COWPEA AS A MULTIFUNCTIONAL LEGUME IN A TEXAS DOUBLE CROPPING SYSTEM

A Thesis

by

MELANIE L. AIOSA

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Chair of Committee,	Francis M. Rouquette, Jr.
Co-Chair of Committee,	Clark B. Neely
Committee Members,	Gerald R. Smith
	Vanessa Corriher-Olson
	Cristine L. Morgan
	Russell W. Jessup
Head of Department,	David D. Baltensperger

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ABSTRACT

Cropping systems involving legumes are increasing due to the costs and environmental concerns attributed to N fertilizer. A green manure cover crop in a cropping system can benefit the soil by improving fertility and disrupting disease, insect or weed cycles. Two studies were conducted during two years at the Texas A&M AgriLife Research and Extension Center at Overton, TX. Cowpea (*Vigna unguiculata* [L.] Walp) was evaluated as a multifunctional legume in a double cropping system with forage rye (*Secale cereale* L.), and 37 cowpea plant introduction (CPI) lines were evaluated for seed and biomass production. These studies measured cowpea biomass production, seed yield, carbon and nitrogen plant concentrations, and soil N contributions. Primary objectives were to 1) maximize rye biomass production through the incorporation of cowpea biomass in combination with different nitrogen rates concurrently; and 2) evaluate new CPI lines for traits needed in the cropping system and for potential seed production.

The green manure cover crop of cowpea showed no impact ($P \geq 0.34$) compared to fallow plots when comparing rye biomass in 2015 and in 2016 ($P \geq 0.26$). Nitrogen rates had a positive linear relationship with rye biomass for 2015 (r^2=0.79) and 2016 (r^2=0.97). The lack of response of soil N from the summer green manure cover crop may be likely due to the soil type. Darco loamy fine sand (loamy, siliceous, semiactive, thermic Grossarenic Paleudult), is an excessively drained soil and has very little nutrient holding capacity.

The CPI lines were evaluated based on biomass production and seed yield by late September to incorporate into a cropping system with forage rye, and nematode resistance. Cowpea biomass production peaked at 3021 kg ha^{-1} in 2015 and at 6009 kg ha $^{-1}$ in 2016. Cowpea seed yield ranged from 0 to 2322 kg ha^{-1} in 2016 and 9 to 351 kg ha $^{-1}$ in 2015. One CPI line was identified with root-knot nematode resistance. Separately the CPI lines exhibited valuable traits such as biomass production, seed yield, and nematode resistance and will be further evaluated for additional breeding programs.

DEDICATION

To my parents, Vicky and Chris. Thank you for everything you do.

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NOMENCLATURE

COMB	Combine
DAP	Days After Planting
FAL	Fallow
IAC	Iron and Clay
NUE	Nitrogen Use Efficiency

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CHAPTER I

INTRODUCTION AND LITERATURE REVIEW

Cropping systems

Cropping systems using multiple crops in rotations or sequences have been used for centuries for the benefits of soil fertility and nutrient cycling. “Double cropping- also known as sequential cropping, is the management of planting a second crop immediately after the harvest of the first, and includes harvesting two crops from the same field in one year” (Nafziger, 2015). Double cropping has shown the potential for benefiting grain cropping systems by incorporating legumes into the system without sacrificing grain production (Martens et al., 2001, 2005). Double cropping requires a season(s) long enough for crops to mature and allow transition time between the two crops. One region with a suitable climate where double cropping is commonly found is in the southeastern U.S. In the East Texas Pineywoods ecoregion (Gould, 1962), common cropping systems include grain and fiber row crops as well as forage crops with pasture (Neely, 2013).

Cropping systems are employed because of the benefit one crop may have when rotated with another. Yields of non-leguminous crops can be greater when paired with a legume crop. Such benefits to yield have been shown with wheat (*Triticum aestivum*) and soybean (*Glycine max*) in the northern Great Plains (Badaruddin and Meyer 1994). A green manure crop has been defined as “plant material incorporated into the soil while green or at maturity” (Fageria, 2007). A common purpose of green manure crops in a cropping system is to improve soil health, and act as a nutrient source for the subsequent crop (Cherr, 2006; Fageria, 2007; Kasper and Singer, 2011). When green manure crops are incorporated into the soil, plant tissue is mineralized over time and nutrients become available for subsequent crops. Green manure cropping is not a new concept. For example, the Greeks would turn under faba beans (*Vicia faba* L.) for soil improvement (Fageria, 2007). Research has been conducted on the prospect of green manure crops as early as 1917 in the U.S. (Pieters, 1917). Due to high energy costs and rising environmental concerns the application of green manure crops has become increasingly popular (Evers, 2000; Fageria, 2007).

Cattle production in the southeastern U.S. is a vastly important industry, thus forage production is crucial to stocking rates. A cropping system that incorporates a crop for livestock can have economic benefits by providing N and, environmental benefits by decreasing pollution (Franzeluebbers, 2007). Small grains used as a winter pasture are common in the southeastern U.S. to extend the grazing seasons while warm-season perennial grasses are dormant (Altom et al., 1996; Newell, 2012). Rye (*Secale cereale* L.) is one of the small grain species that thrive in east Texas winters and can tolerate low pH soils in the region (Kim, 2001; Newell, 2012). Hardiest of cereals (Corriher-Olson and Redmon, 2015), rye can be seeded later in the fall compared to other crops and still produce considerable amounts of biomass (Nelson, 1981). Yield values for ‘Maton’ rye, a commonly used forage in the southeastern U.S., average more than 8,000 kg ha^{-1} and ‘Elbon’ rye yields averaging about 7,000 kg ha^{-1}from multiple harvests per year at Texas A&M AgriLife Research Center, Overton, Texas (Neely, 2015). Both Elbon and Maton rye are later forage producers when the climatic conditions are more favorable for growth and they typically gain an advantage over earlier rye varieties (Baker, 2004). In prior studies by Rouquette et al., (1990) rye has been incorporated into cropping rotations with cowpea (*Vigna unguiculata* [L.] Walp) and succeeded as a winter grazing option without added N and based on average daily gains of stocker cattle.

Soil properties

Nutrient cycling within a cropping system is largely dependent on the soil type; different types of soil can hinder nutrient retention. Soil properties specifically clay content versus sand content depict nutrient retention. Sandy soils are common in the southeastern U.S. and are generally well drained. Such well drained soils contributing to leaching of nitrate especially during the spring time heavy rains and lack the binding properties involved in nutrient retention (Appel and Mengel, 1993). Leaching of N occurs more often in a course texture soil as opposed to fine-textured soil where the clay particles are closer together and restrict downward movement (Sogbedji, 2000; Geleta et al., 1994; Havlin, 2014a). The mobility of N makes tracking and evaluation of use, difficult in field situations.

Nitrogen

Nitrogen is one of the most crucial nutrients for plant growth and, also the most common limiting soil factor in plant production. Sustainable practices can benefit producers economically and also benefit soil health (Liu et al., 2003; Turmel et al., 2014). Nitrogen use efficiency (NUE) is important in sustainable agriculture systems, since N is a costly factor in high production systems (Ta et al., 1989; Redmon et al., 1995; Evers, 2000). One way to improve NUE is through cropping systems (Raun and Johnson, 1999). Cereal crops have a greater NUE when paired with a legume species in a rotation. Raun and Johnson (1999), found that wheat following a legume had a greater efficiency than wheat following fallow. Cropping systems that involve a legume can be very beneficial to the subsequent crop that will be grown due to their symbiotic N fixation and N transfer. “The ability to convert atmospheric N_2 to plant available form is second only to photosynthesis in importance for maintenance of life on earth” (Hons, 2015). In the U.S., approximately 20% of N supplied to crops is from biological fixation and crop residues (Havlin et al., 2014).

Similar to N, C is a driving factor in production systems as plant residue is comprised of 40-45% C (Havlin et al., 2014). When paired with N, C:N ratios are generally a predictor of net N mineralization (Neely, 2013), and important in understanding decomposition rates. When the C:N ratios are wider N is limiting and residue decomposes at a slower rate such as grasses with C:N ratios ranging from 50-90:1. Legumes typically have a narrow C:N ratio, and rapidly decompose becoming readily available for the subsequent crop with C:N ratios ranging from 10-30:1 (Quemada and Cabrera, 1995; Neely, 2013). Quemada and Cabrera (1995) showed selective properties of four cover crops including legumes and non-legumes species. The legumes had a lower C:N ratio due to greater N content.

Legumes

Legumes have an increasingly important role in cropping systems due to: an immediate seed crop for human consumption; increased soil fertility with nutrient rich residues; and a vital role in livestock feed (Sinclair, 2012). Legume species have been utilized in cropping systems as green manure crops or intercropping (Cherr, 2006; Fageria, 2007; Rouquette and Smith, 2010) and can provide a better synchronization of plant uptake of N residues when compared to inorganic N sources (Cherr et al., 2006). Warm season legumes contribute large amounts of N to the soil, but with limitations as the warm growing season is the time that most primary crops are grown. Incorporating a warm-season legume into a cereal cropping system is ideal for a winter forage grazing system. The addition of legumes to the system is beneficial as a N input. The specific *Rhizobium* bacteria infect the root hairs of the legume host plant and form nodules. These nodules, provided by the plant serve as a home for the bacteria, and in turn the rhizobia use the enzyme nitrogenase to break the triple bond of N^2 from the atmosphere to ammonium for the plant to use (Rouquette and Smith, 2010; Bauer, 1981; Stern 1993; Sinclair, 2012; Havlin, 2014a). The fixation of atmospheric N can occur with no fertilizer N added to the soil, and under low soil N conditions legumes derive much of their N through symbiotic fixation of atmospheric N_2 (Evers, 2000). A legume growing in a sandy soil is more likely to fix N from the atmosphere compared to a legume growing on a clay loam soil where nutrients are more abundant in the soil (Evers, 2000). Estimates of biological N fixation range from 20-200 kg N ha^{-1} per year depending upon species, intercrop, nodulation productivity, duration of crop growth and soil fertility

(Brophy, 1987; Sinclair, 2012; Silveira et al., 2014). Unless there is available N in the soil or it is applied as an inorganic fertilizer, the fixation from a legume is the most important source of N in a cropping system (Stern, 1993).

There are several factors that can influence the amount of N fixation by legume: 1) High soil N, either from commercial application of N or from continuous growth of legumes, can cause a delay in the nodule formation. 2) Improper inoculation or no inoculation can restrict nodule formation or performance. If the proper bacterium is not present in the soil there may not be an initiation of nodule formation on the plant; 3) Any type of stress that affects the plant will immediately affect and or inhibit the nodulation process; including drought, low temperatures, disease or low nutrient status (Evers, 2000; Haby et al., 2006; Peoples et al., 2009).

Cowpea

Cowpeas were extensively grown in the southern U.S. for the use as green manure crops, seasonal forage or cover before the development and adaptation of inorganic N fertilizers (Harrison et al., 2006). Originating in the South African region, cowpeas are a versatile and widely adapted legume grown from Africa and Asia to the Americas. They can be used for many different purposes such as fodder or pulse crop due to their high protein content (Singh, 2014). In the U.S., cowpeas are commonly grown in California and Texas and are used for wildlife forage, a dry pulse crop, or a fresh vegetable for human consumption. Cowpea research is not a new concept in Texas as William Brittingham was the first to initiate a cowpea improvement system in 1942 (Miller, 1979). Cowpeas are excellent warm season cover crops because they are well adapted to drier humid environments; thrive in a well-drained soil; can withstand alkaline and acidic conditions; tolerate heat and drought stress; and produce high biomass with little to no fertilizer N applied (Elowad and Hall, 1987; Fery, 1990; Grichar et al., 1996; Ehlers and Hall, 1997.; Singh, 2003; Harrison, et al., 2006).

The primary threat to grain production of cowpea are pests such as aphids, nematodes, and wildlife. Aphids, specifically the cowpea aphid (*Aphis craccivora),* is widely distributed in cowpea growing regions and affects the host plant as they remove assimilates and increase the respiration rate (Singh and Van Emden, 1979). Aphids are especially damaging in drought prone areas and are prevalent from seedling through pod developement stages. When infested severely with aphids, they can completely destroy a crop (Singh, 2014). Nematodes are especially damaging to cowpeas, particularly four species of root knot nematode belonging to the genus *Meloidogyne* (Singh, 2014). With the infection of root knot nematodes, roots become infected by the juvenile nematodes as early as 6 days after planting. In the case of severe infection, root growth is affected and the plant cannot efficiently absorb water from the soil. Cowpea varieties that exhibit some resistance to pests are beneficial in production but also in regions where pesticide application is not an option or in heavy sandy soils which are common for most of the Southeastern U.S. (Ehlers and Hall, 1997; Ehlers et al., 2009). Cowpeas are planted for supplemental browse for white-tailed deer all through the southern US. Because of the high nutritive value deer selectively graze cowpea, especially in the summer months when other forms of browse are not as readily available (Redmon and Rouquette, 2000).

Cowpeas vary widely in pod type, seed color, growth habit and time of maturity. Seed type preference varies in different parts of the world based on intended use. Cowpea varieties that are early maturing are useful in a double-cropping system since they produce seed and contribute to the system in sufficient time to plant the subsequent crop. In proper growing conditions, cowpea can fix up to160 kg ha^{-1} N in 60 days, meeting most of its own requirements and contributing up to 40 kg ha^{-1}N to the soil (Singh, 2014). In production operations where the objective is to provide forage for wildlife or livestock, a later maturing, indeterminate type cowpea such as ‘Iron and Clay’ would be beneficial (Redmon and Rouquette, 2009). Iron and Clay is an old cultivar that is a varietal mix and produces large amounts of biomass and is resistant to root-knot nematode (Redmon et al., 1992; Smith et al., 2006; Neely, 2013). It grows through the summer and remains vegetative into early fall at which time it produces seed. Iron and Clay is the recommended cowpea cultivar for east Texas (Redmon et al., 1992; Smith et al., 2010). ‘Combine’ cowpea; however, is a determinate, early maturing type cowpea that is grown for seed production and ground cover through the summer months in Texas. Combine matures and produces seed in about 60 days, and can be useful in a cropping system as a green manure crop and incorporated into the soil in a timely manner. The concept of the 60-day cowpea was originally for incorporation into a maize (*Zea mays*) cropping system. Cowpea grows and produces seed through the short rainy season as the maize could not withstand the drought type conditions, and the maize is grown during the long rainy season (Singh, 2014). In the East Texas Pineywoods region (Gould, 1962) cowpea has been shown in previous research by Redmon et al.,

(1992) and Rouquette et al., (2006) to be well adapted and beneficial to cropping systems as a green manure crop.

The incorporation of legumes into cropping systems play a major role in the U.S. since commercial fertilizers are costly inputs in crop production systems. Legumes have the potential to replace commercial N fertilizer inputs, and improve soil health. Evaluation of the contribution of cover crops in the form of legumes is crucial in determining their impact. Specific legumes and their adaptations to different regions of the U.S. are important to consider when incorporating a legume into a cropping system. Soil type, moisture availability, nutrient status, and growing characteristic can hinder the beneficial impacts of green manure legume. Rye as winter forage is commonly grown and adapted to colder temperatures, and when paired with cowpeas as a green manure crop in East Texas, could serve as a winter grazing option.

Different cultivars of cowpeas have been used in the southeastern U.S. for decades and have served as forage for deer, a green vegetable crop, or a pulse crop. A specific cultivar that has the ability to produce seed in mid to late Sept., and accumulate a significant amount of biomass while being resistant to nematodes and other pests in the area is needed. Such a variety would simultaneously serve as a N contributing, green manure cover crop while also providing an opportunity for a cash crop from seed production. The main focus of this study was to develop a cropping system involving cowpea used as a dual purpose green manure cover crop-grain crop, combined with rye as winter forage for cattle. Results from this study could initiate a double-cropping system where both crops are beneficial to the producer and the N fertilizer inputs are decreased.

CHAPTER II

A DOUBLE CROPPING SYSTEM EVALUATING THE N TRANSFER FROM COWPEA TO FORAGE RYE

Synopsis

Cowpea (*Vigna unguiculata* [L.] Walp) is a legume commonly grown in the southeastern U.S. as a fresh vegetable, wildlife browse, or seed crop. In East Texas, the use of cowpea as a green manure has potential to provide N to a cool season forage or grain crop. The objective of this study was to evaluate two cowpea cultivars as green manure crops in a double-cropping system to supply N to forage rye (*Secale cereale* L.). The 2-year study was conducted at the Texas A&M AgriLife Research and Extension Center at Overton, TX on a Darco soil (loamy, siliceous, semiactive, thermic Grossarenic Paleudult) during 2014 - 2016. Summer cover crop treatments included an early maturing cowpea ‘Combine’, a late - maturing cowpea ‘Iron and Clay’, and summer a fallow site without a crop in 6 by 12 m plots. The cover crop treatments and four N rates 0, 34, 67, 101 kg ha^{-1} were applied in the fall in a factorial arrangement using a split-plot design with 4 replicates for rye biomass. Cowpea biomass incorporated as green manure combined for both years was 6913 kg ha^{-1} and 3856 kg ha^{-1} for Iron and Clay and Combine, respectively. A cool season annual grass ‘Elbon’ rye was grown in 2015 and ‘Maton’ rye in 2016. Rye biomass was harvested with a mechanical harvester. Data were analyzed using PROC MIXED and PROC REG in SAS® 9.4. The summer

green manure crop, cowpea, was not effective as an N source on rye forage biomass after two years of cropping. Soil N data were analyzed and showed no difference ($P \geq 0.40$) between plots of cowpea or N rate. Green manure cover crop of cowpea had no effect on the soil N; thus there was no impact on rye biomass. The fertilizer N rate did show a positive linear relationship measurably with rye biomass both years ($r^2 = 0.79$; $r^2 =0.97$)($P < 0.0001$). Darco is a deep, sandy soil and more time is needed to impact the organic matter content. This cropping system may benefit the soil in a long term production setting; however, short-term measurements did not reveal detectable differences for soil N among cowpea varieties versus fallow.

Introduction

Cover crops are a more sustainable practice for agriculture as they improve soil properties, reduce erosion, and also can help reduce weed invasion (Harrison et al., 2006). Desirable aspects of a cover crop are biomass accumulation and N fixation potential, which also aids in weed control and nutrient inputs into the soil (Martens et al., 2005). Cowpea as a green manure crop can contribute to the soil by incorporating plant residue and allowing N transfer from legumes to a non-legume crop (Evers, 2000; Cherr, 2006; Rouquette and Smith, 2010; Neely, 2013). Cowpeas are a widely adapted legume in the southern U.S. because they are drought-tolerant and grow well in acidic soil. A healthy crop of cowpea has potential to fix up to 160 kg of N per ha and contribute up to 40 kg ha^{-1} N in the soil (Singh, 2014).

Cowpeas are a genetically diverse species with diverse growth habits, seed production rates, and maturity ranging from 60 to 120 d. A maturity of 60 to 100 days for a warm season cover crop is crucial in a double-cropping system for the southeastern U.S. if seed production is desired. A longer maturing cowpea that provides adequate ground cover would also be useful in a cropping system for weed suppression; however, seed production may be limited due to timing of maturity. Early maturing, warm-season legumes allows adequate time between crops for decomposition of plant matter and field preparation prior to subsequent crop (Martens et al., 2001).

Climates in the southeastern U.S. are well suited for double-cropping systems since the growing season is long and rainfall is ample for both crops (Martens et al., 2001). Rye (*Secale cereale* L.) is a regularly used cool-season annual grass grown in East Texas and the southeastern states. It is cold hardy, tolerant to acidic soils, and will produce large amounts of biomass (Nelson, 1981; Moyer and Coffey, 2000; Neely, 2015). Grazing rye in the winter months can offer opportunity for economic gain while the soil receives additional organic matter and has not been left fallow (Schomberg et al., 2014). A double-cropping system incorporating both cowpea and rye has potential to reduce commercial fertilizer inputs and provide ground cover throughout the year.

At the Texas A&M AgriLife Research and Extension Center at Overton, TX, research has been conducted evaluating commonly grown cowpea ‘Iron and Clay’as a cover crop (Rouquette et al., 1990), forage for deer (Redmon and Rouquette et al., 2000; Evers et al., 2002), and also for nematode resistance (Smith et al., 2006). A cropping system including cowpea as a green manure cover crop and rye as a winter grazing forage has shown beneficial average daily gain for grazing cattle in East Texas (Rouquette et al., 1990). Rouquette and Smith (1998) have also evaluated the acceptance of cattle to grazing cowpea. The legume provides higher nutritive value forage in the diet; however, palatability limits intake from cattle. When incorporating rye into a no-till cotton system, Schomberg et al. (2014) showed that grazing winter forage offered economic gains for the producer in years where the cotton crop was not profitable. A double-cropping system with a grazing component would be beneficial. The objectives of this research were to 1) evaluate rye forage yield when preceded by a warm season green manure crop; and 2) quantify soil N contributions from cowpea as a green manure in combination with fertilizer N rates.

Methods and materials

Overall experiment

This experiment was located at the Texas A&M AgriLife Research and Extension Center at Overton, TX (32° 18’22.18”N 94°58’23.14”W) on Darco loamy fine sand (loamy, siliceous, semiactive, thermic Grossarenic Paleudult). Land preparations included rotary tillage and 0-20-20 fertilizer (6 S - 3.75 Mg - 0.19 B) applied at 385 kg ha^{-1} on 18 June in 2014 and 2 June in 2015. The experimental design was a split-plot with four replicates. Main plots included fallow, Iron and Clay (IAC) and ‘Combine’ (COMB) cowpea with dimensions of 6 by 12 m. Subplots included four N rates. Soil samples were taken at 0-15 cm and 15-30 cm depths at the beginning of the experiment on 10 June in 2014, and at termination of the experiment on 17 May in 2016. Soil samples were dried at 52°C, and soil C and N were measured using combustion analysis (Kirsten, 1979).

Cowpea cover crop green manure

Before cowpeas were planted, *trifluralin (*presented as the butoxyethyl ester; Treflan ™) was applied 19 June, 2014 and 1 June, 2015 at 1.17 L ha^{-1} as a pre-emergent for weed suppression. After herbicide application, the soil was rotary-tilled and packed using a roller-packer. All crops were planted using a JD BD 1110 grain drill with 15 cm row spacing. Cowpea planting depth was 1.9 to 3.2 cm. Before planting, cowpeas were inoculated with commercial *Bradyrhizobium spp.* Cowpeas were planted 24 June in 2014 and 9 June in 2015. Due to different seed size, calibrations were used for each variety. In 2014, IAC was planted at 52 kg ha^{-1} and COMB at 40 kg ha $^{-1}$. In 2015, IAC was planted at 53 kg ha $^{-1}$ and COMB at 27 kg ha $^{-1}$. Seed population was about 80,000 ha^{-1} for each variety. An adjacent plot area was planted to cowpeas with the same preparations and planting depth, to allow for plant height measurements and biomass sampling and prevent traffic on the green manure cowpea treatments. This plot area was 3-m wide by 30-m long with two replicates each of COMB and IAC. Cowpeas received no additional N fertilizer. After germination, plant heights were taken weekly for seven consecutive weeks in 2014, and five consecutive weeks in 2015. Along with heights, developmental plant staging was recorded (Southern IPM, 2016). Two harvests for biomass were taken on 7 Aug. and 5 Sept. in 2014. The area harvested included two rows and 1 m in length and heights of harvest included 0, 7.6, and 15 cm. Plant heights were selected to assess cowpea re-growth potential. In 2015 a biomass was taken at the end of the season on 23 July to document total biomass incorporated into the soil as green manure. Leaf and stem separations occurred after each biomass sampling to document characteristics of leaf to stem ratios for each cultivar. Plant samples were dried at 52° C, weighed, and ground to 1-mm particle size for N concentrations using the Dumas total combustion method (Leco FP-528, Leco corporation, St. Joseph, MI, AOAC (2000) [procedure 990.3]).

Rye forage

Rye was planted on 15 cm row spacing at 113 kg ha^{-1}. 'Elbon' rye was planted on 3 Oct. 2015 and 'Maton' on 28 Oct. 2016. Four N rates were randomly applied by hand to sub-plots (3.0 by 6.0 m) within each main plot as ammonium nitrate (34-0-0). In the first year, fertilizer N rates 0, 34, 67, 101 kg ha^{-1} were applied by hand on 31 Oct. 2014. In the second year, total fertilizer N rates 0, 67, 134, 202 kg ha^{-1} were split applied on 15 Dec. 2015 and 26 Feb. 2016.

Three rye harvests were made on 6 Feb., 17 Mar., and 20 Apr., 2015, and on 26 Feb., 17 Mar., and 8 Apr., 2016, using a mechanical harvester (Swiftcurrent, Sask, CA). Rye biomass from plots were subsampled for subsequent forage analysis. Before each harvest, plant height, percent ground cover, and rye growth stage were measured in all plots. All biomasssamples were weighed and dried at 52°C ground to 1 mm and tested for N concentration (Leco FP-528, Leco corporation, St. Joseph, MI, AOAC (2000) [procedure 990.3]). Following termination of winter rye growth, soil was rototilled in preparation for planting cowpeas for green manure.

Statistical analysis

All data were analyzed using SAS® 9.4 (SAS Institute, Cary, NC). Relationships among variables were examined using the PROC REG procedure. All response variables were analyzed using the MIXED procedure. Summer treatment and N rate were used as fixed effects, and replicate as the random effect. Years were analyzed separately due to different rate of N fertilizer. Results were reported as least square means, and mean separations were conducted using the PDMIX800 macro. Significance was declared when $P < 0.05$.

Climate

Weather conditions have a major impact on cropping systems, especially in East Texas where temperature can remain above 37°C for weeks during the summer, and with no precipitation. These climatic conditions can cause major stresses on crops. A summer cover crop that is drought tolerant and not substantially effected by high temperatures may be needed for use in the southeastern U.S. Weather conditions that occurred during this two year experiment were reflective of normal conditions in East Texas with droughty summer months (Texas A&M AgriLife Research-Overton Center, 2016)(Fig. 2.1). In 2015, less than 1 mm of rain fell during the month of July. Summer temperatures were average in 2014; however, in 2015 temperatures were above average (Texas A&M AgriLife Research-Overton Center, 2016)(Fig. 2.2). Sparse rainfall may have increased stress on cowpea. The conditions for forage rye were normal for 2014-2015 with heavy rains providing rye an early emergence and growth. In the second year, 2015-2016, warm temperatures paired with the late planting date deterred the initial growth of rye.

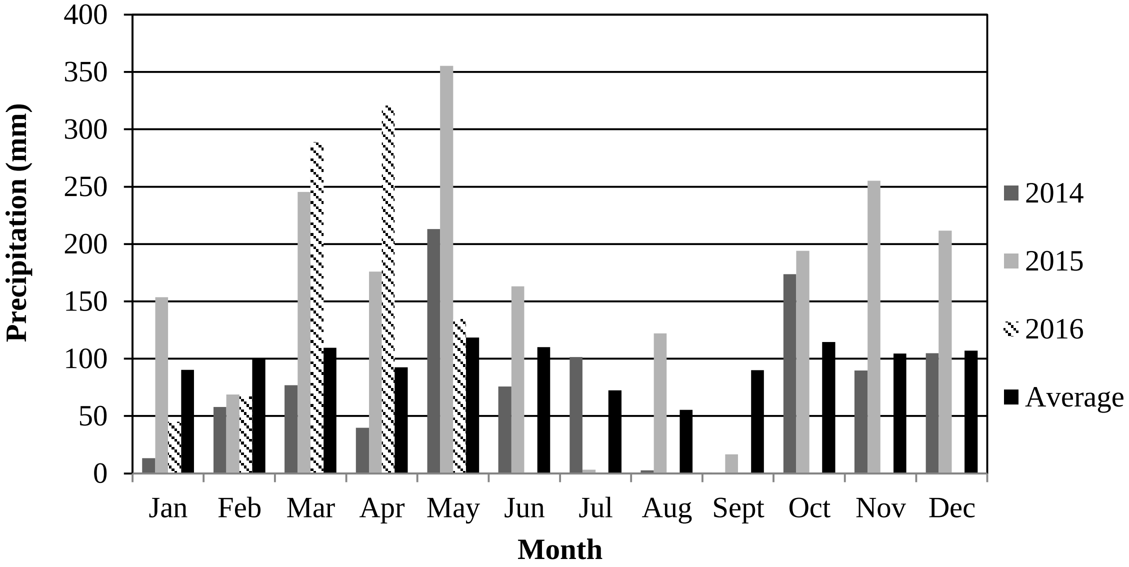

Figure 2.1 Monthly precipitation for Overton, TX for three years of the study, and the 30-year average.

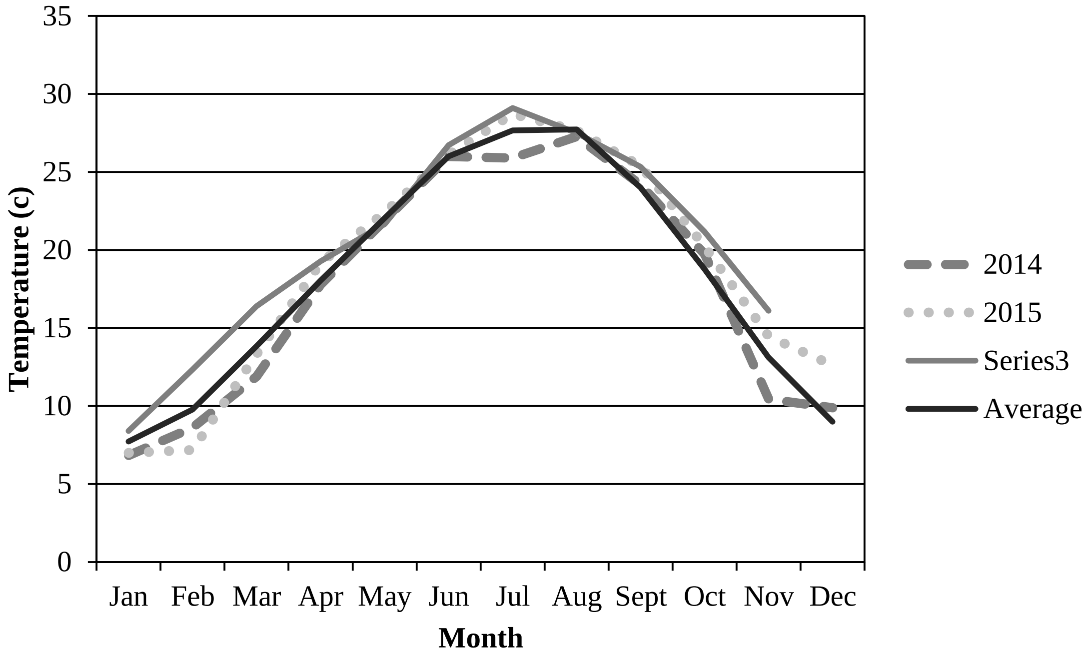

Figure 2.2 Average monthly temperatures for Overton, TX for the three years of the study and the 40-year average.

Results and discussion

Cowpea green manure impact on rye biomass

Cowpea cultivars had significantly different N ($P < 0.0001$) and C ($P < 0.0001$) yields at all harvest dates ($P < 0.0001$)(Table 2.1). Differences may be attributed to plant maturity rate. The early maturing variety COMB matures in 60-d and has larger N content during the earlier part of the growing season. The late maturity of IAC of 120-d infers the variety is still growing vegetative through most of the season at the Overton location. The C:N ratios for the two varieties were different ($P < 0.004$) at the harvest date 5 Sept., 2014. At this date the IAC variety is still growing in a vegetative stage compared to COMB that has set seed and started to drop leaves.

Table 2.1. Cowpea N and C yields separated by harvest date and cultivar.

Harvest	**Cultivar**	**C**	**N**	**C:N**
		kg ha^{-1}		
Aug., 7 2014	COMB	577 a†	42 a	14 a
	IAC	533 b	39 b	14 a
Sept., 9 2014	COMB	1016 a	24 b	22 a
	IAC	434 b	66 a	15 b
July, 23 2015	COMB	337 b	24 b	14 a
	IAC	424 a	33 a	13 a

Combine cowpea (COMB); Iron and Clay cowpea (IAC).
†Different letters represent significant differences ($P < 0.05$) between cultivars within each planting date and column.

The impact of cowpea as a green manure was documented by the biomass of rye. There was no difference between the two cowpea treatments or fallow plots when comparing the rye biomass in 2015 ($P \geq 0.34$) as well as in 2016 ($P \geq 0.26$). Nitrogen fertilizer rates and rye biomass separated by the cowpea green manure treatments are shown for 2015 rye in (Figure 2.3) and for 2016 in (Figure 2.4). Rye biomass within the summer treatments, showed no positive impact on N release from cowpea green manure on the soil. Similar results were recorded by Schroeder (1998), where cowpea was planted as a warm season cover crop preceding broccoli and did not provide sufficient N for the broccoli crop. Rainy weather conditions in the fall after cowpea incorporation may have accelerated decomposition of plant material and possible leaching or volatilization from the cowpeas before initial rye planting. In a study by Franzluebbers

(1994) where cowpea plant parts were analyzed on the rate of decomposition in moist soil versus repeatedly dried and wet soil. Their study indicated that soil that was repeatedly wet and dried showed smaller percentage of plant material decomposed when compared to the continuously moist soil. Since the soil in this experiment was not continuously moist the results of this study were similar to the findings by Franzluebbers (1994).

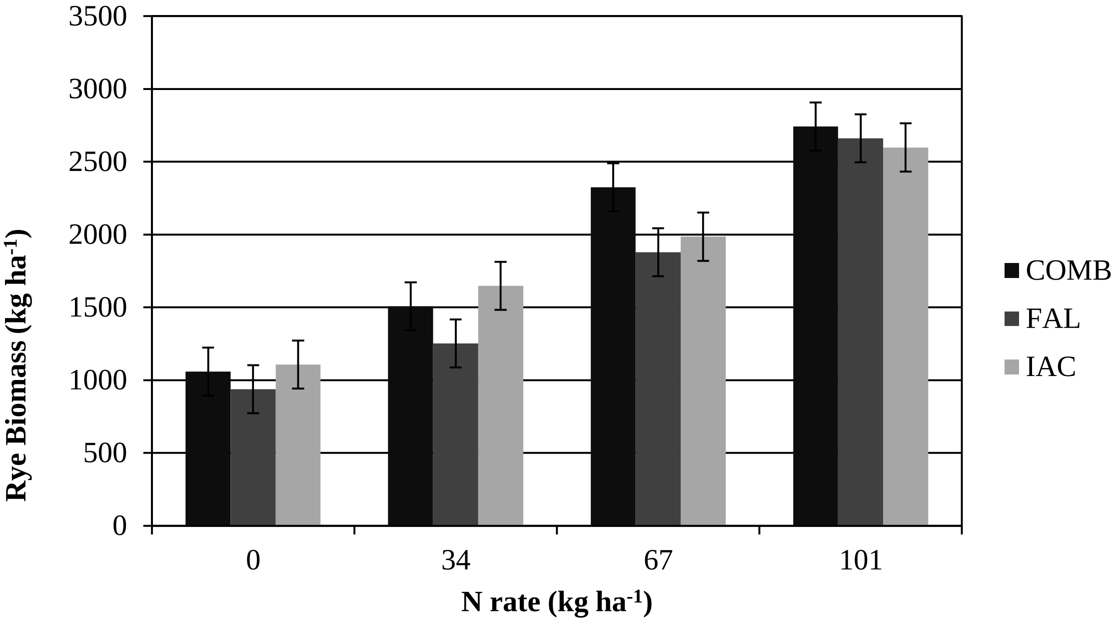

Figure 2.3 Rye biomass separated by N fertilizer rate and summer treatment for 2015. Combine cowpea (COMB); Iron and Clay cowpea (IAC); fallow plots (FAL).

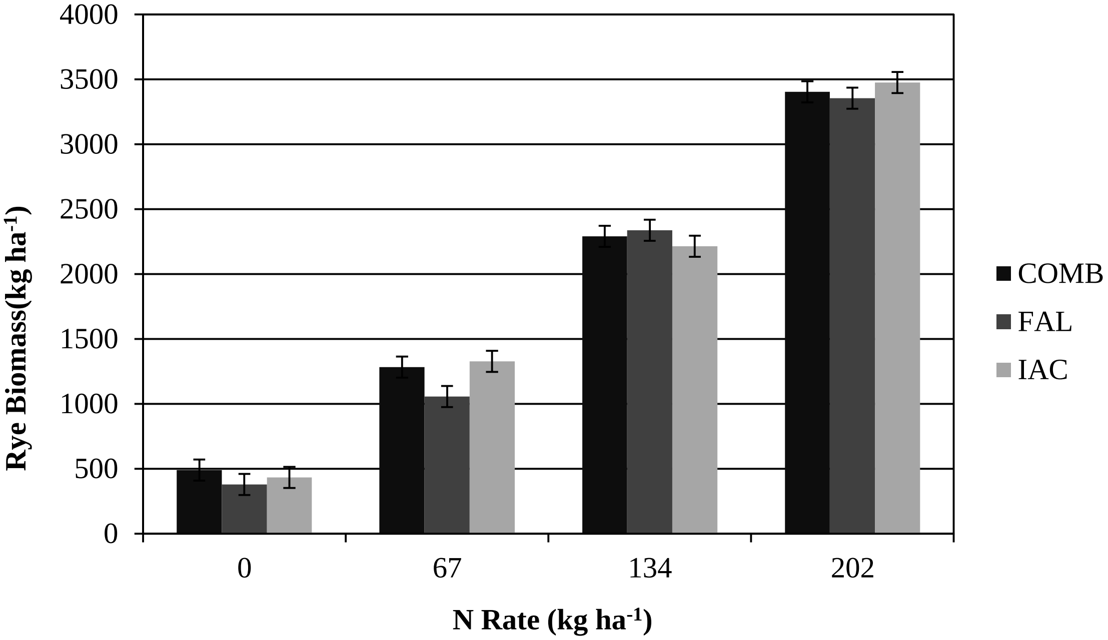

Figure 2.4 Rye biomass affected by N fertilizer rate and summer treatment for 2016. Combine cowpea (COMB); Iron and Clay cowpea (IAC); fallow plots (FAL).

Rye biomass

The rye biomass was significantly different *(P* < 0.0001)(Table 2.2) at each N rate for both years of cropping. There was a positive, linear relationship between rate of N fertilizer and rye biomass for 2015 (r^2=0.80) (P < 0.0001)((Fig. 2.5) and 2016 (r^2=0.97) (P < 0.0001)(Fig. 2.6). These results were similar to a study conducted with varieties of tall fescue (*Festuca arundinacea*) as N rate increased, biomass production increased (Collins, 1991). The rye biomass was not affected (*P* ≥ 0.34) by the green manure - cowpea treatment compared to the control fallow both years. This may be attributed to the shallow rooting nature of rye which may have restricted uptake of nutrients after rainfall events. Rooting depth and volume can aid in moisture and nutrient capture in leachable soils such as the Darco (Dunbabin et al., 2003). Evaluating nitrate and ammonium concentrations in the Darco soil, Haby and Leonard (2006) showed that Tifton 85 bermudagrass *(Cynodon dactylon)* has a rooting depth well-equipped with retrieving Nitrate-N at least to 1.2 m deep in this deep sandy soil.

Table 2.2. Effect of N rate on rye biomass yields rate in 2014-2015 and 2015-2016 cropping seasons in Overton TX.

N Rate	**Rye biomass**		**N Rate**	**Rye Biomass**	
2014-2015			*2015-2016*		
	kg ha^{-1}			**kg ha^{-1}**	
101	2667	a†	202	3412	a†
67	2063	b	134	228	b
34	1469	c	67	1223	c
0	1034	d	0	434	d

†Different letters represent significant differences (*P* < 0.05).

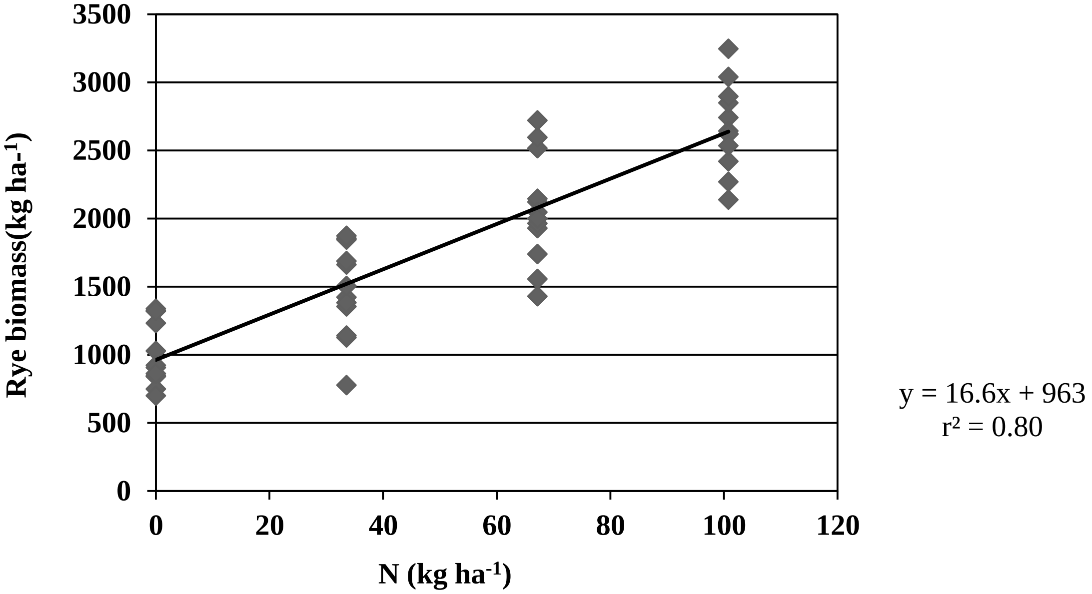

Figure 2.5 Effect of N rate on rye biomass yields in 2014-2015 cropping season in Overton, TX.

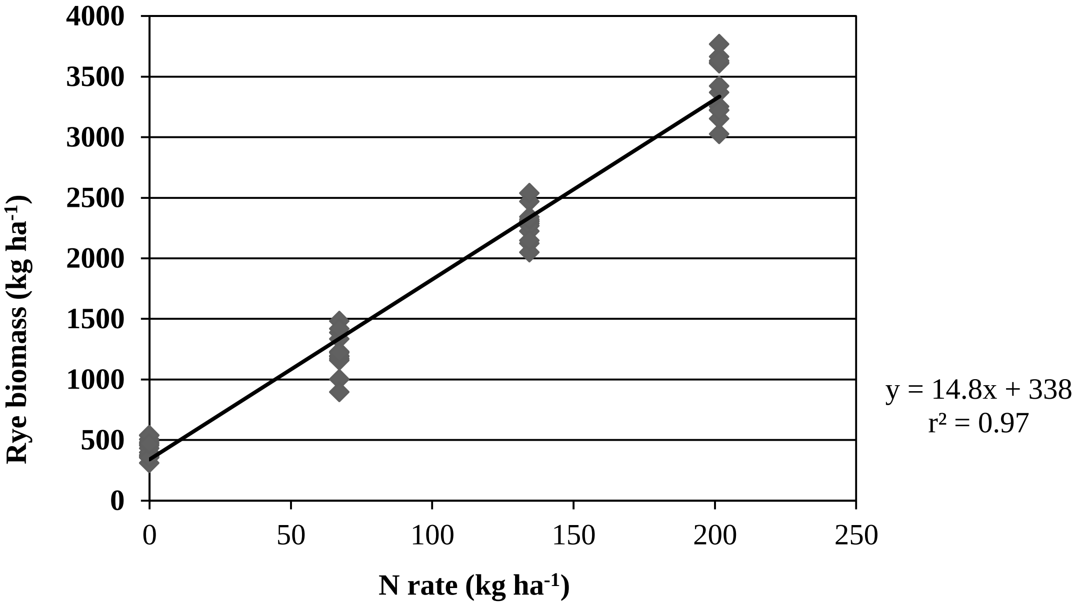

Figure 2.6 Effect of N rate on rye biomass yields in 2015- 2016 cropping season in Overton, TX.

Soil C and N

Total soil C and N were analyzed by the Dumas total combustion method (Kirsten, 1979). Soil samples were taken in 2014 before the initiation of the cropping system, and again after the rye forage crop in 2016. The results from the analysis show there were no differences ($P \geq 0.40$) between soil C or N among treatments of cowpea from 2014 to 2016 within a soil depth (Table 2.3). Table 2.4 illustrates the soil N from the 2016 soil sampling, there were no significant differences among treatments within a soil depth, however; the soil depth was significant ($P < 0.0001$) with almost five times as much N at the 0-12 cm depth. Soil N changes are more often a gradual change as opposed to a drastic change when a cropping system is altered (Smith and Sharpley, 1990). Sandy soils are generally well drained, therefore nutrients-specifically N are leached out in the spring during the rainy season, making retention of nutrients for the next crop difficult (Appel and Mengel, 1993). Rouquette and Keisling (1983) conducted a trial on two soil types (Darco and Cuthbert) and compared N rates as used in hay production. The Darco soil consistently lacked nutrient holding capacity when compared to other soils such as Cuthbert (fine, mixed, semiactive, thermic Typic Hapludult) a local soil for the area. Cuthbert is a heavier soil with roughly 11% more clay content. A study by Johnson et al., (1995) looking at the nutrient removal of N and P on the Darco soil and showed similar results with little to no nutrient retention in the Darco soil. Poultry litter and dairy effluent were applied as the fertilizer N source and lysimeters and buffer strips were put in place to measure leaching in the Darco soil. In the study, there were low soil nitrate concentrations indicating that nitrates were not accumulating in the coarse-textured soil.

Table 2.3. Change in soil C, soil N, and soil C:N ratio over two years in a cowpea green manure-rye forage cropping system as affected by green manure treatment at different depths.

Depth	Treatment	C		N		C:N ratio	
cm		Δ g kg^{-1}					
0-12	COMB	487	a† *	36	a	11	a
	IAC	228	a	25	a	12	a
	FAL	137	a	15	a	11	a
12-24	COMB	228	a	35a	a	10	a
	IAC	194	a	32	a	10	a
	FAL	97	a	41	a	9	a

Combine cowpea (COMB); Iron and Clay cowpea (IAC); fallow plots (FAL).

*Values represent difference between initial samples taken in spring 2014 and final samples taken after the second year of rye forage in spring 2016.

†Different letters represent significant differences ($P < 0.05$)

Table 2.4. Differences in 2016 soil N by summer treatment at two depths.

Depth	**Treatment**	**N**	
cm		**g kg^{-1}**	
0-12	IAC	4698	a†
	COMB	4607	a
	FAL	4428	a
12-24	IAC	95	a
	COMB	95	a
	FAL	95	a

Combine cowpea (COMB); Iron and Clay cowpea (IAC); fallow plots (FAL).
†Different letters represent significant differences (*P* < 0.05).

Nitrogen budgeting

With N being the most important nutrient for grass dry matter production, and to avoid negative impacts on the environment, a N budget may enhance sustainable production. A N budget was estimated by plot for the 2-year growing season from May 2014 to May 2016 based on a N budget consistent with Liu et al., (2003). Inputs to the cropping system included cowpea above ground biomass and commercial fertilizer N in the form of ammonium nitrate (34-0-0). Outputs measured included rye forage biomass. Soil samples were taken at the initiation of the system (2014), and at the end of the system (2016) and were analyzed for total N and C. Table 2.6 shows the estimated N budget by cowpea summer treatment and N rate treatment. Previous results suggested that soil N was not altered by the green manure cropping system. When observing the N budget (Table 2.6); initial soil N and gain in soil N for all plots suggested soil N slightly increased in all plots except for the fallow plots.

These results are consistent with knowledge of this soil type since the Darco soil is a deep sand that is excessively drained (Soil Survey Staff, 2016). With the Darco, nutrient retention is difficult especially after rain events. In a permanent pasture situation, Johnson et al., (1994) showed nitrate leaching through the Darco soil and very little N concentration in the soil after periodic soil sampling. They proposed that N was leached and not captured in the soil. The overall cropping system did not accumulate N in the soil from the green manure cover crop or the added N fertilizer.

Table 2.5. Plant and soil N data arranged by summer treatment plot and N fertilizer rate for each cropping system year.

2014-2015 double cropping system (kg ha^{-1})	IAC	IAC	IAC	IAC	COMB	COMB	COMB	COMB	FAL	FAL	FAL	FAL
	1	2	3	4	1	2	3	4	1	2	3	4
Initial soil N (0-6)in	786	786	786	786	786	786	786	786	786	786	786	786
N input (cowpea)	38	38	38	38	14	14	14	14	0	0	0	0
N input (fertilizer)	0	34	67	101	0	34	67	101	0	34	67	101
N uptake (rye)	21	45	32	58	17	44	33	64	18	35	29	58
Residual soil N (calculated)	803	813	859	867	783	790	834	837	768	785	824	829

2015-2016 double cropping system (kg ha^{-1})	IAC	IAC	IAC	IAC	COMB	COMB	COMB	COMB	FAL	FAL	FAL	FAL
	1	2	3	4	1	2	3	4	1	2	3	4
Initial soil N (calculated)	803	812	859	867	783	790	834	837	768	785	824	829
N input (cowpea)	61	61	61	61	36	36	36	36	0	0	0	0
N input (fertilizer)	0	67	134	202	0	67	134	202	0	67	134	202
N uptake (rye)	8	40	6	42	8	41	7	39	9	37	6	38
Residual soil N (calculated)	856	833	914	886	811	785	863	834	759	748	818	791
Ending soil status	878	812	875	823	913	846	870	856	775	859	841	830
Soil N gains (calculated)	92	26	89	37	127	60	84	70	-11	73	55	44

Combine cowpea (COMB); Iron and Clay cowpea (IAC); fallow plots (FAL).

Conclusion

Cowpea as a green manure crop in a double-cropping system with rye as a winter forage on a predominately sandy soil had no measurable impact on N during this two year period. The Darco soil is well drained and low in clay, and did not retain N. The cowpea green manure data showed that either IAC or COMB cowpeas used as a green manure crop had a positive impact on the forage yield of rye on Darco soils. There was no effect of rye biomass by cowpea or fallow. Weather conditions and the time between incorporation of cowpeas and rye planting provided favorable conditions for rapid decomposition and mineralization of cowpea biomass. These conditions may have accelerated the rate of decomposition, and thus decreased nutrient availability for the rye crop. Without measuring decomposition rates exact reasoning on the lack of influence from the cowpea is unsure. Additionally, the soil sampling depth may not have captured N that may have leached below the soil sampling depth of 30 cm.

This two-year cropping system study documented the use of cowpea as a green manure crop for winter forage rye. The results presented from this experiment warrant an alteration to the system. During the two years of this experiment on a very sandy soil and in a humid climate, and with the amount of biomass incorporated, there was no impact on soil N, and thus no effect on rye biomass from a cowpea green manure despite substantial biomass and N contributions from the cowpea.

Future research evaluating effectiveness of cowpea as a green manure and N source for subsequent crops may include the addition of cattle for grazing of the rye. We suspect grazing could impact soil fertility because cattle recycle nutrients to the soil by excreta. Other options could include using a different form of commercial fertilizer paired with a new cowpea variety. A cowpea variety with early seed production and large biomass would contribute by adding a cash crop to the system. Other management strategies that include higher biomass, such as forage sorghum, and or broiler litter may offer appropriate plant residue to effect N retention.

CHAPTER III

COWPEA PLANT INTRODUCTION LINES EVALUATED FOR DOUBLE CROPPING SYSTEM

Synopsis

Cowpea (*Vigna unguiculata* [L.] Walp) is a diverse legume as the species varies by seed color, seed size, maturity, physical appearance, and production traits. The objective of this experiment was to identify cowpea plant introduction (CPI) lines with improved seed and biomass production traits in a double-cropping system with forage rye (*Secale cereale* L.). The target for cowpea maturity for this investigation in northeast Texas and the Southeastern U.S. was mid- late Sept. In the first of two years of the experiment, 37 CPI lines were grown in a greenhouse at the Texas A&M AgriLife Research, Overton, TX, and were screened for maturity and seed yield. Based on these traits, 16 CPI lines were selected for subsequent field evaluations. 'Iron and Clay' and 'Combine' cowpea were used as known indeterminate and determinate cultivar checks, respectively, and 1 breeding line for direct comparison of the 16 CPI lines. The lines were planted on a Darco loamy fine sand (loamy, siliceous, semiactive, thermic Grossarenic Paleudult) in a randomized complete block design. Data collected included biomass, seed yield, and maturity stage by days after planting (DAP). Data were analyzed using PROC MIXED in SAS® 9.4. Least square means were separated using LSD's. When comparing the lines, there were differences in biomass production ($P <$

0.01) in both years. The largest biomass production was by entries 10 at 3021 kg ha^{-1} and 30 at 4351 kg ha $^{-1}$ respectively in 2015 and 2016. The largest seed yield produced was by entry 15 at 2322 kg ha^{-1} in 2016. In 2016 differences ($P < 0.0001$) among entries were detected. In a separate greenhouse study, 15 CPI lines and a cowpea breeding line, TX-505, were evaluated for resistance to southern root-knot nematode under greenhouse conditions. Cowpea plant introduction line number seven and TX-505 were rated as nematode resistant, and the other fourteen CPI lines were rated as susceptible. Iron and Clay and Combine did not produce high seed yields at the target harvest dates compared to other entries and had lower biomass contributions. The CPI line that showed tolerance to nematodes was entry number 7. Cowpea plant introduction lines were identified with potential to improve Texas cowpea seed production and double-cropping systems.

Introduction

Cowpeas are a widely adapted legume that are drought tolerant, and adapted to the acidic soils common to the southeastern U.S. (Redmon et al., 1992; Singh, 2014). The use for cowpeas varies since the legume is very diverse and ranges from use as a food crop (green vegetable and dry peas) to a cover crop; a forage and hay crop for cattle; and a browse in food plots for whitetail deer. While cowpeas can offer many benefits as a cover crop, genotypes vary greatly in growth habit (Harrison, 2006; Singh, 2014). Cowpea genotypes commonly used for forage are typically indeterminate in growth habit and remain vegetative through the summer months and into early fall. Cowpea varieties with resistance to pests benefit production as insect damage is the primary limiting factor for grain production (Singh and Van Embden, 1979; Ehlers and Hall, 1997).

Commercially available varieties such as ‘Iron and Clay’ (IAC), a two variety mix, have vigorous growth and nematode resistance (Smith et al., 2006).The southern root-knot nematode (*M. incognita*) is an important pest of cowpeas on sandy soils of the southeast and southwest US (Ehlers and Hall, 1997). Root-knot nematode infections on susceptible varieties results in damaged root systems, reduced biomass and seed yield, and reduced drought tolerance (Cook and Evans, 1987; Roberts et al., 2005). Resistance to root-knot nematode in cowpea is conditioned by two genes (RK and RK ^{2})(Ehlers et al., 2000; Petrillo et al., 2006), and can be detected using greenhouse seedling inoculation techniques (Boerma and Hussey, 1992). Cowpea used for green manure cover cropping should have resistance to root-knot nematode as this can limit nematode populations in the soil (Roberts et al., 2005).

Cowpeas in symbiosis with *Bradyrhizobium* also contribute N to the cropping system (Neely, 2013) which makes them a desirable cover crop. Cowpeas also perform well in scenarios where the main purpose may be forage for wildlife or grazing purposes (Redmon et al., 1992; Redmon and Rouquette, 2009). The late maturity (120-d) of ‘Iron and Clay’ cowpeas; however, makes incorporation into a cropping system challenging. A cowpea variety with high forage yield and seed at harvest maturity by September is not currently available. Varieties such as ‘Combine’ (COMB) provide seed production in a short amount of time, but lack nematode resistance and forage production qualities that IAC provides. Neely (2013) reported significantly more total N yield from IAC (104 kg ha^{-1}) compared to COMB (41 kg ha^{-1}), in a study from the Texas A&M AgriLife Research and Extension Center at Overton. The objective of this research was to identify cowpea plant introduction lines with one or a combination of the following traits: 1) high biomass production; 2) high dry seed yield by mid to late September; 3) and resistance to southern root-knot nematode (*M. incognita*). Lines identified with these valuable traits will be used in a breeding program to develop improved cowpea cultivars for double-cropping systems in Texas and the Southeastern U.S.

Materials and methods

Greenhouse screening

This experiment was performed in a greenhouse at the Texas A&M AgriLife Research and Extension Center at Overton, TX. The Germplasm Resources Information Network (GRIN) component of the National Genetic Resource Program (NGRP) was used to identify 34 cowpea plant introduction lines by utilizing the following search criteria: APHID equal to '3'; SEEDPROD equal to '1'; and CORE equal to 'y'. In this study, this search protocol identified 34 CPI lines. These CPI lines and 2 check cultivars; IAC and COMB, were planted in 7570 ml, (216 by 216mm) pots in LC1 peat/vermiculite mix (Table 3.1). Three seeds were planted on 23 June 2014 and if all germinated one was eliminated. The CPI lines were arranged in the greenhouse in a completely random design with three replications. A commercial inoculant (N-Dure™, Kentland, IN) of *Bradyrhizobium* was applied at the recommended rate of 5 g to 50 ml of water, and pots were watered as needed. Between 60-d and 90-d, mature seed were collected as available, and lines with no mature seed at 90-d were eliminated from further consideration. Seed harvested in the greenhouse were the basis for further field evaluation later on.

Field evaluation 2015

In 2015, 16 CPI lines, 2 check cultivars and 1 breeding line were evaluated at the Texas A&M AgriLife Research and Extension Center at Overton for suitability in a double-cropping system with forage rye (Table 3.1). Plots were located on a Darco loamy fine sand (loamy, siliceous, semiactive, thermic Grossarenic Paleudults). Soil preparation prior to cowpea planting included: rototilling, herbicide application and incorporation of 1.17 L ha^{-1} of *trifluralin (*presented as the butoxyethyl ester; Treflan ™), and roller-packed to prepare a firm seed bed. The CPI lines for the experiment were hand-planted with 40 seed into 3 m single row plots with three replicates on 12 June 2015. A commercial inoculant (N-Dure™, Kentland, IN) of *Bradyrhizobium* was applied at the recommended rate of 5 g to 50 ml of water at planting. Heights and growth staging were taken for nine consecutive weeks during the growing season from June to September in 2015. Growth staging was scored based on a cowpea growth staging code by Legume IPM (Southern IPM, 2016)(Appendix A-1). Biomass was collected as lines matured on three dates: September 4, 10, and 28 of 2015. Biomass sampling date was determined by harvesting CPI when they exhibited harvest maturity (80% of pods changed to mature color) (Southern IPM, 2016). The seed harvested from 2015 was rated based on appearance and quality. Seed that was under-developed or moldy seed was given a rating of 1 whereas, plump and fully developed seed was given a 9.

Fifteen CPI lines and one cowpea breeding line (TX-505) were evaluated for resistance to southern root-knot nematode. The CPI entries numbers 11 and 25 were not included in this screening due to lack of seed available. Seed of each entry was planted 7 September, 2015 in a greenhouse at Lonoke, Arkansas. After germination and emergence, each seedling was inoculated with 5000 eggs of *M. incognita* race 3. The CPI lines were arranged in a completely random design with five replications. Single plants were grown in SC10 containers (3.8 cm diameter by 21 cm depth; Stuewe and Sons, Tangent, OR) using washed builders sand as media.

At sixty days post-establishment, cowpea plants were removed from the containers, and root systems washed to remove sand. Root galling was visually rated on a scale of 1 to 8 where 1 = 1% galling and 8 = 100% galling. Root-knot nematode eggs were extracted from the individual root system using methods developed by Hussy and Barker (1973). Nematode reproduction was expressed in eggs per gram of root.

Field evaluation 2016

In 2016, 13 CPI lines, 2 check cultivars and 1 breeding line were evaluated at the same location as 2015 with the same field preparations. Entries 11 and 24 were not planted in 2016 due to limited seed availability. Cowpeas were planted on 14 June, 2016. Heights and growth staging were taken for nine consecutive weeks during the growing season from June to Sept. Biomass was collected at two dates 12 Aug and 3 Oct. The first date was conducted mid-growing season and the second was at the termination of the plots. The seed was harvested as pods exhibited R8 maturity (Southern IPM, 2016) and were harvested four consecutive weeks until plots were terminated. The first seed harvest of 2016 was 9 Sept. this was after some predation occurred on the plots. Entries 30 and 37 had major damage and did not regain seed during the time of the seed harvest.

Table 3.1. Description of 34 cowpea plant introduction lines from the National Genetic Resource Program, two check cultivars and one Texas A&M University breeding line that were evaluated in Overton, TX for biomass, seed yield, maturity, and root-knot nematode resistance.

Entry	**Accession**	**Plant ID**	**Country/ Origin**	**Exp. 1 2014**	**Exp. 2 2015**	**Exp. 3 2016**
1	PI354553	P734	India	Y	N	N
2	PI354801	P1308	India	Y	N	N
3	PI354823	P1337	India	Y	N	N
4	PI354833	PI1351	India	Y	N	N
5	PI354837	PI1356	India	Y	N	N
6	PI354838	PI1357	India	Y	N	N
7	PI35863	V.67-05	India	Y	Y	Y
8	PI367921	NAVAJA	Mozambique	Y	Y	Y
9	PI406290	IFH 27-8	Nigeria	Y	Y	Y
10	PI175963	6097	Turkey	Y	Y	Y
11	PI176796	TVu1537	Turkey, Erzincan	Y	Y	N
12	PI183251	TVu2329	Egypt	Y	N	N
13	PI186460	TVu1551	Nigeria, Lagos	Y	N	N
14	PI200867	TVu1975	Myanmar	Y	N	N
15	PI208845	TVu2377	Costa Rica, Cartago	Y	Y	Y
16	PI209971	TURU NASHI WASE SASAGE	Japan	Y	Y	Y
17	PI214354	TVu1570	India, Punjab	Y	N	N
18	PI220851	LOBIA-I-SAFADE	Afghanistan, Herat	Y	N	N
19	PI221731	TVu2396	South Africa, Transvaal	Y	Y	Y

Table 3.1. Cont.

Entry	**Accession**	**Plant ID**	**Country/ Origin**	**Exp. 1 2014**	**Exp. 2 2015**	**Exp. 3 2016**
20	PI227830	TVu1577	Guatemala	Y	N	N
21	PI257463	TVu1930	South Africa	Y	Y	Y
22	PI292898	TVu1890	Hungary	Y	N	N
23	PI292912	TVu2483	U.S.	Y	N	N
24	PI292913	TVu2484	Hungary	Y	Y	N
25	PI293477	CALIFORNIA BLACKEYE	U.S.	Y	Y	Y
26	PI293525	JACKSON PURPLEHULL	U.S.	Y	Y	Y
27	PI339587	TVu1924	South Africa	Y	Y	Y
28	PI339582	TVu2656	South Africa	Y	Y	Y
29	PI339613	TVu2003	Tanzania	Y	N	N
30		Iron & Clay	East Texas Seed, Tyler, TX	Y	Y	Y
31		Combine	Adams-Briscoe Seed, Jackson, GA	Y	Y	Y
32	PI339592-3	TVu2656	South Africa	Y	Y	Y
33	PI339592-1	TVu2656	South Africa	Y	N	N
34	PI200867-1	TVu1975	Myanmar	Y	N	N
35	PI208845-1	TVu2377	Costa Rica, Cartago	Y	Y	Y
36	PI354838-1	PI1357	India	Y	N	N
37		TX-3	Texas A&M AgriLife Research, Overton, TX	N	Y	Y

Statistical analysis

Data were analyzed using SAS® 9.4 (SAS Institute, Cary, NC). Relationships among variables were examined using the REG procedure. All response variables were analyzed using the MIXED procedure. Entry was used as a fixed effect and replicate used as a random effect. Results were reported as least square means, and mean separations were conducted using the PDMIX800 macro. Significance was declared when $P < 0.05$.

Climate

The weather conditions during the growing season for the CPI lines in 2015 were relatively normal for east Texas (Texas A&M AgriLife Research-Overton Center, 2016). The temperatures (Fig. 3.1) were slightly above the 40-year average with below average rainfall (Fig 3.2) during the growing season. These conditions had a major impact on crop growth, and limited biomass growth and seed production.

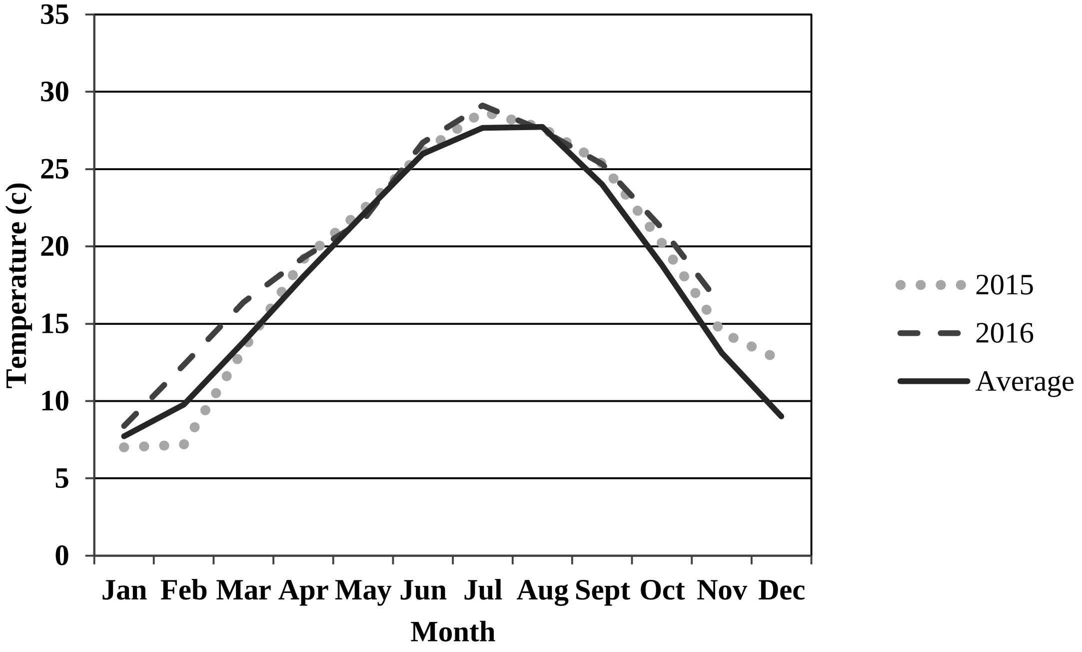

Figure 3.1 Temperature during growing season for CPI lines in 2015, 2016, and the 40-year average.

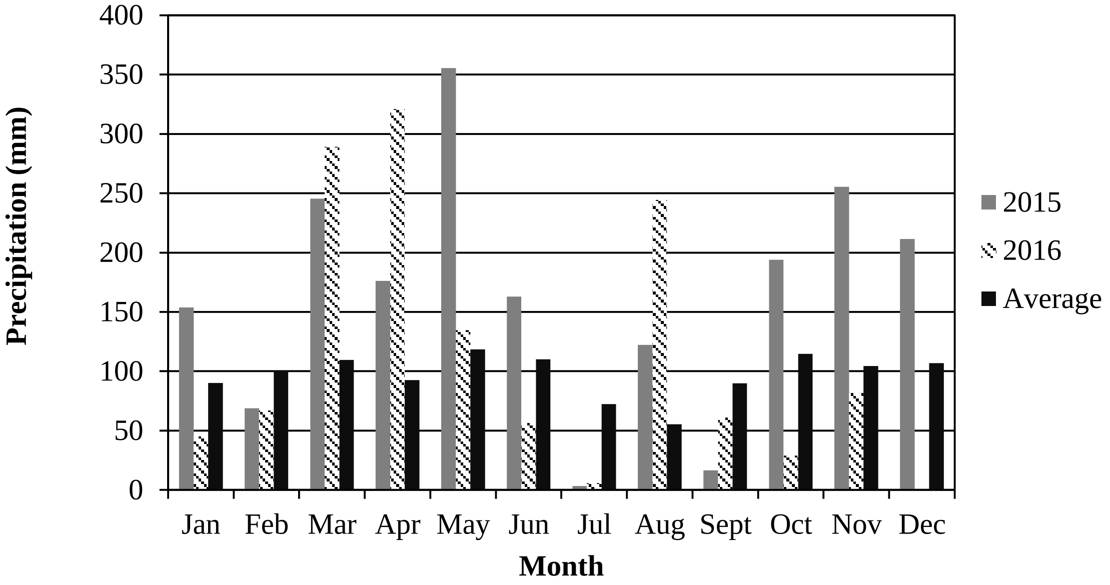

Figure 3.2 Monthly rainfall in 2015, 2016, and 30-year average in Overton, TX.

Results and discussion

Biomass production

The results for 2015 biomass field screening are presented in Table 3.2. The CPI with the highest biomass yield was entry number 10 with 3021 kg ha^{-1}. Entry number 9 followed with the next highest biomass at 2396 kg ha^{-1} ($P < 0.01$). The check cultivar IAC (entry 30) biomass data was not analyzed due to replication loss of the plots. In previous research (Neely, 2013), IAC yielded higher biomass (3330 kg ha^{-1}) when compared to COMB (1440 kg ha^{-1}). Butler (2014) showed biomass for IAC ranging from 2094 to 340 kg ha^{-1} across four locations in Texas. The COMB (entry 31) biomass was 492 kg ha^{-1} for this study and was much less than previous research. The reduced yield for COMB was likely due to limited rainfall during the growing season. In 2015, not all entries were analyzed due to severe predation on the plots by birds which resulted in replication loss and a poor stand. When calculating the biomass production, a scoring system was initiated to account for the poor stands within the plots. A percent cover score (100= full stand, and 50= half stand, etc.) was taken into account for the stand loss and incorporated into the biomass data. Percent cover data are presented in Appendix B-1.

Table 3.2. Biomass production of 14 entries from 2015 field trial in kg ha^{-1}.

Entry	**DMY**	
	kg ha^{-1}	
10	3021	a†
9	2396	ab
26	1592	bc
19	1479	bcd
15	1296	bcde
35	1118	bcde
32	1073	bcde
28	1059	cde
27	1054	cde
37	992	cde
25	964	cde
11	739	cde
31	492	cde
7	275	de
24	149	d

†Different letters represent significant differences ($P < 0.05$).

Seed production

Seed yields taken in greenhouse from Exp. 1 were used in the screening process to decide which CPI lines were selected for the second year of the trial. In Table 3.4, all reported CPI lines seed weights proceed to Exp. 2 except entry 23 which had the lowest seed weight at 6 g. The amount of seed produced by entry 23 was not enough to advance to the second year of the study. Entry 23 did not perform well in an “ideal” environment in the greenhouse, there was no need to continue evaluation in a field setting.

Table 3.3. Seed weights from year one in the greenhouse, evaluated for seed increase purpose and whether advancement in the trial will occur.

Entry	**Seed wt.**	
	g plant^{-1}	
7	22	a†
8	15	ab
27	15	b
10	14	b
21	14	bc
32	13	bc
9	13	bc
28	13	bc
19	13	bc
15	13	bcd
25	13	bcd
16	12	bcd
24	11	bcd
26	11	bcd
35	9	bcd
11	7	cd
23	6	d

† Different letters represent significant differences ($P < 0.05$).

Seed yield results from 2015 (Appendix B-2) were inconclusive as there was bird predation immediately after planting. Overall seed yield was negatively affected by environmental factors such as high temperatures (Figure 3.1) during early stages of pod fill and seed setting in combination with little to no precipitation (Figure 3.2). Studies have been conducted on the relationship between drought and seed production for cowpea and Turk et al., (1980) discussed some findings linking heat stress with seed yield reduction through abscission in flowers. The single seed weight of all the entries is represented in Table 3.4.

Table 3.4. Single seed weights of 19 entries from 2015 seed yield**.**

Entry	**Single Seed Wt.**	
	mg	
21	757	a†
24	752	ab
15	394	abc
30	341	abc
28	317	abc
27	317	abc
25	292	abc
26	292	abc
11	168	bc
32	133	bc
31	131	bc
7	131	bc
19	97	c
16	90	c
37	73	c
10	73	c
35	73	c
9	60	c
8	30	c

† Different letters represent significant differences ($P < 0.05$).

Plant maturity

Cowpea maturity was measured using the Southern IPM (2016) physiological maturity scale. The CPI lines that were rated R8 were concluded to be at the harvest maturity stage at which 80% of the pods had changed to mature color. During the growing season, cowpea pods changed colors from green (immature) to tan/white (mature). This indicated fully mature and developed seed. Heights and staging is illustrated in Table 3.5 from the 2015 growing season.

Table 3.5. Weekly heights in cm and growth staging for 2015 growing season for all entries.

	27 July		*7 Aug.*		*13 Aug.*		*18 Aug*		*28 Aug.*		*4 Sept.*		*11 Sept.*	
Entry	Ht.	stage†	Ht.	stage	Ht.	stage	Ht.	stage	Ht.	stage	Ht.	stage	Ht.	stage
7	18	V9	26	R1	25	R7	28	R7	25	R5	21	R4	26	R8
8	25	R2	31	V7	32	R7	28	R8	37	R8	31	R5	H*	
9	30	R3	31	R6	34	R8	30	R8	34	R8	30	R6	H	
10	32	V13	37	V13	38	R1	42	R2	51	R6	40	R5	H	
11	23	V7	24	V8	26	V9	37	V11	51	R1	44	R2	44	R3
15	31	V5	32	V9	32	V11	28	R1	33	R3	28	R3	34	R2
16	37	R2	38	R4	34	R7	40	R8	32	R6	36	R8	H	
19	26	V12	35	V12	33	R1	35	R1	47	R2	49	R2	45	R5
21	19	V6	26	V6	24	R1	24	R2	40	R3	42	R3	40	R3
24	20	V6	22	V9	23	V8	30	R1	33	R3	38	R2	37	R3
25	27	V10	38	V15	41	V18	43	R1	50	R2	45	R4	51	R6
26	28	R3	28	R6	29	R8	27	R8	31	R8	24	R8	H	
27	23	V7	29	R1	31	R1	32	R2	40	R3	41	R4	39	R5
28	29	R2	32	R4	32	R3	35	R4	37	R7	36	R7	H	
30	21	V7	29	V14	28	V14	32	V12	46	V16	52	V16	52	R1
31	17	R1	23	R1	28	R4	27	R5	36	R6	34	R6	H	
32	23	V6	33	R1	35	R3	40	R3	48	R5	44	R4	50	R7
35	24	V7	29	R1	36	R3	36	R2	41	R4	36	R4	47	R7
37	23	V7	31	V11	34	V10	68	V15	61	V15	77	R1	84	R2

*H- plots harvested (biomass and seed)

† Refer to Appendix Table A-1 for staging code.

The values associated with the entries in Table 3.6 represent the percent of dry pods at each harvest date by entry. The harvest dates consisted of three harvests in the month of September. The first and second harvest dates were dependent upon the visual appraisal, and were aimed at harvest maturity (R8). The last harvest date was taken at the end of September to serve as a deadline for seed production in a specific time frame. Entry 9 (93.7%) had the largest percent of dry pods at harvest compared to entry 30 (16.9%) which had more green pods at the last harvest.

Table 3.6. Percent of dry pods at each harvest date by entry**.**

Entry	% Mature Pods	Harvest date
9	93.7	10-Sep
28	82.3	10-Sep
10	80.1	10-Sep
26	78.1	4-Sep
31	61.5	4-Sep
37	60.5	28-Sep
25	57.8	28-Sep
7	57.1	28-Sep
32	45.9	28-Sep
35	45.9	28-Sep
27	34.1	28-Sep
19	33.6	28-Sep
24	24.7	28-Sep
11	17.9	28-Sep
15	17.8	28-Sep
30	16.9	28-Sep

Root-knot nematode resistance

Average percent root galling and nematode reproduction ranged from 13% to 1.2% and 47,696 to 2275 eggs g^{-1} root (Table 3.7). In this study, both CPI lines 7 and TX-505 were resistant to root-knot nematode based on both galling response and reproduction (Smith et al., 2006). This is a common method for nematode evaluations as Ehlers et al., (2009) used similar methods in known nematode resistant cowpea line registered as ‘California Blackeye 50’. The TX-505 was known to be a root-knot nematode resistant breeding line (personal communication, G.R. Smith, 2016). The CPI line 7 had moderate galling with low nematode reproduction. The other fourteen CPI lines were susceptible to infection by nematode based on eggs per gram of root and the fact that the samples were inoculated with 5000 eggs and entries 7, and TX-505 had less than 5000 eggs g^{-1} root.

Table 3.7. Nematode resistance scoring by cowpea entry on percent galling values and eggs per gram of root.

Entry	**Percent Galling**		**Egg/g root**	
21	13	a†	47696	ab
9	13	a	67774	a
19	10	ab	33923	bc
24	10	ab	49308	ab
27	8.4	abc	41604	b
15	6.6	bcd	33178	bc
35	6.6	bcd	59834	ab
10	5.2	bcde	25380	bcde
28	5	cde	27938	bcd
8	5	cde	15953	cde
37	4.8	cde	6987	de
16	4.8	cde	25626	bcde
26	3.4	de	48552	ab
32	3.2	de	13685	cde
TX-505	1.2	e	4044	de
7	1.2	e	2275	e

† Different letters represent significant differences ($P < 0.05$).

2016 Results

Less problems with bird and deer predation were noted in 2016. Table 3.8 shows the biomass production of the entries from 2016. Entry 31-COMB (468 kg ha^{-1}) is among the lower producing entries and entries 30-IAC (4351 kg ha $^{-1}$) and 37 (3475 kg ha $^{-1}$) had the largest production of biomass.

The seed yield results for 2016 (Table 3.8) show a difference ($P < 0.0001$) among entries. Entry 15 had the largest seed yield at 2322 kg ha^{-1} and entry 25 with smallest yield at 47 kg ha^{-1}. Seed yield data was not collected on entries 30 and 37 due to both late maturity and deer predation. Heights and growth staging was taken during the growing season for 7 consecutive weeks and are illustrated in Table 3.9.

Table 3.8. Biomass harvested on 3 Oct., seed yield and harvest date from 2016 entries.

Entry	Seed Yield		Biomass		Harvest Date
	kg ha^{-1}		kg ha^{-1}		
15	2322	a†	2378	bcd	23 Sept.
8	2048	ab	1631	cdefg	15 Sept.
27	1539	ab	2609	bc	23 Sept.
35	1906	ab	2497	bcd	23 Sept.
10	1502	abc	1910	bcde	23 Sept.
7	1460	abc	2895	b	23 Sept.
9	1022	bcd	318	g	23 Sept.
31	792	bc	468	g	23 Sept.
19	676	c	1488	cdefg	23 Sept.
21	603	c	1091	defg	23 Sept.
16	543	c	997	efg	9 Sept.
26	346	c	368	g	23 Sept.
28	190	c	299	g	23 Sept.
25	47	c	2299	bcde	9 Sept.
30	0*		6009	a	-
37	0*		4707	b	-

† Different letters represent significant differences ($P < 0.05$).

*Entries 30 and 37 did not have a seed yield due to deer predation and late maturity.

Table 3.9. Weekly heights in cm and growth staging for 2016 growing season for all entries.

	14 July		*21 July*		*28 July*		*1 Aug.*		*11 Aug.**		*25 Aug.*		*1 Sept.*	
Entry	Ht.	stage †	Ht.	stage	Ht.	stage	Ht.	stage	Ht.	stage	Ht.	stage	Ht.	stage
7	28	V9	33	V15	41	R2	43	R3	44	R4	56	R3	54	R5
8	30	V10	34	V15	41	R2	46	R4	49	R4	60	R3	60	R4
9	21	V15	21	R1	19	R2	20	R4	21	R6	51	R2	41	R4
10	32	V11	43	V14	49	R1	56	R2	46	R3	65	R3	57	R3
15	32	V9	41	R1	45	R1	49	R3	46	R4	58	R3	53	R5
16	32	V8	44	R1	43	R3	41	R4	41	R6	58	R3	54	R3
19	39	V12	49	V14	69	R1	64	R2	59	R3	71	R1	67	R2
21	35	V9	40	V13	61	R1	61	R2	60	R3	75	R2	69	R2
25	32	V10	42	V11	63	V14	62	V19	62	R2	60	R5	82	R5
26	23	V9	30	R2	22	R4	23	R6	21	R8	44	R6	44	R3
27	29	V12	33	V15	37	R1	49	R2	45	R3	63	R3	61	R3
28	27	V6	32	R1	41	R3	43	R4	30	R5	52	R2	54	R3
30	33	V12	45	V11	55	V19	63	Vn**	67	Vn	73	Vn	74	Vn
31	32	V8	41	R1	48	R2	47	R4	47	R6	61	R3	52	R6
35	28	V6	38	R1	55	R1	51	R3	51	R3	65	R4	56	R4
37	28	V9	37	V14	52	V17	60	Vn	64	Vn	75	Vn	78	Vn

*Plots altered by deer consumption

**Vn; stage is greater than V20

† Refer to Appendix Table A-1 for staging code .

Conclusion

This two year study has identified CPI lines with traits which may be useful in plant breeding efforts. New cowpea varieties could then be useful in double-cropping systems for the Southeastern U.S. The growing season with above average temperatures and near normal rainfall tested the cowpeas' adaptation to heat and drought resistance in 2015. With little rainfall during pod fill seed yields were reduced. Seed production was not as high as noted in other studies and this was attributed to the high temperatures during early pod set in the summer. Seed yields in some of the entries exceeded the seed yield of the control lines IAC and COMB in the 2015 growing season. In 2016, entries showed a better stand and had larger biomass and seed yield values.

The CPI entries in this study varied widely for the desired traits. Entries number 9 and 10 produced significantly more biomass by the Sept. harvest than the eight least productive lines for the 2015 growing season. Check entries number 30 and 37 had the largest biomass production in 2016. In 2016, entries number 15 and 35 had the largest seed yield. The CPI entries in 2015 with 80% or more dry pods at the pre-determined harvest date of Sept. were numbers 9, 28, 10, and 26. The CPI line 7 was resistant to southern root-knot nematode based on root galling and egg counts. No single CPI line had the full complement of all traits identified in our original objectives but at least one source of each trait was identified. The CPI lines 9, 10, 37, 15 and 7 will be used in a future breeding program to produce breeding lines for evaluation. The importance of nematode resistance in a cropping system is crucial as the green manure cover crop can aid in the decrease of reproduction of the nematodes present for other crops. These two experiments were the initiation of an evaluation process to develop multifunctional cowpeas. To be multifunctional, these cowpeas must be adapted to the southeastern U.S. for seed production for a grain crop; biomass for a green manure crop; and an acceptable maturity time to provide a seed crop and incorporate into a cropping system.

CHAPTER IV

SUMMARY AND FUTURE RESEARCH

Summary

This experiment showed no positive effect on the soil N or preceding crop biomass when a green manure cover crop of cowpea was introduced into the cropping system. The soil type for this experiment being excessively drained retains little nutrients. The green manure cover crop of cowpea grows well in the Southeastern U.S. and produces large amounts of biomass. A cowpea variety with an early maturity and seed yield by late Sept. would be beneficial in a double cropping system while providing cover cropping benefits and a possible seed yield. The two varieties IAC and COMB that were in this experiment thrive well in the climate; however, do not prove beneficial as a N source to a cropping system with forage rye on the Darco soil.

Future research

Several CPI lines were identified in these experiments that showed the desirable traits including high biomass production, seed yield by Sept. and nematode resistance. To continue on the evaluation of cowpea as a multifunctional cover crop in a double cropping system, a cowpea variety with those previously stated desirable traits is crucial. Nematode resistance is especially important in a green manure cover crop as a susceptible cover crop provides a host for reproduction and infection in the soil. A cowpea with seed production by Sept. would provide a possible cash crop option for the southeastern U.S. producer. Overall, cowpea as a whole is a diverse legume that can serve many purposes and further research on the legume is needed for incorporation into a cropping system.

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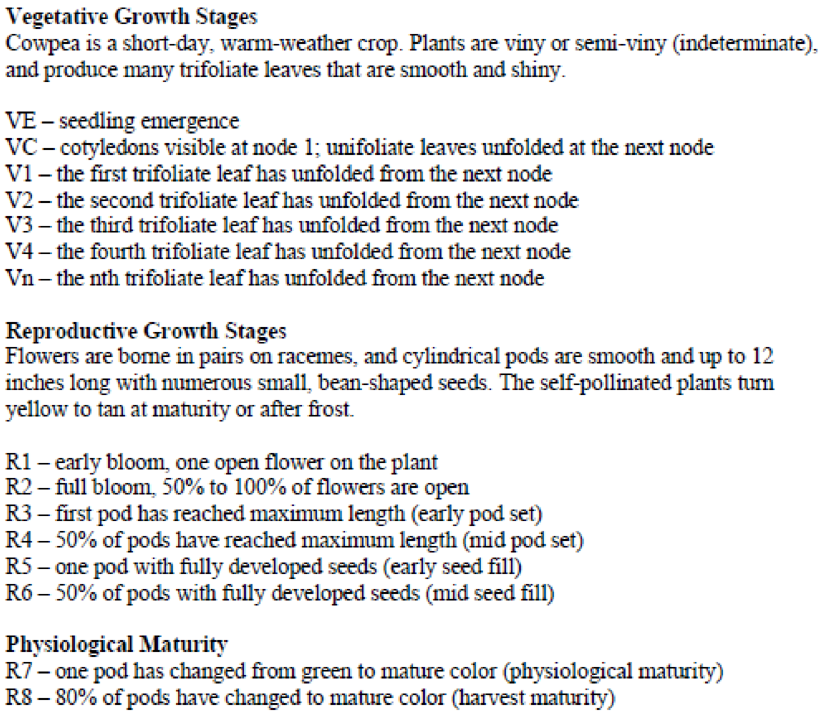
APPENDIX



Vegetative Growth Stages

Cowpea is a short-day, warm-weather crop. Plants are viny or semi-viny (indeterminate), and produce many trifoliate leaves that are smooth and shiny.

VE – seedling emergence
VC – cotyledons visible at node 1; unifoliate leaves unfolded at the next node
V1 – the first trifoliate leaf has unfolded from the next node
V2 – the second trifoliate leaf has unfolded from the next node
V3 – the third trifoliate leaf has unfolded from the next node
V4 – the fourth trifoliate leaf has unfolded from the next node
Vn – the nth trifoliate leaf has unfolded from the next node

Reproductive Growth Stages

Flowers are borne in pairs on racemes, and cylindrical pods are smooth and up to 12 inches long with numerous small, bean-shaped seeds. The self-pollinated plants turn yellow to tan at maturity or after frost.

R1 – early bloom, one open flower on the plant
R2 – full bloom, 50% to 100% of flowers are open
R3 – first pod has reached maximum length (early pod set)
R4 – 50% of pods have reached maximum length (mid pod set)
R5 – one pod with fully developed seeds (early seed fill)
R6 – 50% of pods with fully developed seeds (mid seed fill)

Physiological Maturity

R7 – one pod has changed from green to mature color (physiological maturity)
R8 – 80% of pods have changed to mature color (harvest maturity)

Figure A-1 Cowpea growth staging (Southern IPM , 2016)

Analysis of Variance tables for Chapter II

Table A-1 Analysis of variance table for rye biomass by summer treatment and N rate for 2015 and 2016.

Response variable	DF	F value	P value
2015			
Summer TRT	2	1.31	0.34
N rate	3	85.35	0.0001
TRT*N rate	6	1.02	0.44
Residual		3.67	0.0001
2016			
Summer TRT	2	1.39	0.26
N rate	3	755.75	0.0001
TRT*N rate	6	1.14	0.3591
Residual		4.24	0.0001

Table A-2 Analysis of variance table for cowpea C and N of plant material, by date.

Response variable	DF	F value	P value
C			
Cultivar	1	8.74	0.0051
Date	2	12.88	0.0001
Residual		4.58	0.0001
N			
Cultivar	1	13.31	0.0007
Date	2	5.06	0.0108
Residual		4.58	0.0001
C:N			
Cultivar	1	40.78	0.0001
Date	2	69.23	0.0001
Residual		2.74	0.0031

Table A-3 Analysis of variance table for cowpea C content by cultivar at each harvest date.

Response variable	DF	F value	P value
8/07/2014			
Cultivar	1	172.28	0.0001
Residual		2.45	0.0072
9/05/2014			
Cultivar	1	52144.2	0.0001
Residual		2.12	0.0169
7/23/2015			
Cultivar	1	2109.42	0.0001
Residual		2.12	0.0169

Table A-4 Analysis of variance table for cowpea N content by cultivar at each harvest date.

Response variable	DF	F value	P value
8/07/2014			
Cultivar	1	15.38	0.0035
Residual		2.12	0.0169
9/05/2014			
Cultivar	1	1387.1	0.0001
Residual		2.12	0.0169
7/23/2015			
Cultivar	1	211.91	0.0001
Residual		2.12	0.0169

Table A-5 Analysis of variance table for cowpea C:N ratio by cultivar at each harvest date.

Response variable	DF	F value	P value
8/07/2014			
Cultivar	1	0.06	0.8250
Residual		1.22	0.1103
9/05/2014			
Cultivar	1	67.19	0.0038
Residual		1.22	0.1103
7/23/2015			
Cultivar	1	6.33	0.0864
Residual		1.22	0.1103

Table A-6 Analysis of variance table for change in soil C, and soil N from 2014 to 2016.

Response variable	DF	F value	P value
C			
Depth	1	12.92	0.0001
TRT	2	2.83	0.0655
N rate	3	3.32	0.0245
Residual		6.00	0.0001
N			
Depth	1	695.45	0.0001
TRT	2	0.49	0.6176
N rate	3	0.65	0.5835
Residual		5.87	0.0001

Table A-7 Nitrogen budget formulas

“Initial soil 2014-2015”	From soil samples in June 2014
”Initial soil 2015-2016”	From “residual soil N”
“N input (cowpea)”	From plant sample analysis
“N input (fertilizer)”	N applied as fertilizer
“N uptake (rye)”	N in rye from plant sample analysis
“NUE”	Fertilizer applied/N uptake (rye)
“Residual soil N”	[Initial soil N + N input (cowpea, fertilizer)]-N uptake
“Ending soil status”	From soil samples in May 2016
“Soil N gains”	Ending soil – initial soil

Table A-8 Nitrogen Use Efficiency (NUE) across all plots and fertilizer N treatments.

2014-2015 double cropping system (kg ha^{-1})	**IAC**	**IAC**	**IAC**	**IAC**	**COMB**	**COMB**	**COMB**	**COMB**	**FAL**	**FAL**	**FAL**	**FAL**
	1	2	3	4	1	2	3	4	1	2	3	4
N input (fertilizer)	0	34	67	101	0	34	67	101	0	34	67	101
N uptake (rye)	21	45	32	58	17	44	33	64	18	35	29	58
NUE	0	0.76	2.09	1.74	0	0.77	2.03	1.58	0	0.97	2.31	1.74

2015-2016 double cropping system (kg ha^{-1})	**IAC**	**IAC**	**IAC**	**IAC**	**COMB**	**COMB**	**COMB**	**COMB**	**FAL**	**FAL**	**FAL**	**FAL**
	1	2	3	4	1	2	3	4	1	2	3	4
N input (fertilizer)	0	67	134	202	0	67	134	202	0	67	134	202
N uptake (rye)	8	40	6	42	8	41	7	39	9	37	6	38
NUE	0	1.68	22.33	4.81	0	1.63	19.14	5.18	0	1.81	22.33	5.32

Combine cowpea (COMB); Iron and Clay cowpea (IAC); fallow plots (FAL)

Analysis of Variance for Chapter III

Table B-1 Percent cover of each individual entry and replicate taken on August 18, 2015.

Entry	Rep	Percent Cover
7	1	70
7	2	10
7	3	*
8	1	*
8	2	*
8	3	80
9	1	*
9	2	80
9	3	90
10	1	100
10	2	100
10	3	100
11	1	60
11	2	20
11	3	*
15	1	90
15	2	10
15	3	30
16	1	10
16	2	90
16	3	10
19	1	10
19	2	90
19	3	40
21	1	50
21	2	*
21	3	*
24	1	70
24	2	40
24	3	*
25	1	80

Table B-1 Cont.

Entry	Rep	Percent Cover
25	2	100
25	3	40
26	1	50
26	2	80
26	3	100
27	1	50
27	2	60
27	3	50
28	1	70
28	2	30
28	3	80
30	1	10
30	2	10
30	3	90
31	1	40
31	2	60
31	3	10
32	1	10
32	2	50
32	3	70
35	1	70
35	2	90
35	3	90
37	1	70
37	2	70
37	3	80

*Represent replicate loss, no data taken on that plot

Table B-2 Seed yield of 15 entries from 2015 field trial.

Entry	**Seed Yield**
	kg ha^{-1}
37	351.7 a†
10	342.5 a
26	253.0 ab
19	252.0 ab
7	240.1 ab
27	218.4 ab
25	197.1 ab
15	155.8 ab
9	150.5 ab
28	110.7 ab
31	109.8 ab
35	90.3 ab
11	85.8 ab
32	67.5 ab
24	9.4 b

† Different letters represent significant differences ($P < 0.05$).

Table B-3 Analysis of variance table for biomass and seed yield of cowpeas separated by entry for 2015.

Response variable	**DF**	**F value**	**P value**
Biomass			
Entry	14	3.00	0.0112
Residual		3.24	0.0006
Seed yield			
Entry	14	1.01	0.4726
Residual		3.39	0.0003

Table B-4 Analysis of variance table for seed weight of cowpeas from 2014 in greenhouse separated by entry.

Response variable	**DF**	**F value**	**P value**
Entry	16	2.08	0.0355
Residual		4.12	0.0001

Table B-5 Analysis of variance table for cowpea, percent of dry pods at harvest date**.**

Response variable	**DF**	**F value**	**P value**
Entry	15	9.21	0.0001
Residual		3.54	0.0002

Table B-6 Analysis of variance table for cowpea weight per single seed separated by entry.

Response variable	**DF**	**F value**	**P value**
Entry	18	1.26	0.2972
Residual		3.37	0.0004

Table B-7 Analysis of variance table for cowpea entries, percent galling and eggs per gram of root**.**

Response variable	**DF**	**F value**	**P value**
Percent Galling			
Entry	15	3.19	0.0007
Residual		5.48	0.0001
Eggs per gram of root			
Entry	15	4.34	0.0001
Residual		5.48	0.0001

Table B-8 Analysis of variance table for cowpea entries biomass and seed yield for 2016.

Response variable	**DF**	**F value**	**P value**
Biomass			
Entry	15	11.07	0.0001
Residual		3.60	0.0002
Seed yield			
Entry	15	5.82	0.0001
Residual		3.37	0.0004

Grazing notes from half-acre plots

2015

Field preparations the same as the small plots with the exception of no fertilizer N was applied. Four pastures were included in this study: 14-1 IAC; 14-3 COMB; 23-2 IAC; 23-3 COMB. ‘Elbon’ rye was planted 3-Oct. 2014. In 2014-2015 season grazing was initiated on 11 Feb. 2015.

<u>Grazing days</u>

- 14.1(IAC): 42 grazing days
- 14-3(COMB): 35 grazing days
- 23-2(IAC): 42 grazing days
- 23-3(COMB): 35 grazing days

Cattle on pasture were Brahman steers.

2016

Field preparations the same as the small plots with the exception of no fertilizer N was applied. Four pastures were included in this study: 14-1 IAC; 14-3 COMB; 23-2 IAC; 23-3 COMB. ‘Maton’ rye planted 16-Nov. 2015. Pasture planted later and poor stands because of heavy rains. In 2015-2016 season grazing was initiated on 2 Mar. 2016.

<u>Grazing days</u>

- 14.1(IAC): 6 grazing days
- 14-3(COMB): 4grazing days

- 23-2(IAC): 2 grazing days
- 23-3(COMB): grazing days

-cattle on pastures were F1 cow calf pairs