass, it is a difficult cultivar to harvest for grain or seed expansion in the southeastern USA as the first frost in late October to early November often damages the plants before the pods are harvestable.

Total digestible nutrients (TDN) were influenced by cultivar ($p < 0.01$), harvest ($p < 0.01$), and the interaction between harvest and cultivar ($p < 0.001$) but not soil P ($p > 0.05$; Table 5). IAR7/8-5-4-1, KVx396, and Coronet contained the highest TDN at 750, 744 and 726 g TDN kg^{-1}, respectively. IT82E-18 and IT97K-556-4 had the lowest TDN at 716 and 709 g TDN kg^{-1}, respectively. The first harvest (733 g TDN kg^{-1}) on average contained more digestible nutrients than the second harvest (720 g TDN kg^{-1}). Cultivar ($p < 0.01$), harvest ($p < 0.01$) and the interaction between harvest and cultivar ($p < 0.001$) but not soil P ($p > 0.05$; Table 6) influenced estimated net energy content (ENE). IAR7/8-5-4-1, KVx396, and Coronet had the highest means at 645, 639 and 623 g ENE kg^{-1}, respectively, while IT82E-18 and IT97K-556-4 contained the lowest energy at 614 and 607 g ENE kg^{-1} respectively. The three net energy parameters (NEG, NEL, NEM) were all influenced by cultivar ($p < 0.01$), harvest ($p < 0.01$) and the interaction between harvest and cultivar ($p < 0.001$) and not soil P ($p > 0.05$; Table 6). IAR7/8-5-4-1, KVx396, and Coronet all had the highest means for each. IT82E-18 and IT97K-556-4 had the lowest values (Mcal kg^{-1}) in all three energy parameters.

Conclusions

Cultivars Iron & Clay and Speckled Purplehull produced the greatest biomass over the two sites, suggesting that they offer the greatest potential for forage or cover crop use in regional organic and low-input systems of the cultivars evaluated. Both cultivars display indeterminate growth, high biomass, and are high in protein. Indeterminate cultivars were more competitive with weeds than determinate cultivars because they were able to cover more surface area in the plot. They both produced relatively high stand densities, suggesting that they are potentially more resistant to endemic seedling diseases. Soil P amendments can have conflicting effects in an organically managed system. Many cowpea accessions are not screened for P use efficiency and cultivars screened in this trial did not respond to P fertilization in low P soils. Our results also suggest that P amendment may increase relative competitiveness of weeds with cowpea in these low P soils under organic management.

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Appendix A:
Tables and Figures

Table 1. Mixed models analysis of variance for all response variables as affected by soil P, cowpea cultivar, harvest (where applicable) and their interactions

	Soil P	Cultivar	Cult. x soil P	Harvest	Soil P x harvest	Cult. x harvest	Soil P x cult. x harvest
	------------------------------------*p*-value------------------------------------						
Stand Count	NS[†]	<0.001	NS	n/a	n/a	n/a	n/a
Annual cowpea biomass	NS (0.16)	<0.001	NS	n/a	n/a	n/a	n/a
Annual weed biomass	NS (0.26)	NS (0.14)	NS (0.21)	n/a	n/a	n/a	n/a
Cowpea biomass	NS	<0.001	NS	NS	NS	NS	NS
Weed biomass	NS	NS	NS	<0.001	NS	NS	NS
Protein (kg ha^{-1})	NS	<0.001	NS	<0.001	NS	NS	NS
Protein (%)	NS	<0.001	NS	<0.001	NS	NS	NS

[†]NS= not significant, $p > 0.05$
n/a= Not applicable

Table 2. Cowpea cultivar descriptors collected from visual field observation (Verbree; unpublished data).

Cultivar Name	Origin	Days to flowering	Days to maturity	Seed weight (100-seeds; g)	Photo-sensitivity	Growth habit	Determinacy
Iron & Clay	U.S. Department of Agriculture-Agricultural Research Service Georgia, USA	83	110	11.3	Yes	Semi-prostrate	Indeterminate
Speckled Purplehull	Georgia, 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
KVx396	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 3. Cowpea cultivar and harvest effects on forage quality fiber parameters acid detergent fiber (ADF), neutral detergent fiber (NDF) and lignin as a proportion of biomass and on a mass basis. Within columns, means followed by the same letter are not significantly different, $p > 0.05$.

Cultivar	Harvest	Biomass (kg ha^{-1})	ADF (g kg^{-1})	NDF (g kg^{-1})	Lignin (g kg^{-1})
Iron & Clay	1st	2431 ab	284 ab	361 abc	30.6 de
	2nd	2229 abc	258 def	334 def	36.3 abc
Speckled Purplehull	1st	2424 ab	263 cde	336 cdef	28.5 ef
	2nd	2469 a	267 bcde	344 bcde	38.2 abc
IT97K-556-4	1st	1826 bcd	286 ab	373 a	30.5 de
	2nd	1730 cdef	270 bcde	358 abcd	38.6 ab
KVx396	1st	1503 defg	238 fg	307 gh	27.0 ef
	2nd	1802 cde	256 def	326 efg	35.9 abc
IT85F-867-5	1st	1761 cde	249 efg	315 fgh	24.7 f
	2nd	1425 defg	279 abc	359 abc	38.6 ab
IAR7/8-5-4-1	1st	1335 defgh	232 g	294 h	27.8 ef
	2nd	1269 defgh	251 def	316 fgh	34.0 cd
IT82E-18	1st	1170 fgh	249 efg	324 efg	26.0 f
	2nd	998 gh	293 a	364 ab	39.5 a
Coronet	1st	1210 efgh	254 def	321 efg	34.8 bcd
	2nd	757 h	271 bcd	340 bcdef	37.5 abc
		----------------------------*p*-values-----------------------			
Soil P		NS†	NS	NS	NS
Cultivar		<0.05	<0.01	<0.001	NS
Cultivar x soil P		NS	NS	NS	NS
Harvest		NS	<0.01	<0.01	<0.001
Soil P x harvest		NS	NS	NS	NS
Cultivar x harvest		NS	<0.001	<0.01	<0.01
Soil P x cultivar x harvest		NS	NS	NS	NS

†NS, not significant, $p > 0.05$

Table 4. Cowpea cultivar and harvest effects on forage calcium, magnesium, phosphorus, and potassium as a proportion of biomass and on a mass basis. Within columns, means followed by the same letter are not significantly different, $p > 0.05$.

Cultivar	Harvest	Ca ($g\ kg^{-1}$)	Mg ($g\ kg^{-1}$)	P ($g\ kg^{-1}$)	P ($kg\ ha^{-1}$)	K ($g\ kg^{-1}$)
Iron & Clay	1st	9.85 a	4.56 abc	3.15 bcd	7.8 a	22.6 bcd
	2nd	8.29 de	3.77 fg	2.82 fg	6.3 abc	21.1 ef
Speckled Purplehull	1st	9.28 b	4.44 bc	3.23 ab	7.9 a	23.3 ab
	2nd	8.16 e	3.88 efg	2.87 f	7.1 ab	21.9 de
IT97K-556-4	1st	9.79 a	4.90 a	3.23 ab	6.0 abc	22.6 abcd
	2nd	8.18 de	3.94 efg	2.88 f	5.0 cde	21.8 def
KVx396	1st	9.05 bc	4.21 cde	3.20 bc	5.0 cde	23.5 ab
	2nd	8.48 de	3.94 efg	3.03 e	5.5 bcde	23.0 abc
IT85F-867-5	1st	9.30 b	4.37 bcd	3.09 cde	5.6 bcd	22.1 cde
	2nd	8.24 de	4.01 efg	2.74 g	3.9 defg	19.9 gh
IAR7/8-5-4-1	1st	9.00 bc	4.17 cdef	3.25 ab	4.6 cdef	23.6 a
	2nd	8.58 cde	4.02 defg	3.06 de	3.9 defg	22.5 bcd
IT82E-18	1st	9.18 b	4.68 ab	3.35 a	4.0 defg	23.7 a
	2nd	8.65 cd	4.67 ab	3.03 e	3.0 fg	20.8 fg
Coronet	1st	8.24 de	3.71 g	2.88 f	3.6 efg	21.7 def
	2nd	8.18 e	3.92 efg	2.79 fg	2.1 g	19.7 h
		---------------------------------*p*-values----------------------------				
	Soil P	NS†	NS	NS	NS	NS
	Cultivar	<0.05	<0.01	<0.001	<0.001	<0.001
	Cultivar x soil P	NS	NS	NS	NS	NS
	Harvest	<0.001	<0.001	<0.001	<0.01	<0.001
	Soil P x harvest	NS	NS	NS	NS	NS
	Cultivar x harvest	<0.001	<0.001	<0.01	NS	<0.001
	Soil P x cultivar x harvest	NS	NS	NS	NS	NS

†NS, not significant, $p > 0.05$

Table 5. Cowpea cultivar and harvest effects on forage quality parameters water soluble carbohydrates (WSC) and total digestible nutrients (TDN) as a proportion of biomass and on a mass basis. Within columns, means followed by the same letter are not significantly different, $p > 0.05$.

Cultivar	Harvest	WSC ($g\ kg^{-1}$)	TDN ($g\ kg^{-1}$)
Iron & Clay	1st	129.2 ef	702 fg
	2nd	169.7 ab	731 bcd
Speckled Purplehull	1st	142.7 def	726 cde
	2nd	168.0 abc	721 cdef
IT97K-556-4	1st	119.6 f	700 fg
	2nd	157.5 bcd	718 cdef
KVx396	1st	157.3 bcd	754 ab
	2nd	159.2 bcd	733 bcd
IT85F-867-5	1st	161.0 bcd	742 abc
	2nd	171.3 ab	707 efg
IAR7/8-5-4-1	1st	157.9 bcd	761 a
	2nd	159.0 bcd	739 bcd
IT82E-18	1st	147.0 cde	741 abc
	2nd	143.3 de	691 g
Coronet	1st	188.9 a	735 bcd
	2nd	176.5 ab	716 def
		------------*p*-values-------	
	Soil P	NS†	NS
	Cultivar	<0.001	<0.01
	Cultivar x soil P	NS	NS
	Harvest	<0.01	<0.01
	Soil P x harvest	NS	NS
	Cultivar x harvest	<0.05	<0.001
	Soil P x cultivar x harvest	NS	NS

NS, not significant, $p > 0.05$

Table 6. Cowpea cultivar and harvest effects on forage quality parameter estimate net energy (ENE), net energy for lactation (NEL), net energy for gain (NEG), and net energy of metabolism (NEM) as a proportion of biomass and on a mass basis. Within columns, means followed by the same letter are not significantly different, $p > 0.05$.

Cultivar	Harvest	ENE ($g\ kg^{-1}$)	NEL ($Mcal\ kg^{-1}$)	NEG ($Mcal\ kg^{-1}$)	NEM ($Mcal\ kg^{-1}$)
Iron & Clay	1st	600 fg	1.60 fg	1.03 fg	1.64 fg
	2nd	628 bcd	1.68 bcd	1.12 bcde	1.74 bcd
Speckled Purplehull	1st	623 cde	1.67 cde	1.10 cde	1.72 cde
	2nd	618 cdef	1.65 cdef	1.09 cdef	1.70 cdef
IT97K-556-4	1st	599 fg	1.60 fg	1.03 fg	1.64 fg
	2nd	616 cdef	1.65 cdef	1.08 cdef	1.70 cdef
KVx396	1st	649 ab	1.73 ab	1.18 ab	1.81 ab
	2nd	629 bcd	1.68 bcd	1.12 bcd	1.74 bcd
IT85F-867-5	1st	637 abc	1.70 abc	1.15 abc	1.77 abc
	2nd	606 efg	1.62 efg	1.05 efg	1.66 efg
IAR7/8-5-4-1	1st	655 a	1.75 a	1.20 a	1.83 a
	2nd	635 bcd	1.70 bcd	1.14 abcd	1.76 abcd
IT82E-18	1st	637 abc	1.70 abc	1.15 abc	1.77 abc
	2nd	591 g	1.58 g	1.00 g	1.61 g
Coronet	1st	631 bcd	1.69 bcd	1.13 bcd	1.75 bcd
	2nd	614 def	1.64 def	1.08 def	1.69 def
		---*p-values*---			
	Soil P	NS†	NS	NS	NS
	Cultivar	<0.01	<0.01	<0.01	<0.01
	Cultivar x soil P	NS	NS	NS	NS
	Harvest	<0.01	<0.01	<0.01	<0.01
	Soil P x harvest	NS	NS	NS	NS
	Cultivar x harvest	<0.001	<0.001	<0.001	<0.001
	Soil P x cultivar x harvest	NS	NS	NS	NS

†NS, not significant, $p > 0.05$

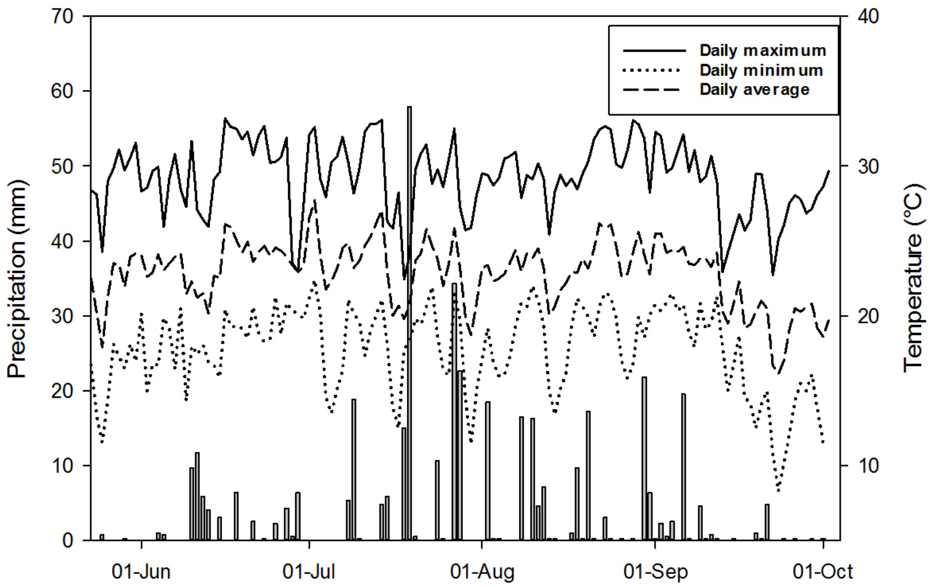

Figure 1a. Organic Crops Unit (OCU) temperature data recorded as daily maximum, daily minimum and daily average (˚C) and precipitation recorded as rain (mm). Represents data from planting on May 23, 2014 to harvest October 2, 2014.

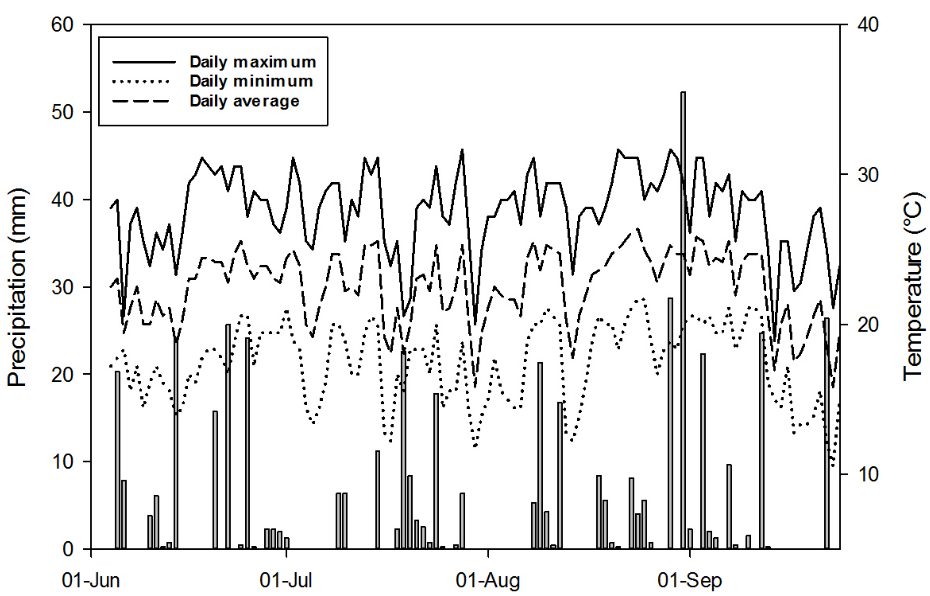

Figure 1b. Plateau Research and Education Center (PREC) temperature data recorded as daily maximum, daily minimum and daily average (˚C) and daily precipitation recorded as rain (mm). Represents data from planting on June 4, 2014 to harvest September 24, 2014.

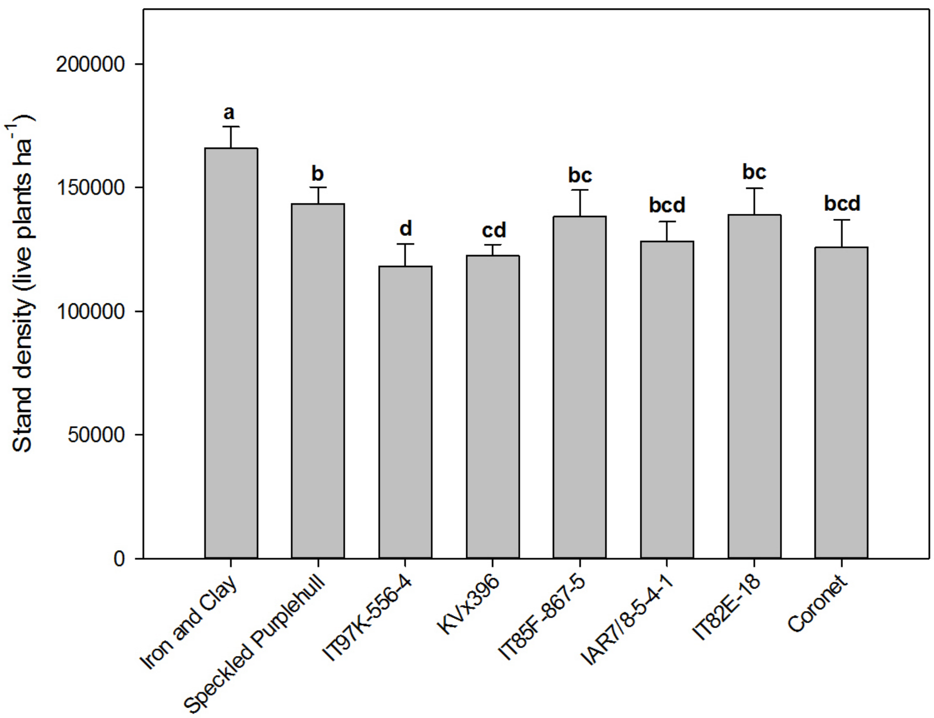

Figure 2. Stand density at 4-weeks post planting as influenced by cultivar, averaged over location. Means followed by the same letter are not significantly different, $p > 0.05$. Error bars represent raw standard error of the mean.

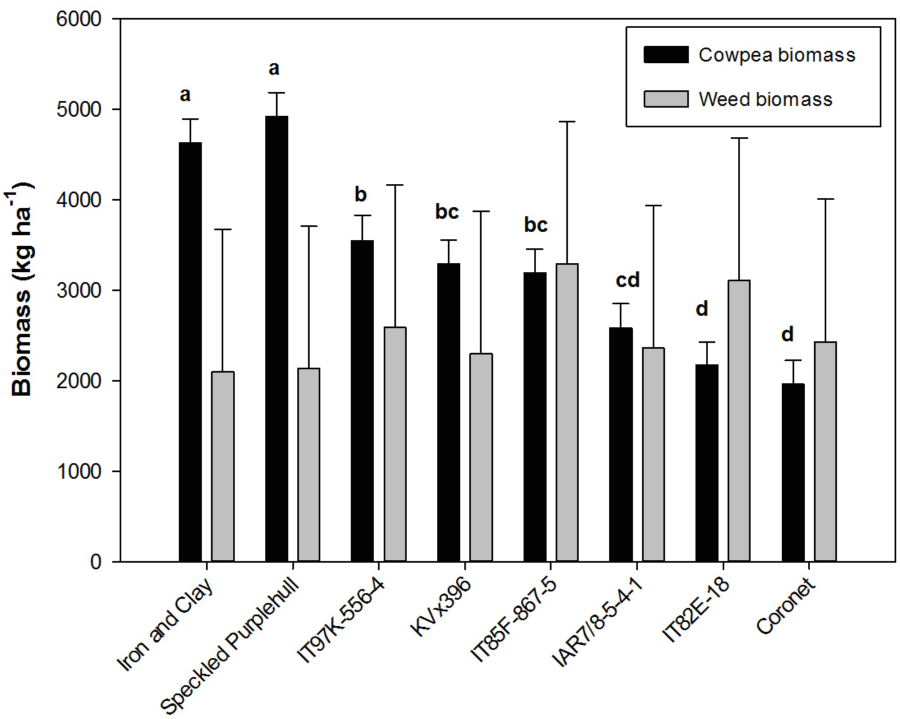

Figure 3. Total annual cowpea and weed biomass as influenced by cultivar, averaged over location. Means indicated by the same letter or no letters are not significantly different ($p > 0.05$). Error bars represent standard error of the mean.

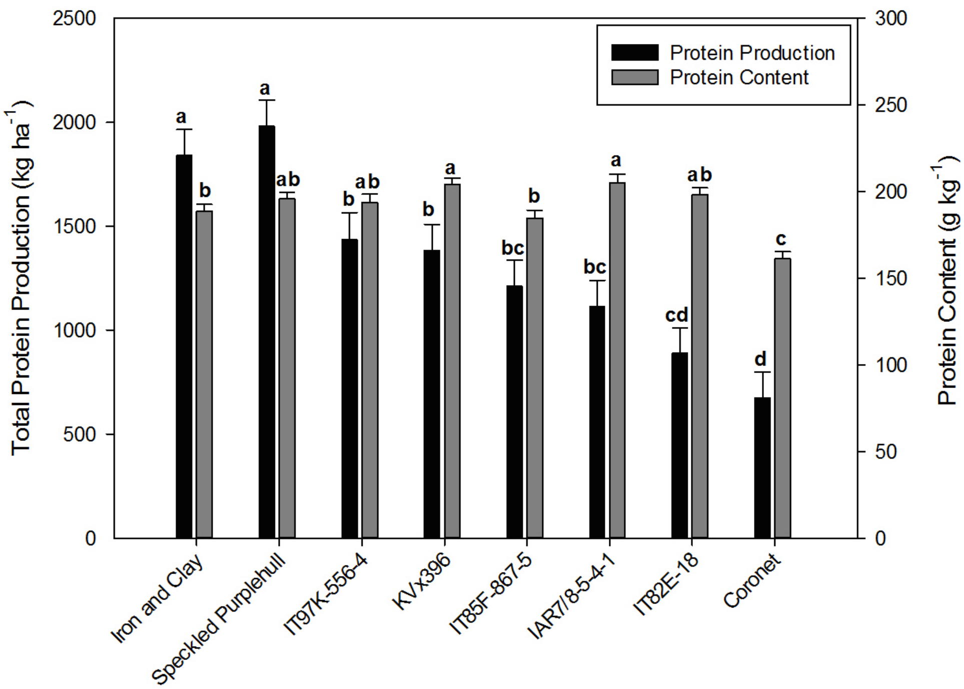

Figure 4. Total protein production and the protein content per cultivar as influenced by cultivar and averaged over location. Means followed by the same letter or no letters are not statistically different ($p > 0.05$). Error bars represent standard error of the mean.

Chapter 2

Screening cowpea cultivars for resistance to charcoal rot (*Macrophomina phaseolina*)

Screening cowpea cultivars for resistance to charcoal rot (*Macrophomina phaseolina*)

Samantha Hill, Alemu Mengistu, David Verbree, David M. Butler

Abstract

Charcoal rot [*Macrophomina phaseolina* (Tassi) Goid.] is a fungal disease that is economically important to many host plant species. High temperatures and drought conditions favor disease development making it difficult to predict when disease outbreak will occur. Cowpea (*Vigna unguiculata* L. Walp.) is an important crop for many regions of the globe and is one of the host species for *M. phaseolina*. Efforts have been made to breed genetic lines that are resistant to *M. phaseolina* but little research has been done to screen many popular cowpea cultivars for resistance. Our result indicated that two of the 26 cultivars, IT85F-867-5 and IT98K-589-2 displayed the highest stand densities in both the field trial and the greenhouse study, suggesting they may be resistant to *M. phaseolina*. Later maturing cultivars, such as Iron & Clay and US1136, may also withstand infection from *M. phaseolina* to produce grain or forage yields due to known genetic resistance or physiological mechanism involved in plant aging. C.T. Pinkeye and Coronet displayed the highest numbers of CFU at maturity and were amongst the highest in visual RSS ratings indicating that their physiology may provide a more desirable environment for microsclerotial growth later in the season. Correlation analysis however, showed that field and greenhouse studies on cultivar resistance to *M. phaseolina* did not seem to be correlated indicating that cultivar responses differed under the two environments.

Abbreviations: Colony-forming unit, CFU; Colony-forming unit index, CFUI; Root and stem severity, RSS; potato dextrose agar, PDA; SCN; soybean cyst nematode; potato dextrose broth, PDA; polymerase chain reaction, PCR

Introduction

Macrophomina phaseolina (Tassi) Goid. is the causal fungal agent of charcoal rot a soil-borne pathogen that causes infection in over 500 different host plant species globally (Afouda *et al.*, 2008; Pearson *et al.,* 1984; Su *et al.,* 2000; You *et al.,* 2011). It is common in subtropical and tropical countries with a semiarid climate and is severe in arid regions that often have sustained drought periods (You *et al.*, 2011). Drought stress causes negative effects to host plant physiology, weakening plant tissues and predisposing crops to infectious facultative parasites such as *M. phaseolina* (Mayek-Perez *et al.*, 2002). This fungus survives in the soil as sclerotia embedded in organic debris or free in soil and can persist due to the high number of species in it's host range (Abawi and Pastor-Corrales, 1988; Songa *et al.*, 1997). There is a strong association between the occurrence of drought and susceptibility to *M. phaseolina* (You *et al*., 2011). Host crops show a variety of disease symptoms that can coincide with any stage of development. In seedlings, *M. phaseolina* can cause pre- or post-emergent damping off, black cotyledonary lesions at varying degrees of severity or it can persist in a crop showing little to no disease symptoms. In soybean (*Glycine max* (L.) Merr.), aboveground symptoms are typically not apparent until after flowering and reproductive growth has occurred (R5-R7) (Fehr *et al.,* 1971; Mengistu *et al.,* 2007); while in common bean (*Phaseolus vulgaris* L.) damage is mainly significant in the early stages of development (Mayek-Perez *et al.,* 2002).

Cowpea (*Vigna unguiculata* L. Walp.) is an important food crop for many developing countries and is highly adapted to many agro-ecological environments. *M. phaseolina* is important to cowpea where it undergoes moisture stress and can cause seedling damping-off in early growth stages or losses in grain production in adult plants (Adekunle *et al.*, 2001). Currently there are limited chemical or effective cultural controls to combat the fungus; thus identifying genetic resistance in cultivars is a priority (Pearson *et al*., 1984). Some cowpea cultivars have already been identified as having genetic resistance to charcoal rot (e.g., Iron & Clay) and earlier maturing cultivars may have the ability to still produce several pod flushes before the fungus ultimately cause high disease severity in later maturity stages (Singh *et al*., 1997; Afouda *et al*., 2008). However, plants remain susceptible to infection at any growth stage, particularly if there are environmental stressors such as drought or high temperatures (Afouda *et al*., 2008). Identifying cowpea genotypes that show resistance to *M. phaseolina* is important for producers as host resistance may be the only viable method for control, as with soybean (Mengistu *et al.*, 2007).

The objectives of this study were (i) to screen 26 cowpea cultivars for resistance or susceptibility to *M. phaseolina* in a naturally-infested field using two methods of verification, (ii) to evaluate these same 26 cultivars under a controlled greenhouse environment for seedling disease resistance.

Materials and Methods

Field screening

Field plots were established at the West Tennessee Research and Education Center (WTREC) in Jackson, TN. The experimental design was a randomized complete block design with four replications. Twenty-six cowpea cultivars were selected based on their commercial popularity or existence in other ongoing cowpea trials (Table 8). Plots were planted on May 21, 2014 with a cone planter (ALMACO, Nevada, IA, USA) at a rate of 430,600 seeds ha^{-1} (200 seeds $plot^{-1}$) at a 2-cm depth. Each plot was a 6.1-m single row and rows were 76-cm apart. The field was known to be naturally infested with *M. phaseolina* (A. Mengistu, personal communication). In order to reduce plot to plot variability plots were further inoculated with charcoal rot infested Japanese millet (*Echinochloa frumentacae* L.) seed at a rate of 1.6-g inoculum m^{-1} of row. Plots were maintained weed free with Round-Up (Isopropylamine salt of N-(phosphonomethyl) glycine (56)) + Zidua (Pyroxasulfone) as a pre-emergent herbicide and Prefix (S-metolachlor + fomesafen) was used as a post-emergent herbicide. Plots were not irrigated. Stand density was evaluated on June 3, 2014 and July 10, 2014 by counting every germinated, live cowpea in each plot. Data on rainfall and temperature averages were collected from a weather station at WTREC equipped with precipitation gauges and temperature sensors (CR3000 datalogger, Campbell Scientific, Logan, UT, USA).

Field plot disease assessment protocols were used according to Mengistu *et al*. (2007). On September 19, 2014, five random individuals were sampled from each plot and branches and axillary roots were removed. Plants progressed to the R7 growth stage before being collected for sampling. The stems included the taproot and were washed thoroughly of excess soil, then bundled and placed in a burlap bag to air dry, and then stored at room temperature until processed. This period of time allowed any existing microsclerotia to develop within the root-stem system. Stems were then assessed for root and stem severity (RSS) by longitudinally splitting the stem and taproot of each plant and visually rating the intensity of discoloration from microsclerotia development. The ratings were on a scale of 1 to 5 where 1 = no discoloration and

5 = highly discolored (Figure 5). The RSS scale ratings were used to categorize cultivar resistance or susceptibility as described for soybean by Paris *et al.* (2006) and Mengistu *et al*. (2007): resistant (values of 1), moderately resistant (values > 1 and ≤ 2), moderately susceptible (values > 2 and < 3) and susceptible (values of 3 to 5).

The five plant samples per plot that were used for the RSS assessment were also used to determine colony-forming units (CFU) of *M. phaseolina* present in the stem. Each stem was cut at the cotyledonary node and a lower portion of the stem and root were ground with a laboratory cyclone mill (Thomas Model 4 Wiley Mill, Thomas Scientific, Swedesboro, NJ, USA) and passed through a 1-mm mesh screen. The mill was thoroughly cleaned between each sample to avoid sample-to-sample contamination.

Each plant tissue sample was then weighed to 0.005 g into a microcentrifuge tube and 1 mL of 10% sodium hypochlorite solution was then added to each tube. Using a vortex shaker, the samples were washed and shaken in 1-min intervals 3 times with 15 s between each shake period. Tubes were filled with sterile distilled water to dilute the sodium hypochlorite solution and poured into a 45-µm sieve. Samples were then gently rinsed from the sieve into 15 mL tubes using sterile water. 250-mL bottles containing 50 mL of autoclaved potato dextrose agar (PDA) was cooled from 50˚C and the ground stem samples, 0.05 g of rifampicin, and 15 drops of tergitol were added to the bottles. Media was shaken by hand until evenly mixed, poured evenly into five Petri dishes, and allowed to solidify. The plates were incubated at 30˚C for 3 days. The numbers of *M. phaseolina* colonies per plate were counted and data converted to CFU g^{-1} of tissue. A colony forming unit index (CFUI) was calculated by dividing the CFU g^{-1} of each cultivar with the highest average CFU g^{-1} of a susceptible cultivar within the experimental plot. C.T. Pinkeye had the highest average CFU g^{-1} thus all reported data for CFUI are based on CFU from C.T. Pinkeye. The genotypes were then classified in percentage based on CFUI as resistant (0 to < 10), moderately resistant (10 to ≤ 30), moderately susceptible (> 30 to 60) and susceptible (> 60), in accordance with the classification system of Schmitt and Shannon (1992) developed for SCN (Mengistu *et al*., 2007).

Greenhouse screening

The same twenty-six cultivars were evaluated in a greenhouse at seedling stage. Sterile potting soil (indicate the composition, company producing it, city and state) was placed in 15 cm pots and organized in a completely randomized design with three replications. Eight seeds were planted in each pot at a 2 cm depth and then inoculated with charcoal rot infested millet seed at one of 3 levels of inoculation (0 g (control), 1 g, and 3 g)/pot by placing the appropriate density directly adjacent to the cowpea seed. Pots were hand-watered every other day to maintain soil moisture content.

Pot stand density was recorded 14 days after planting and was rated for disease severity. Pots were then thinned to 4 plants per pot to assess disease severity over time. Aboveground infection of seedlings was rated at 20 days and 25 days according to an adapted *M. phaseolina* rating scale provided by Abawi and Pastor-Corrales (1990) for common bean (Fig. 6a). This visual scale was used based on disease severity exhibited below the cotyledonary node (Fig. 6b). Fifteen samples of seedlings displaying various stages of disease were collected for verification of causal disease organism. Sampled tissue was cut 1 cm above and 1 cm below the infected cotyledonary node, disinfected in 10% ethanol solution, rinsed in deionized water and then placed on water agar plates. Fungal hyphae were allowed to grow for 10 days. After growth on the media had been established, a heat sterilized wire wand was used to transfer small samples of hyphal growth to rifampicin amended PDA plates and incubated at room temperature (21 ˚C) for 10 additional days.

DNA extraction of fungal isolates was completed using Qiagen plant extraction kits. The fungal pathogens were grown in potato dextrose broth (PDB) for 7 days. The extraction product was amplified by polymerase chain reaction (PCR) with transcribed ITS1 and ITS4 internal regions for *Macrophomina*. PCR was carried out in a 50 µl reaction mixture containing 50 ng genomic DNA, 10 µl 5X buffer, 1 µl of 10 mM dNTPs, 2.5 µl of 10 µM of each primer and 0.25 µL of Taq polymerase (Hot master mix). The following protocols were used for PCR reaction: 1 cycle of initial denaturalization at 94°C for 2 min, 30 cycles of de-naturalization at 94°C for 1 min, 56°C for 30 s for primer annealing, 72°C for 1 min for extension and 1 cycle of final extension at 72°C for 5 min. The amplification was analyzed in agarose gel at 1% through electrophoresis. PCR resulting product was purified using ExoSAP. Base pairs obtained were compared with the sequences reported in the database of NCBI's gene bank (National Center for Biotechnology Information, www.ncbi.nih.gov).

Analysis of variance was performed using mixed models (PROC GLMMIX, SAS 9.4, Cary, NC, USA) and least squares means computed and separated with LSD. Differences between means were considered significant at $p \leq 0.05$. Field screening was analyzed using a randomized complete block design. Cultivar was considered as fixed effects in the model and block was considered random. The greenhouse screening was analyzed using a completely randomized design with cultivar, inoculation level, and the interaction between cultivar and inoculation level as fixed effects. Inoculation levels of 1 g pot^{-1} and 3 g pot^{-1} were different from the control (0 g pot^{-1}) but not from each other; therefore means were analyzed to distinguish differences between inoculated pots and the control. Percent data was transformed using the arcsin of the square root. Differences in germination and disease ratings of inoculated pots versus the control were log transformed to improve normality. Data was then back transformed and presented.

Results and Discussion

Field study

The average temperature during the growing season was 24˚C. Precipitation totaled 720 mm from planting on May 21, 2014 to the sampling date on September 19, 2014 (Fig. 7); which was nearly double the total precipitation recorded for the same time period in the previous year (data not shown). Mayek-Perez *et al.* (2002) reported that drought stress was an important contributor to charcoal rot development in common bean. There was a short period (13 d) in July where there was no precipitation recorded at WTREC, but drought periods were limited for the majority of the season (Fig. 7). Many of the existing plants in this study did not show any visual signs of charcoal rot infection until late in the season when sampling occurred and pods were nearly dry. Likely due to high soil moisture, two late maturing US cultivars, Iron & Clay and US1136, did not show any visual symptoms in the field and also did not mature to R7 due to photosensitivity before the sampling date, therefore they were not sampled for the other disease assessment methods. Iron & Clay is the most commercially available forage and cover crop cowpea cultivar in the U.S. and is known to contain a single gene pair that could be resistant to *M. phaseolina* and *Fusarium spp.* (Singh *et al*., 1997). For this reason, Iron & Clay and US1136 are not included in RSS visual ratings or in the CFUI assessment.

Cultivar was significant for all tested parameters ($p < 0.001$, Table 7). The first stand count taken 2 weeks after planting (growth stage V1 to V3) showed the highest stand density from IT85F-867-5 (81,000 plants ha^{-1}), IT98K-589-2 (80,000 plants ha^{-1}) and IT82E-18 (79,600 plants ha^{-1}). Stand density was least for C.T. Pinkeye (56,000 plants ha^{-1}), IT98K-205-8 (50,000 plants ha^{-1}) and Melakh (38,000 plants ha^{-1}). The second stand count taken 7 weeks after planting (growth stage R1 to R3) showed similar results with IT85F-867-5 and IT98K-589-2 having the highest stand density (78,500 and 76,000 plants ha^{-1}) and C.T. Pinkeye, IT98K-205-8, California Blackeye 27 and Melakh having the lowest plant populations (from 51,000 to 36,500 plants ha^{-1}) (Fig. 8). Differences in plant populations from the first stand count to the second stand count could indicate seedling damping off and plant mortality from *M. phaseolina* (Afouda *et al*., 2008). Stand density changes from the vegetative stage to the reproductive stage were significantly influenced by cultivar ($p < 0.05$, Table 7). Mississippi Silver and Early Acre had the highest stand density loss (18 to 19%) while stands differed least for KVx403 (3%), KVx396 (2%) and UCR288 (2%) (Fig. 9).

Cultivar also significantly affected visual RSS ratings ($p < 0.01$). Means from RSS ratings show that no cultivars were classified as resistant or moderately resistant. Thirteen cultivars were classified as moderately susceptible and eleven were susceptible according to this scale. IAR7/8-5-4-1, C.T. Pinkeye and Coronet had the highest RSS ratings of 3.7, 3.5 and 3.5 respectively. IT98K-1069-6, UCR288 and IT97K-499-35 had the lowest RSS ratings of 2.2, 2.2 and 2.0, respectively (Fig. 10). Average values for CFU g^{-1} were highest for C.T. Pinkeye, therefore, C.T. Pinkeye was labeled the most susceptible and was used as an indicator for disease resistance. CFUI ranged from 1.6% to 71% among genotypes and was also significant for cultivar ($p < 0.001$). Six cultivars were classified as resistant (2% to 8%), eight cultivars were moderately resistant (11% to 29%), seven cultivars were moderately susceptible (31% to 56%) and two cultivars were susceptible (61% to 71%) relative to C.T. Pinkeye (100%) (Fig. 11).

Coronet and IT98K-476-8 were highest for CFUI percentage at 71% and 61% compared to C.T. Pinkeye and were labeled susceptible. Lowest CFUI percentage was from Melakh, IT97K-1069-6, and KVx403 at 1.6%, 2% and 3.8%, respectively; these cultivars were labeled resistant. CFUI was moderately positively correlated to RSS ratings ($p < 0.001$; $r = 0.536$), which could be due to both methods being based on rating the intensity of microsclerotial infection as the indicator of disease severity (Mengistu *et al*., 2007).

Greenhouse study

Disease severity was highest for the first rating date (14 days after planting), and this date includes the germination and rating of 8 plants. After pots were thinned to 4 plants, there was no change in disease severity for the remaining plants from 20 to 25 days after planting (data not shown), thus the data presented are reflective of the first rating date only.

Cultivar ($p < 0.001$) was highly significant for percent emergence in the inoculated pots, but neither the inoculation levels nor the interaction differed ($p > 0.05$). Emergence percentage in inoculated pots showed highest stand density from IT98K-589-2 (100%; 8 plants pot^{-1}), IT85F-867-5 (97%), Early Acre (95%) and IT90K-277-2 (95%), and lowest stand density from UCR288 (44%), Mississippi Silver (50%) and IT98K-476-8 (33%; data not shown). Similarly, average ratings for infected cotyledonary nodes were related to cultivar ($p < 0.001$) but inoculation level (1 or 3 g pot^{-1}) and the interaction were not significant ($p > 0.05$). Ratings were highest from IT98K-476-8 (7.0), UCR288 (6.1) and Mississippi Silver (5.8). Lowest ratings were from IT90K-277-2 (1.4), Early Acre (1.3), and IT98K-589-2 (1.2; data not shown).

To account for potential differences related to seed lot (e.g., seedborne pathogens, seed viability, seed lot vigor), differences in emergence percentage and disease severity ratings between inoculated (1 or 3 g pot^{-1}) and uninoculated (i.e., control) were assessed. Cultivar was significantly related to differences in emergence ($p < 0.01$) but inoculation level (1 or 3 g pot^{-1}) and the interaction were not related to emergence. UCR288 had the highest difference in emergence with 40% less emergence in inoculated pots compared to respective uninoculated pots, followed by Colossus (14%) and IT98K-476-8 (14%; Fig. 12). Emergence differences were least for Mississippi Silver (0.7%) while IT98K-589-2 and IT97K-1042-3 had no difference from inoculated pots to the control. Differences in disease severity ratings were also significantly related to cultivar ($p < 0.001$), but not inoculation or the interaction ($p > 0.05$), with UCR288 being rated 3.6 points higher in inoculated pots compared to the uninoculated control and US1136 and Colossus rated 2.0 points higher in inoculated pots than in the control. Mississippi Silver, IT98K-589-2 and IT82D-889 were the least different from the control and rated 0.06 points higher than the control pots.

Based on results of field and greenhouse studies, cowpea genotype resistance to *M. phaseolina* in seedling stages compared to reproductive stages do not seem to be correlated. In the field screening, the best performing cultivars for stand density at both stand counts were IT85F-867-5 (81,752 and 78,525 plants ha^{-1}) and IT98K-589-2 (80,676 and 75,863 plants ha^{-1}; Fig. 5). They were also among the least in stand density loss between stand counts (4% and 7%, respectively). These two cultivars also had the highest stand density in the greenhouse screening in inoculated pots and were two of the best performing cultivars for emergence differences in inoculated pots versus control pots (2%, IT85F-867-5; 0%, IT98K-589-2). These results suggest that these two cultivars may be resistant to *M. phaseolina* at this growth stage. Adekunle *et al.* (2001) states that the greatest losses in cowpea production occur due to seedling damping off and *M. phaseolina* is often the causal agent where moisture stress is involved. In another study Hill *et* *al*. (2015, unpublished data) experienced seedling disease losses for cowpea due to both *Fusarium spp.* and *M. phaseolina* (Shrestha *et al*., unpublished data). Moisture stress in this study was much higher than at the location in Jackson, TN and stand losses from seedling damping-off were significant. The ability for a cultivar to escape the seedling disease stage without anti-fungal seed treatment could indicate genetic resistance to *M. phaseolina* and these cultivars could still produce grain or forage yields before *M. phaseolina* infects mature plant tissues. The greenhouse screening showed that those plants that escaped the seedling disease stage, even plants infected with slight cotyledonary lesions, did not show any change from growth stage V3 to V5.

Because *M. phaseolina* can infect plant tissues at any maturity stage depending on environmental conditions, conclusive decisions about the resistance of these two cultivars cannot be made without identifying genetic markers for resistance (Afouda *et al*., 2008). In a highly cited study, Short *et al*. (1978) hypothesized that the populations of sclerotia in roots and stems of a host may indicate compatibility between the host plant and *M. phaseolina*. They showed that there was extreme variability of the amount of sclerotia present in root tissues of soybean (*Glycine max*) cultivars and that the variability may be due to a combination of genetic, physiological, and environmental factors. They concluded that there could also be differences in multiple host genes in individual plants that result in different levels of compatibility with the fungus. If environmental stressors are not present, *M. phaseolina* may persist in plant tissues without showing any symptoms of infection even in highly inoculated environments (Afouda *et al*., 2008).

However, visual ratings of microsclerotia growth are still good indicators for disease resistance. C.T. Pinkeye and Coronet displayed the highest percentages of CFUI and were among the highest in visual RSS ratings. UCR288 performed well in the field (74760 plant ha^{-1}; Fig. 8) with little stand losses between the first and second stand counts (2%; Fig 9) and had relatively low RSS and CFUI means (2.15 RSS; 21% CFUI; Fig 10 and 11), but in the greenhouse study it was the worst in emergence losses (40%; Fig. 12) and rating differences between inoculated and uninoculated pots (3.6; Fig. 13); thus this cultivar may be more contagious to infection in seedling disease stages than in maturity. Some cultivars did not display any visual symptoms in the field (e.g., Iron & Clay and US1136) but they cannot be labeled as resistant solely based on lack of visual symptoms due to the absence of highly favorable conditions for *M. phaseolina* in the field (Afouda *et al*., 2008). Collins *et al*. (1990) showed that water hindered microsclerotial growth and development of *M. phaseolina* by limiting the exchange of O_2 and CO_2 where microbiological activity was occurring. In the greenhouse, Iron & Clay performed comparably to the best and worst cultivars in all tests and US1136 had high differences between inoculated and uninoculated pots in both emergence and ratings (Fig. 12 and 13). Afouda *et al*. (2008) states that many stress factors are involved in the development of *M. phaseolina* including plant age, high temperatures, and drought stress. One genetic mechanism that could be involved in charcoal rot development is the ability of the cultivar to maintain internal water turgor pressure during water stress (Mayek-Perez *et al*., 2002). Drought stress causes plant tissues to weaken and allows space for microsclerotia to infect the internal plant structure blocking xylem vessels and causing plants to wilt (You *et al*., 2011; Mayek-Perez *et al*., 2002). Mayek-Perez *et al*. (2002) studied the mechanisms involved in common bean resistance to *M. phaseolina* and concluded those cultivars that showed higher water and turgor potentials were more resistant to *M. phaseolina* than susceptible cultivars; thus, cultivars that are resistant to drought stress may also be resistant to root rot pathogens and vice versa.

Conclusions

IT85F-867-5 and IT98K-589-2 displayed the highest stand densities in both the field trial and the greenhouse study, suggesting they may be resistant to *M. phaseolina* in the seeding disease stage. In the greenhouse, UCR288 was the worst performer in stand losses and had the highest rating differences from inoculated to uninoculated pots indicating that this cultivar may be susceptible in the seedling disease stage. Later maturing cultivars, such as Iron & Clay and US1136, may be able withstand infection from *M. phaseolina* to produce grain or forage yields due to known genetic resistance or physiological mechanism involved in plant aging. C.T. Pinkeye and Coronet displayed the highest numbers of CFU at maturity and were amongst the highest in visual RSS ratings indicating that their physiology may provide a more desirable environment for microsclerotial growth later in the season. Environmental conditions are an important factor when screening for *M. phaseolina* due to its high association with moisture stress.

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Appendix B:
Tables and Figures

Table 7. Mixed models analysis of variance for all response variables as affected by cultivar, inoculant level (where applicable; 1 g pot^{-1} versus 3 g pot^{-1}) and their interactions. Colony-forming unit index, (CFUI); Root and stem severity rating, (RSS).

	Cultivar	Inoculant Level	Cult. x Inoc. Level
	-----------------------------*p-value*	-----------------------------	-----------------------------
		Field Screening	
Stand at V1-	<0.001	n/a	n/a
Stand at R1-R3	<0.001	n/a	n/a
Stand loss (V1-3 to R1-3)	<0.05	n/a	n/a
RSS	<0.01	n/a	n/a
CFUI	<0.01	n/a	n/a
		Greenhouse Screening	
Disease rating	<0.001	NS†	NS
Stand density at 14 days	<0.001	NS	NS
Disease rating difference (inoculated – control)	<0.001	NS	NS
Stand density difference (inoculated-control)	<0.01	NS	NS

†NS= not significant, $p > 0.05$
n/a= Not applicable

Table 8. Cowpea cultivar descriptors collected from visual field observation (Verbree; unpublished data).

Cultivar Name	Origin	Days to flowering	Days to maturity	Photo-sensitivity
California Blackeye 27	University of California-Davis, California, USA	37	64	No
California Blackeye 46	University of California-Davis, California, USA	53	85	No
Colossus	U.S. Department of Agriculture-Agricultural Research Service, South Carolina, USA	37	71	No
Coronet	University of Georgia, Georgia, USA	37	83	No
C.T. Pinkeye Purplehull	C.T. Smith Company, Texas, USA	40	83	No
Early Acre	University of Arkansas, Arkansas, USA	52	76	No
IAR7/8-5-4-1	Institute for Agricultural Research (IAR), Nigeria	54	90	No
Iron & Clay	U.S. Department of Agriculture-Agricultural Research Service Georgia, USA	83	110	Yes
IT82D-889	International Institute of Tropical Agriculture (IITA), Nigeria	48	71	No
IT82E-18	International Institute of Tropical Agriculture (IITA), Nigeria	40	64	No
IT85F-867-5	International Institute of Tropical Agriculture (IITA), Nigeria	37	64	No
IT90K-277-2	International Institute of Tropical Agriculture (IITA), Nigeria	53	85	No
IT97K-499-35	International Institute of Tropical Agriculture (IITA), Nigeria	48	83	No
IT97K-1042-3	International Institute of Tropical Agriculture (IITA), Nigeria	40	83	No
IT97K-1069-6	International Institute of Tropical Agriculture (IITA), Nigeria	54	83	No
IT98K-205-8	International Institute of Tropical Agriculture (IITA), Nigeria	53	83	No
IT98K-476-8	International Institute of Tropical Agriculture (IITA), Nigeria	48	83	No
IT98K-589-2	International Institute of Tropical Agriculture (IITA), Nigeria	40	69	No
IT98K-1111-1	International Institute of Tropical Agriculture (IITA), Nigeria	44	70	No
KVx396	Institut de l'Environnement et Recherches Agricoles (INERA), Burkina Faso	52	87	No
KVx403	Institut de l'Environnement et Recherches Agricoles (INERA), Burkina Faso	40	70	No
Melakh	Institut Senegalais de Recherches Agricoles (ISRA), Senegal	40	83	No
Mississippi Silver	Mississippi State University, Mississippi, USA	52	83	No
Speckled Purplehull	Heirloom, Southeastern USA	58	83	No
UCR288	University of California-Riverside, California, USA	55	76	No
US 1136	U.S. Department of Agriculture-Agricultural Research Service, South Carolina, USA	83	110	Yes

Figure 5. Split lower stem and root sections showing *Macrophomina phaseolina* microsclerotia evaluated for root and stem severity (RSS). On a scale of 1 to 5, 1 = no microsclerotia visible in tissue; 2 = very few microsclerotia visible in pith, vascular tissue or under the epidermis, vascular tissue has not discolored; 3 = vascular tissue is partly discolored and microsclerotia have partially covered the tissue; 4 = vascular tissue is discolored with numerous microsclerotia embedded in tissue, microsclerotia are also visible under the outside epidermis in stem and root sections; and 5 = vascular tissue darkened due to high numbers of microsclerotia both inside and outside of the stem and root tissues (rating system adapted from Mengistu *et al*., 2007; Paris *et al*., 2006).

Scale	Description
1	No visible symptoms.
3	Lesions are limited to cotyledonary tissues.
5	Lesions have progressed from cotyledons to about 2 cm of stem tissues.
7	Lesions are extensive on stem and branches. The foliage exhibits chlorosis and necrosis.
9	Most of the stem, petioles, and growing point are infected. A considerable amount of pycnidia and sclerotia is produced.
10	Pre-emergent seedling damping off.

Figure 6a. Rating scale from Abawi and Pastor-Corrales (1990; 1 to 9 scale) for aboveground infections of *Macrophomina phaseolina* on common bean *(Phaseolus vulgaris L.)* and adapted for cowpea *(Vigna unguiculata* (L.) Walp.).

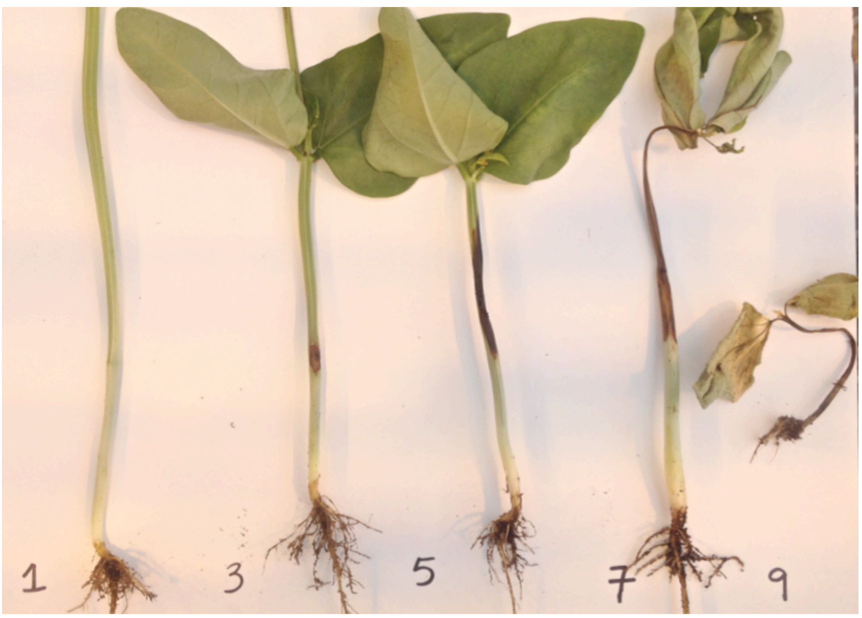

Figure 6b. Cotyledonary nodes of cowpea seedlings displaying symptoms as described by Abawi and Pastor-Corrales (1990) with ratings of 1 (far left) to 9 (far right).

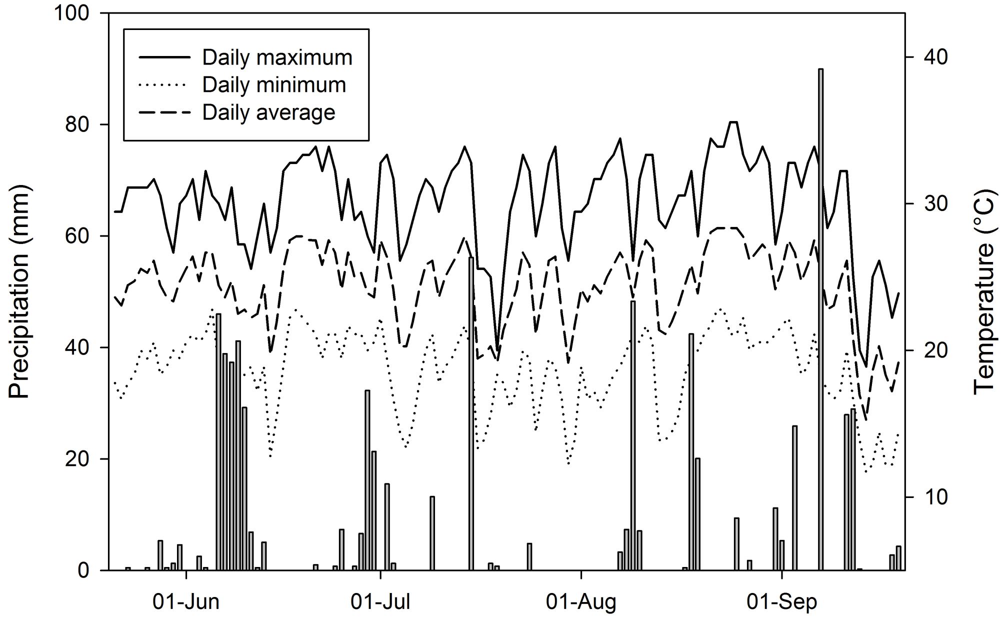

Figure 7. West Tennessee Research and Education Center (WTREC) temperature data recorded as daily maximum, daily minimum and daily average (˚C) and precipitation recorded as rain (mm). Represents data from planting on May 21, 2014 to plant sampling September 19, 2014.

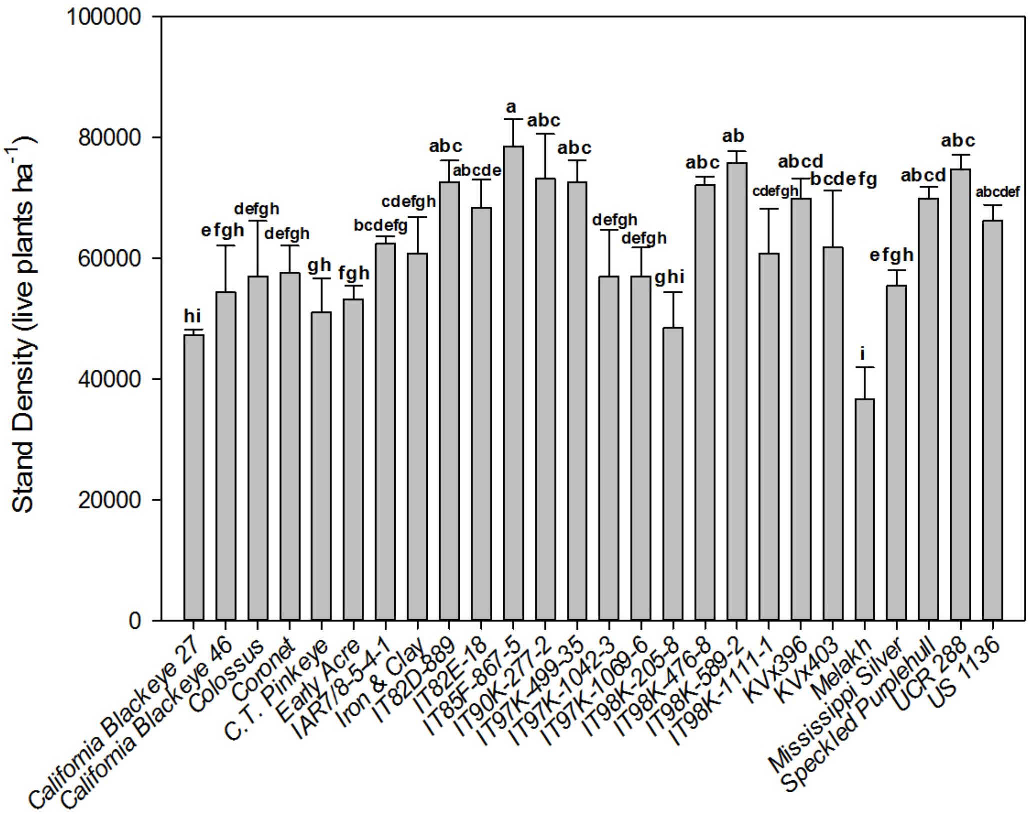

Figure 8. Field study stand density at 7-weeks (flowering) post planting as influenced by cultivar. Means indicated by the same letter are not significantly different ($p > 0.05$). Error bars represent raw standard error of the mean.

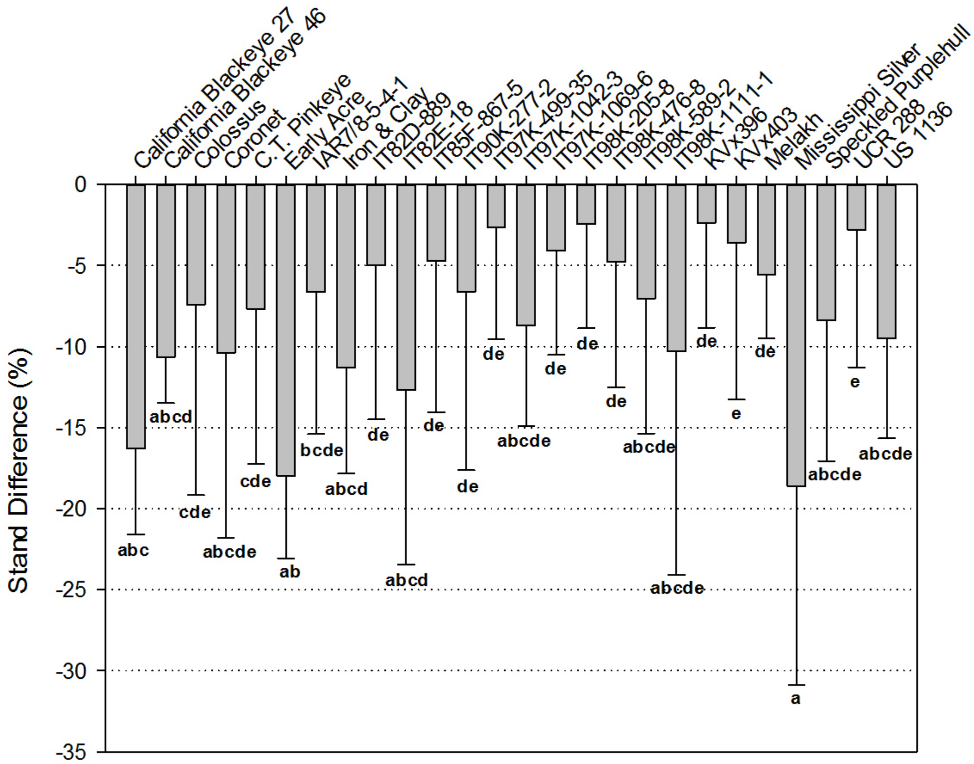

Figure 9. Percentage stand loss from 2-weeks (V2 to V3 growth stage) and 7-weeks (R1 to R3 growth stage) post planting as influenced by cultivar in the field study. Means indicated by the same letter are not significantly different ($p > 0.05$). Error bars represent raw standard error of the mean. Untransformed means are reported.

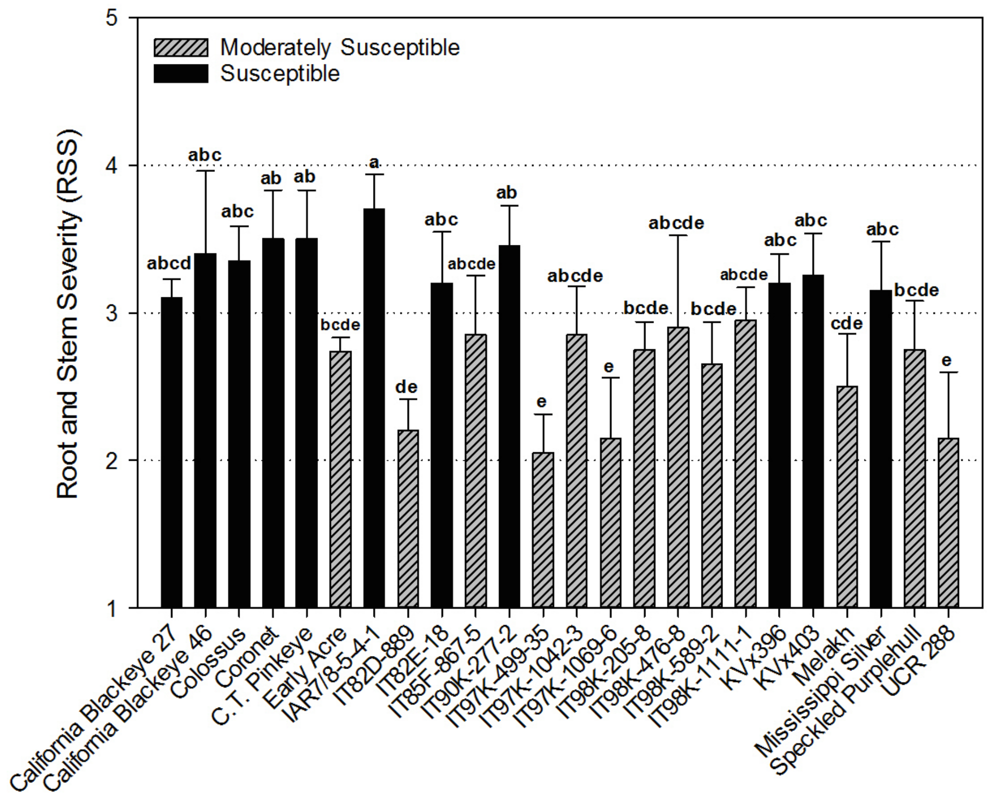

Figure 10. Average root and stem severity rating as influenced by cultivar in the field study. Genotypes were classified on a rating of 1 to 5 based on the intensity of internal stem discoloration (using the rating system of Mengistu *et al*., 2007, Paris *et al*., 2006). Means indicated by the same letter are not significantly different ($p > 0.05$). Error bars represent raw standard error of the mean.

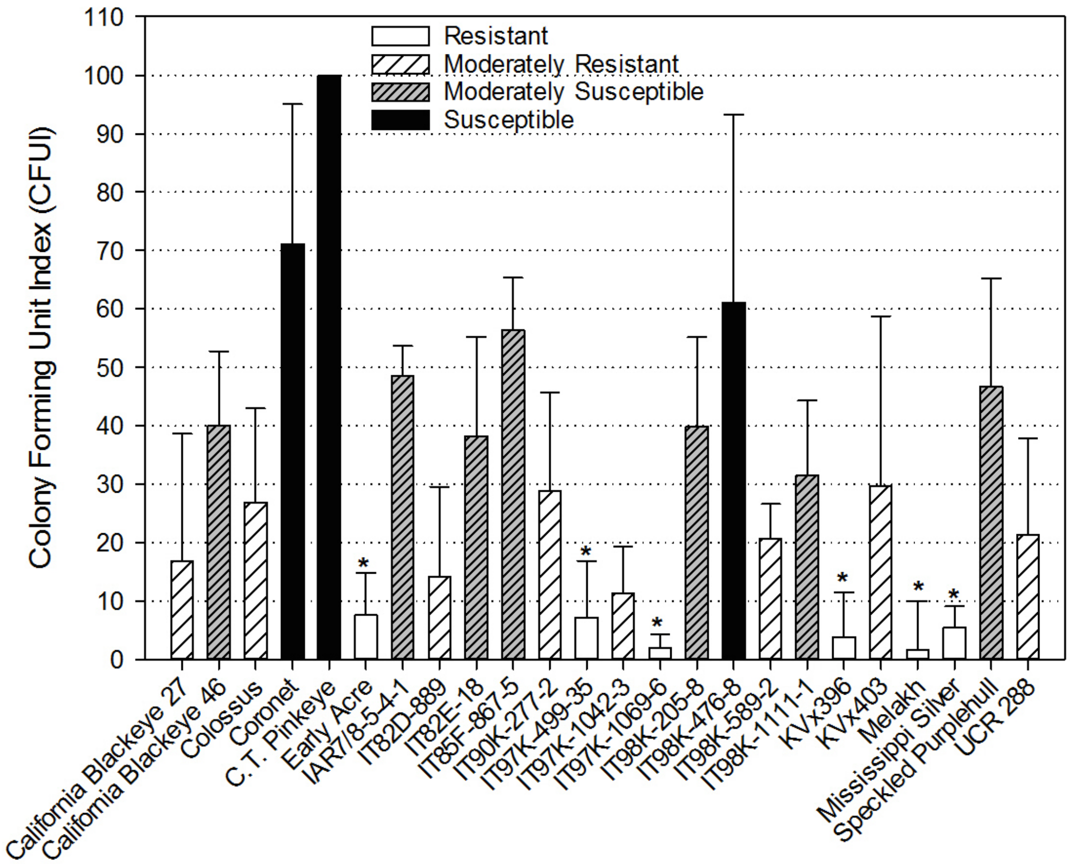

Figure 11. Colony-forming unit index (CFUI) as influenced by cultivar in the field study. CFUI values less than 10 were considered relatively resistant (white bars) as compared to C.T. Pinkeye, between 10 and 30 were considered moderately resistant (striped white bars), between 31 and 60 were considered moderately susceptible (striped grey bars), and greater than 60 were considered susceptible (solid black bars) (Schmitt and Shannon, 1992). Means indicated by asterisk are significantly different ($p > 0.05$) from C.T. Pinkeye, which was the cultivar with the highest average number of CFU per g. Untransformed means are reported. Error bars represent raw standard error of the mean.

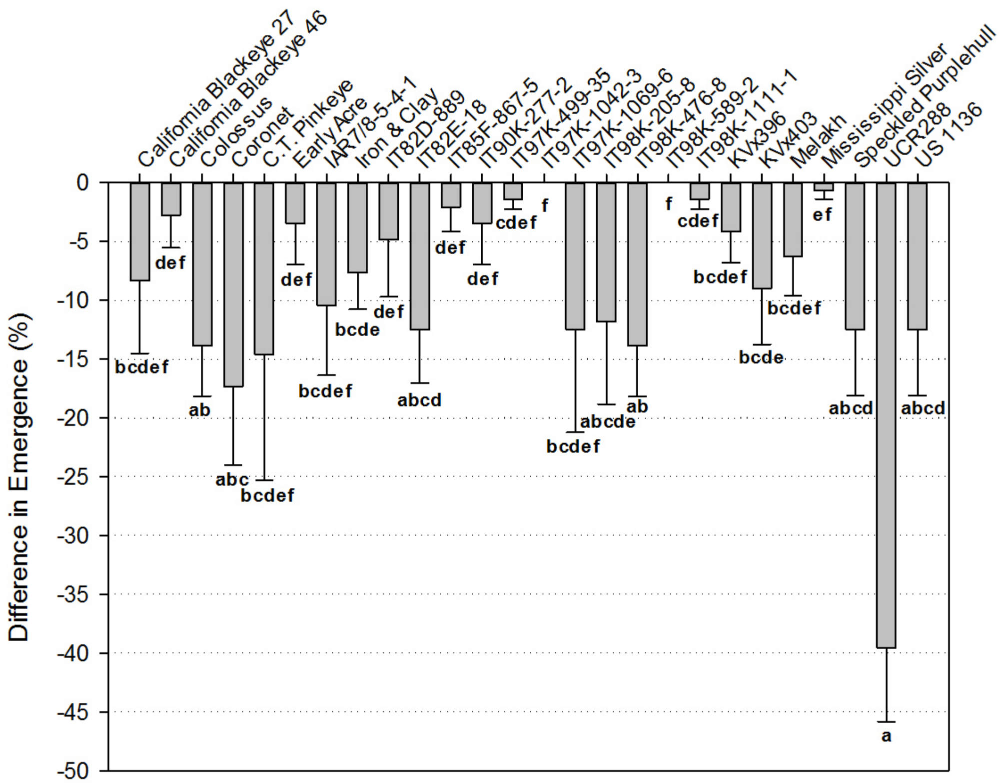

Figure 12. Difference in stand density of inoculated pots versus uninoculated pots as influenced by cultivar in the greenhouse study. Cultivars that had higher inoculated emergence percentages than the control were considered not different at zero percent. Means indicated by the same letter are not significantly different ($p > 0.05$). Error bars represent raw standard error of the mean. Untransformed means are reported.

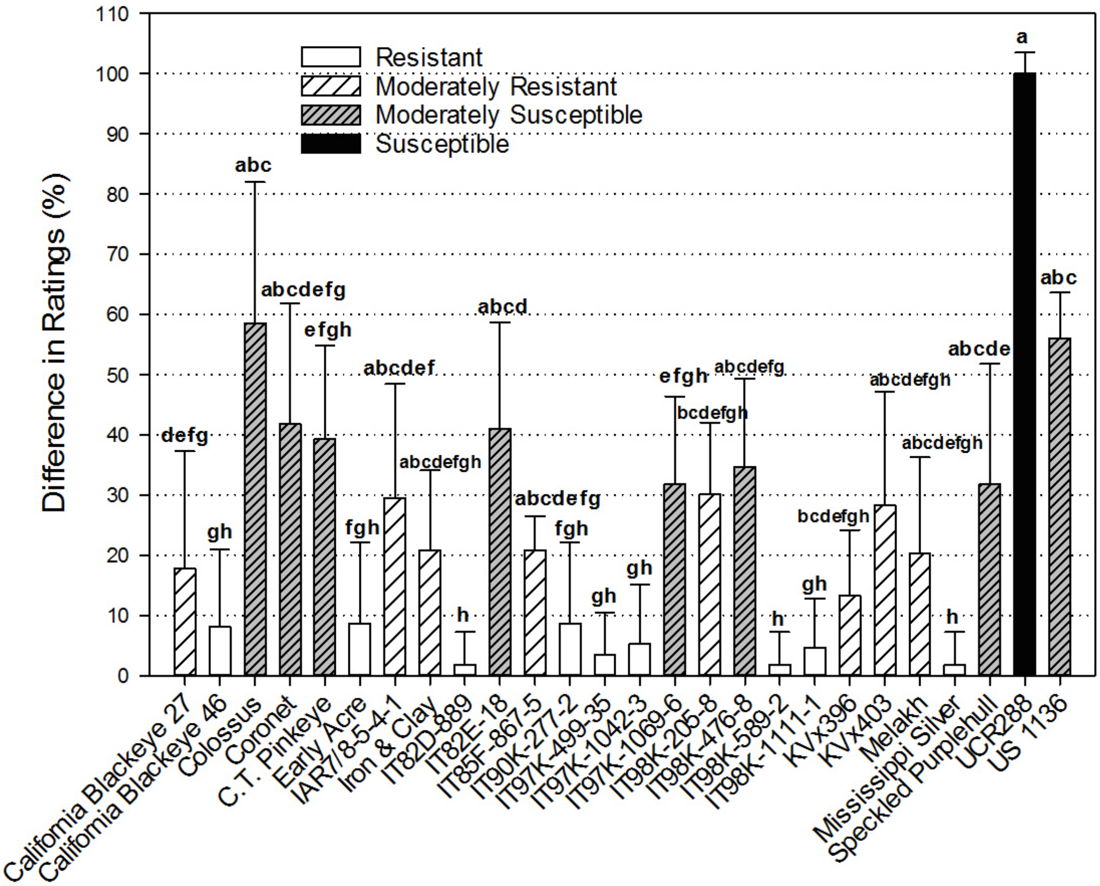

Figure 13. Difference in ratings of inoculated pots versus uninoculated pots as influenced by cultivar in the greenhouse screening. Values less than 10 were considered relatively resistant (white bars) as compared to UCR288, between 10 and 30 were considered moderately resistant (striped white bars), between 31 and 60 were considered moderately susceptible (striped grey bars), and greater than 60 were considered susceptible (solid black bars) (Schmitt and Shannon, 1992). Means indicated by the same letter are not significantly different ($p > 0.05$). Error bars represent raw standard error of the mean. Untransformed means are reported.

Conclusion

Cowpea has many traits that make it an attractive forage or cover crop for integration into organic production systems, including high rates of nitrogen (N) fixation, phosphorus (P) use efficiency, regrowth ability, and high digestibility. Cultivars Iron & Clay and Speckled Purplehull produced the greatest biomass over the two sites, suggesting that they offer the greatest potential for forage or cover crop use in regional organic and low-input systems of the cultivars evaluated. Both cultivars display indeterminate growth, high biomass, and are high in protein. Indeterminate cultivars were more competitive with weeds than determinate cultivars because they were able to cover more surface area in the plot. They both produced relatively high stand densities, suggesting that they are potentially more resistant to endemic seedling diseases. Soil P amendments can have conflicting effects in an organically managed system. Many cowpea accessions are not screened for P use efficiency and cultivars screened in this trial did not respond to P fertilization in low P soils. Our results also suggest that P amendment may increase relative competitiveness of weeds with cowpea in these low P soils under organic management.

Charcoal rot (*Macrophomina phaseolina*) can cause pre- or post-emergent damping off, black cotyledonary lesions at varying degrees of severity or it can persist in a crop showing little to no disease symptoms in many host crop species globally. Cowpea displays similar symptoms to common bean in seedling stages and similar symptoms to soybean in later maturity stages. In this cowpea cultivar screening, IT85F-867-5 and IT98K-589-2 displayed the highest stand densities in both the field trial and the greenhouse study, suggesting they may be resistant to *M. phaseolina* in the seeding disease stage. In the greenhouse, UCR288 was the worst performer in stand losses and had the highest rating differences from inoculated to uninoculated pots indicating that this cultivar may be susceptible in the seedling disease stage. Later maturing cultivars, such as Iron & Clay and US1136, may be able withstand infection from *M. phaseolina* to produce grain or forage yields due to known genetic resistance or physiological mechanism involved in plant aging. C.T. Pinkeye and Coronet displayed the highest numbers of CFU at maturity and were amongst the highest in visual RSS ratings indicating that their physiology may provide a more desirable environment for microsclerotial growth later in the season. Environmental conditions are an important factor when screening for *M. phaseolina* due to its high association with moisture stress.

Cowpeas vary greatly in disease resistance, growth habit, photosensitivity, determinacy and nutritional quality. Cultivar choice is the most important consideration when selecting cowpeas for forage or grain production systems and producers should select an appropriate cultivar to fit production needs and objectives.

Vita

Samantha Hill was born in Miami, FL, to the parents of Larry and Debby Hill. She is the eldest of her two siblings, Victoria and David Hill. She graduated from Oak Mountain High School in 2006 with an Advanced Academic Diploma and continued her education at Millsaps College where she earned a Bachelors of Science degree in Biology in 2010. Shortly after college graduation, Samantha was accepted as a volunteer in the United State Peace Corps where she served as Coastal Resource Extension Management in Manapla, Negros Occidental, Philippines. Her work in the Peace Corps was devoted to coastal clean ups, coastal ecosystem restoration and solid waste management. She recognized that something as simple as domestic plant production for produce items could reduce the amount of waste generated by a local population. This drove her to pursue her education in Plant Sciences at the University of Tennessee, Knoxville where she was granted a graduate assistantship. Samantha graduated from the University of Tennessee, Knoxville in 2015 with a Masters of Science degree in Plant Sciences and plans to work for local farming co-ops in Birmingham, AL to enhance organic fruit and vegetable production.