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Tyler Neal Sandlin

ROW SPACING AND POPULATION DENSITY EFFECT ON SEED YIELD OF

OKRA AND SEED OIL AS A SOURCE OF BIODIESEL

By

Tyler Neal Sandlin

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By

Tyler Neal Sandlin

Approved:

Brian S. Baldwin
Professor of Agronomy/Genetics
(Director of Thesis)

David H. Nagel
Extension Professor
(Committee Member)

Ashli E. Brown
Assistant Professor of Biochemistry
(Committee Member)

Winthrop B. Phippen
Professor of Plant Breeding/Genetics
(Committee Member)

William L. Kingery
Professor of Agronomy/Soils
(Graduate Coordinator)

George Hopper
Interim Dean of College of
Agriculture and Life Sciences

Name: Tyler Neal Sandlin

Date of Degree: December 9, 2011

Major Field: Agronomy

Major Professor: Dr. Brian S. Baldwin

Title of Study: ROW SPACING AND POPULATION DENSITY EFFECT ON SEED YIELD OF OKRA AND SEED OIL AS A SOURCE OF BIODIESEL

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Candidate for Degree of Masters of Science

Okra (*Abelmoschus esculentis*) is a warm weather vegetable crop with seed characteristics similar to cotton. Putative similarities between these crops make okra a potential candidate as a biodiesel feedstock. The objectives of this research are to determine an optimal inter and intra-row spacing combination to maximize seed yield, and determine optimal plant characteristics for seed yield, oil production, and fatty acid profiles. Data indicated treatments of (22.86 x 7.62, 22.86 x 22.86, and 45.72 x 30.48 cm) were better than 91.44 x 15.24 cm with respect to seed yield, although, 45.72 x 30.48 and 91.44 x 15.24 cm are the same plant population. Variety trials indicated that Annie Oakley II produced substantial seed and oil yields of 3547 kg ha^{-1} and 1376 L ha^{-1}, respectively in 2009. Data indicated palmitic, linoleic, and linolenic acids to be the primary constituents of okraseed oil.

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CHAPTER I

INTRODUCTION

Recent instability in fuel prices and the economy have led to the extensive federally sponsored search for alternative liquid fuel and renewable sources of energy. Carbon-neutral sources of energy are of importance with respect to the environment when exploring the realm of alternative fuels. In order to be considered carbon-neutral, a carbon dioxide emission of zero is required. Ethanol and biodiesel can be made from cellulose, biomass, and plant oils and thus would be included in the category of carbon-neutral fuel sources, which makes them of prime interest. Although ethanol and biodiesel are both alternative fuel sources, biodiesel exhibits some advantages over ethanol. In order to produce ethanol, fermentation is required. During fermentation, conversion of sugars to alcohol results in a net loss of approximately half of the fixed carbon dioxide as a gaseous carbon dioxide and thus the net loss of energy previously fixed by photosynthesis. When producing biodiesel, an approximate net loss (from fuel value) of only ten percent occurs through trans-esterification of fatty acids due to the production of the by-product glycerol. Vegetable oils and waste animal fats are commonly used as feedstocks to make biodiesel with greater emphasis generally placed on vegetable oils. Biodiesel is commonly made from vegetable oil by adding simple alcohols to fatty acids of lipids with a catalyst (transesterification) resulting in the biodiesel and a byproduct of glycerol. Rudolph Diesel originally tested peanut oil (not transesterified fatty acids) as fuel in his compression ignition engine over a century ago and only decided to use petroleum-based cetan because it was considered a waste product of distillation for "coal oil" (kerosene) for lamps. Various oilseeds such as: palm (*Elaeis guineensis* J. or *E. oleifera* (Knuth) Cortes), canola *(Brassica rapa* L.), soybean (*Glycine max* L.), and cottonseed (*Gossypium* spp.) are currently used in the production of biodiesel, with soy oil most commonly used.

Okra (*Abelmoschus esculentus* L.) is grown as a vegetable and primarily consumed as immature fruit. Okra (the indeterminate types) have some similarities related to perennial crops, but is grown as an annual in temperate regions. Determinant okra is an annual, produced at latitudes correlating to the southeastern United States, Africa, Brazil, and India. Okra, a warm-season crop with cultivars well adapted to tropic and warm-temperate zones; could have application as a substitute for other oil producing crops in these zones. The Germplasm Resources Information Network (GRIN) lists 2,254 accessions of okra. This diversity allows for potential testing and selection of cultivars with desired characteristics. Planting and harvesting of okra can be completed using conventional equipment, which lends to relative ease of handling. Oil expressed from the mature seed may have the potential for use as biodiesel.

Although little research has been conducted, okra seed characteristics make it a viable source of oil (fatty acids) for biodiesel. Cotton seed is often crushed for oil used in making biodiesel. Research has shown fatty acid profiles between okra seed and cotton seed are similar. Okra seed is reported to contain between 10 to 25 percent oil, which is similar to cottonseed. Cotton seed meal cake, the crushed product of cotton seed, is used as a high nutrient dense protein source in livestock feed as well. However, cotton has a known toxic pigment, gossypol, which is concentrated in the seed meal. This toxin often times irritates the digestive systems of some animals. Okra meal is edible, having no known toxins. High input costs and low economic returns associated with cotton production is quickly diminishing acreage, causing less production of oil and meal from seed. Okra characteristically has few insect- or disease-related problems and could substitute for cotton seed in this case.

Soybean is the most common feedstock used for biodiesel in the U.S. When using soybean for biodiesel production, the oil must be de-gummed in order to prevent engine filter clogging. Soybean is a major food source for the world. Since okra is not listed on the Chicago Board of Trade, it would not be considered a major food source for the world, and therefore would not be in direct competition with the food market. This makes okra seed oil a potentially viable source of fatty acids for biodiesel production.

Cultivars of okra exist that are either indeterminate or determinant and are known to flower under both long and shortened photoperiod, giving okra potential use as a full- or half-season catch crop. If a producer desired, okra could be planted in the spring and grown throughout the entire growing season and harvested in the fall. Oilseeds such as canola, flax *(Linum usitatissimum* L.), and crambe (*Crambe abyssinica* L.), are typically planted in the fall. Soft red winter wheat (*Triticum aestivum* L.) is also a crop grown in the southern U.S. as a winter annual after summer harvest of another crop. If a winter oilseed or wheat were planted in the southern U.S., the crop would most likely be harvested in the early summer months, between May and June. A follow up or “catch crop” would need to be planted to maximize field use. This would allow for planting between late June and early July. A crop with potential to produce a viable yield in a relatively short period of time would be useful. As mentioned previously, some cultivars of okra could potentially have this ability, and if so, would broaden its scale of viability as an oil seed crop.

The objectives of this study are to evaluate okra seed oil content, quality, and yield for the production of biodiesel. Also, determine the optimal row spacing (inter-row) and planting density (intra-row) spacing to maximize seed yield. Another important objective of this study is to evaluate the viability of a full and half-season okra crop in order to determine its sustainability for farmers as a catch crop.

CHAPTER II

LITERATURE REVIEW

Biodiesel Production

ASTM International defines biodiesel as a fuel comprised of monoalkyl esters of long chain fatty acids derived from animal fats or vegetable oils that meet ASTM6751 standards (ASTM 2008). Animal fats and vegetable oils are hydrophobic and primarily consist a glycerol backbone and three moles of fatty acids known as triacylglycerides (Sonntag, 1979). Biodiesel has an extremely low sulfur content, high cetane number, and desirable emission qualities (Canakci and Van Gerpen, 2001). Transesterification is the process most commonly used to produce biodiesel. Transesterification is primarily used due the ease of this process and high energy efficiency (UNH Biodiesel Group, 2005). Transesterification occurs when animal fats or vegetable oils are combined with a monohydric alcohol and a catalyst at increased temperatures (Moser, 2009). Sodium and potassium hydroxide are the most common catalysts used. Various alcohols can be used in transesterification and differ according to the location where the biodiesel is produced and consumed. Transesterification is a gradational process and commences when an alcohol reacts with triacylgylcerides to produce diacylglycerides and fatty acid alkyl esters (FAAE), and then react once more with the alcohol to produce monoacylglycerides and free another mole of FAAE. These monoacylglycerides complete alcoholysis to generate glycerol and FAAE identified as biodiesel (Moser, 2009).

Various factors are considered to be important with regard to biodiesel quality and performance. Fatty acid ester composition and free fatty acid content are known to have a significant impact on the quality of biodiesel (Imahara et al., 2006). Free fatty acid content greater than one percent will require feedstock pretreatment (Kemp, 2006). Fatty acid chain length, degree of unsaturation, double bond orientation, and ester head group can influence properties including cold weather operability, gelling of fuel, injector plugging, coking, engine performance, oxidative storage stability, kinematic viscosity, and cetane number (Ma and Hanna, 1999; Moser, 2009). Low temperature operability, oxidative stability, and kinematic viscosity are considered to be among some of the most crucial properties of biodiesel. ASTM (ASTM D2500, D5773; D97, D5949; D6371) uses cloud point (CP), pour point (PP), and cold filter plugging point (CFPP) to measure operability of biodiesel at low temperatures. CP is incurred when wax crystals are formed in biodiesel due to its inability to remain liquid at below optimal temperatures (Dunn et al., 1996). PP is defined as the lowest temperature at which the fuel is able to be readily poured (Moser, 2008). CFPP is defined as the temperature at which the fuel filter begins to become plugged by the fuel (Imahara et al., 2006). Oxygen exposure, sunlight, and high temperatures cause degradation of biodiesel known as oxidative instability and creates issues with long term storage (Kemp, 2006). Kinematic viscosity of straight vegetable oils is extremely high and can cause deposit build up in engines (Knothe, 2005). Biodiesel kinematic viscosity is much less than straight vegetable oils, but is mildly higher than petrodiesel. Feedstock selection can have a significant impact on all of the previous mentioned factors affecting biodiesel quality.

Numerous feedstocks exist for the production of biodiesel, but vary with respect to the composition of oil they produce. All feedstocks are not desirable nor are they all equal. Biodiesel is an exact copy of the fatty acids and lipids from which it was derived (Moser, 2008). Feedstock selection also is a function of location and availability. Soybean is currently the primary feedstock in the U.S., while rapeseed comprises the majority of biodiesel production in Canada. (Table 1) lists several biodiesel feedstocks and their fatty acid composition by weight.

Okra Botany and Uses

Okra is a member of the Malvaceae family and with origins highly debated between Africa, India, and the Middle East (Zeven and Zhukovsky, 1975). Okra is a self pollinated diploid species with 2x=130 chromosomes. Many recombinations are possible with okra (Martin et al., 1981). Perennial as well as annual varieties of okra are known to exist. Climatic conditions and chromosome number can differentiate perennial from annual varieties (Sinnadurai, 1977). Flowering of okra generally occurs 30 to 60 days after emergence, with seed maturation occurring 30 to 40 days after anthesis (Yamaguchi, 1983).

Okra is seldom thought of as an oilseed, but rather a warm weather vegetable crop produced for its immature pods. The leaves of okra are even consumed for food or ground into powdered flavoring agents in parts of Asia and Africa, and several industrial products have been made using okra including ropes and paper from the stalks (Martin,1982).

Table 1 Typical fatty acid composition (wt.%) of various feedstock oils.

Fatty Acid	Canola	Palm	Soybean	Sunflower	Cottonseed	Coconut	Okraseed
C6:0 ‡						1	
C8:0						7	
C10:0						7	
C12:0						47	
C14:0		1			1	18	
C16:0	4	45	11	6	23	9	24
C18:0	2	4	4	5	2	3	7
C20:0							
C22:0			1				
C16:1					1		
C18:1	61	39	23	29	17	6	27
C18:2	22	11	54	58	56	2	42
C18:3	10		8	1			
C20:1	1						
Other							

† From Gunstone and Harwood (2007).

‡ C6:0 methyl caproate, C8:0 methyl caprylate, C10:0 methyl caprate, C12:0 methyl laurate, C14:0 methyl myristrate, C16:0 methyl palmitate, C18:0 methyl stearate, *C20:0* methyl arachidate, C22:0 methyl behenate, C16:1 methyl palmitoleate, C18:1 methyl oleate, C18:2 methyl linoleate, C18:3 methyl linoleneate

Okra Agricultural Requirements and Maintenance

Nitrogen requirements for okra are approximately 30 lbs per acre (34 kg ha^{-1}) for normal (non-depleted) soil conditions (Nagel, 2000). Dhankhar and Singh (2009) state that phosphorus and potassium fertilization take precedence over nitrogen when growing okra for seed production. Cotton aphids (*Homoptera aphididae*), *Pythium*, *Rhizoctonia*, *Fusarium*, and *Phytophtor*a are listed as possible pests of okra (Yamaguchi, 1983). Few herbicides are labeled for use in okra. Trifluralin and S-metolachlor are two preplant herbicides that can be used for weed control in okra (Nagel, 2000). Soil temperatures between 24 and 32ºC are optimal for seed germination and plant growth is optimal between 18 and 35ºC (Yamaguchi, 1983).

Okra Oil Composition and Seed Yield

Okra seed and oil yield varies with cultivar. Okra seed is similar to cottonseed and is known to contain between 20 and 25 percent oil (Nagel, 1987). Okraseed fatty acid profiles can also differ depending on cultivar and climate, with several noting these differences(Clopton et al., 1948; Crossley and Hilditch, 1951; Martin, 1982). Okra seed oil is primarily comprised of unsaturated fatty acids, with linoleic and oleic acids constituting 70% of the total according to Martin (1982). Crossley and Hilditch (1951) reported that a breakdown of okra seed oil consists of: 24% palmitic, 7% stearic, 27% oleic, and 42% linoleic acid. Clopton et al. (1948) found differing compositions, reporting: 33% palmitic, 0.5% stearic, 41.8% oleic, and 13.2% linoleic. Gelling points for okra seed oil fatty acids have been listed between -25 and 6ºC (Nzikou et al., 2006).

Protein content of okra seed has been determined to vary between 18 and 27% and could easily be supplemented with other protein sources to provide a balanced protein source (Martin, 1981).

One full season study in the Rolling Plains of Texas found okra seed yield to be as much as 2800 kg ha^{-1} (2500 lbs A^{-1}) at 32% oil composition (Nagel, 1987). A yield of this type would result in 900 kg ha^{-1} (800 lbs of oil A^{-1}) and could produce 378.5 liters (100 gallons) of oil. Another full season study conducted at Western Illinois University found yields to be even greater at nearly 3800 kg ha^{-1} (3400 lbs A^{-1}) for 'Green Best' okra (Phippen, 2005) (Figure 1). These yields are similar to soybean at that location . A direct comparison was made for Roundup Ready® soybean and okra, in which soybeans yielded 4370 kg ha^{-1} (3900 lbs A^{-1}) and 'Clemson Spineless' okra yielded 1900 kg ha^{-1} (1700 lbs A^{-1}) (Phippen, 2005). There are obvious differences in yield depending on the cultivar used. Yields similar to 'Green Best' could make okra a potential candidate for biodiesel and an alternative protein source, however, soy fixes its own nitrogen.

U.S. Soybean Production and Uses

Soybean has long been grown in the U.S. with many functions and uses. Soybean is a major food source for the world, with the U.S. accounting for 40 percent of total production. U.S. soybean production was nearly 82 M Mg (3 billion bushels) in 2008. (USDA, 2008). Soybean meal is used as a high protein source for many livestock feeds and the oil is used in various other food and industrial applications. Soybean is the primary source of fatty acids for biodiesel in the U.S. soybean accounted for

Figure 1 Mature pods of the okra cultivar Green Best with seed exposed taken from the 2010 full-season variety trials conducted at Starkville, MS.

approximately 22% of biodiesel feedstock production in 2007 (Moser, 2009). Twenty-seven kilograms (1 bu) of soybean can yield 5.7 liters (1.5 gallons) of biodiesel, and over 1.5 billion liters (400 million gallons) of biodiesel were produced from U.S. soybean in 2007 [American Soybean Association (ASA), 2009]. U.S. soybean average yield in 2008 was roughly 2800 kg ha^{-1} (42 bu A^{-1}) (USDA, 2008). Soybean is also used as the primary protein source and supplement for many livestock animals, with approximately 22 kg of meal produced per 27 kg of soybeans (ASA, 2009). Livestock consumption of soybeans was 32 M Mg in 2007 (ASA, 2009).

Some problems exist with the properties of soybean oil and its use in biodiesel. Soybean oil fatty acids are susceptible to oxidation due to its high degree of unsaturation and location of double bonds (Monyem and Van Gerpen, 2001). This oxidation causes gums that settle out in fuel tanks and can cause injector and filter clogging. Oxidation and gum formation is a potential risk to engine performance that can have negative economic impacts.

Cotton Production, Oil, and Gossypol

Nearly 6.5 million hectares (16 million acres) of cotton were planted in 2000, but has since decreased significantly, with less than 4 million hectares (10 million acres) planted in 2008 (USDA, 2008). Reduction in cotton acreage naturally results in less seed that is harvested and crushed for oil. Previously stated, cottonseed oil and okra seed oil are similar in content due to the law of homologous series. Cottonseed oil is typically comprised of 22% palmitic, 3% stearic, 30% oleic, and 45% linoleic acids (Crossley and Hilditch, 1951). Cottonseed is also known to contain gossypol. Gossypol is a compound known to be toxic to many livestock species, with symptoms of toxicity including lung congestion, hindered growth, and damage to reproductive systems (Randel et al., 1992). This has long been a known and well documented problem with cottonseed meal as a protein source for mammals, with attempted research conducted to further reduce gossypol in cottonseed meal (Hale and Lyman, 1957; Vroh Bi et al., 1999).

Okra Row Spacing and Planting Density

Differing crops require varying row spacings to achieve optimal yields. Several studies have been conducted on effects of okra planting density and row spacing on yield (Amjad et al., 2001; Shrestha, 1983; Wu et al., 2003a,b,c). It has been stated that increased okra yields can be achieved through increased planting densities (McFerran et al., 1963). The majority of these studies focus on repeated mechanized harvest impacts and plant populations for fruit production and not seed production, although correlations between the two may be likely.

Research on the effects of nitrogen fertilization and plant spacing of okra were studied in Nepal by Shrestha (1983). Studies were conducted in the summers of 1980 and 1981 and consisted of three plant spacings (45x15, 45x30, and 45x45cm; 150,000; 75,000; and 50,000 plants ha $^{-1}$, respectively) and four nitrogen rates (0, 30, 60, and 90 kg ha $^{-1}$). No significant interactions between plant spacing and nitrogen rate were found. Pods per plant were found to be greatest at the widest row spacing, while total yield was greatest at the closest row spacing. Pod length and size were greater with 60 or 90 kg treatments in 1981, but significant differences in yield per area were not always apparent. Nitrogen rates of 60 kg ha $^{-1}$ were recommended.

Few studies exist for plant populations affecting okra seed production. Amjad et al. (2001) conducted such a study on phosphorus and planting density effects on seed yield of okra. Three planting densities (37000, 55500, and 111000 plants ha $^{-1}$) and three phosphorus levels (0, 33, and 66 kg P_2O_5 ha $^{-1}$) were tested in main and subplots, while keeping inter-row spacings constant at 60 cm. Lowest planting density resulted in greatest yield of pods per plant, regardless of P_2O_5 treatment. Greatest seed yield per hectare was achieved with the highest planting density combined with 33 and 66 kg ha^{-1} treatment of phosphate. Seed yield per hectare was greatest at the highest planting density across all P_2O_5 treatments.

Ease of planting and harvest are two crucial factors to be considered when preparing for commercial seed production. Optimum planting densities that achieve maximum yield combined with a mechanized harvest are desirable. Both previously mentioned studies were conducted in Nepal and Pakistan, but could prove useful for commercial okra seed production in the U.S. Further research has the potential to improve the uses and yields of okraseed oil.

CHAPTER III

MATERIALS AND METHODS

2009 Field Studies

In 2009 two studies were conducted. The first test consisted of four replications in a randomized complete block design, planted on Jun 3, at the R.R. Foil Plant Science Research Center (North Farm) at Starkville, MS on a Marietta fine sandy loam (Figure 2). The test consisted of four inter-row spacings and four intra-row spacings of 'Clemson Spineless' okra. The inter-row spacings were: 22.86, 45.72, 68.58, and 91.44 cm (9,18,27,and 36 in, respectively) and intra-row spacings were: 7.62, 15.24, 22.86, and 30.48 cm (3,6,9, and 12 in, respectively) (Table 2). Each plot was 3.05m (10ft) long by 3.05m (10ft) wide. The choice of these row spacing configurations allow for comparison of five pair and a triplet of identical populations densities but at different configurations (22.86 x15.24 cm, 45.72 x 7.62 cm = 287,100 plts ha^{-1} ; 22.86 x 22.86 cm, 68.58 x 7.62 cm = 191,300 plts ha^{-1} ; 22.86 x 30.48 cm, 45.72 x 15.24 cm, 91.44 x 7.62 cm = 143,600 plts ha^{-1} ; 45.72 x 22.86 cm, 68.58 x 15.24 cm = 95,600 plts ha^{-1} ; 45.72 x 30.48 cm, 91.44 x 15.24 cm = 71,700 plts ha^{-1} ; 68.58 x 30.48 cm x 91.44 x 22.86 cm = 47,900 plts ha^{-1}) (Table 2).

NORTH 2009

REP IV	68.58 x 15.24	91.44x 15.24	68.58 x 22.86	45.72 x 30.48
	45.72 x 15.24	91.44x 30.48	22.86 x 7.62	45.72 22.86
	91.44 x 7.62	22.86x 15.24	68.58 x 30.48	22.86 x 30.48
	22.86 x 22.86	68.58x 7.62	45.72 x 7.62	91.44 x 22.86
REP III	91.44 x 7.62	68.58x 22.86	22.86 x 30.48	45.72 x 30.48
	22.86 x 15.24	68.58x 7.62	91.44 x 22.86	45.72 22.86
	91.44 x 15.24	45.72x 15.24	45.72 x 7.62	22.86 x 22.86
	22.86 x 7.62	91.44x 30.48	68.58 x 15.24	68.58 x 30.48
REP II	22.86 x 22.86	68.58 x 22.86	68.58 x 30.48	91.44 x 22.86
	68.58 x 15.24	22.86 x 30.48	91.44 x 30.48	22.86 x 7.62
	45.72 x 15.24	91.44 x 15.24	22.86 x 15.24	45.72 x 30.48
	68.58 x 7.62	45.72 x 7.62	91.44 x 7.62	45.72 22.86
REP I	45.72 x 15.24	68.58 x 7.62	22.86 x 30.48	91.44 x 7.62
	91.44 x 22.86	45.72 22.86	68.58 x 30.48	22.86 x 7.62
	91.44 x 15.24	68.58 x 15.24	68.58 x 22.86	45.72 x 7.62
	22.86 x 22.86	22.86 x 15.24	45.72 x 30.48	91.44 x 30.48

Figure 2 Field Map for the 2009 Inter by Intra-Row Spacing (cm) Trial Using Clemson Spineless Okra Planted at Starkville, MS.

Prior to planting, the field was prepared through disking and harrowing. Glyphosate was applied pre-plant burn-down at 1.54 kg ai ha^{-1} (1.375 lb ai A^{-1}). The field was left fallow the previous year. ‘Clemson Spineless’ okra seed was obtained from Wax Seed Co. (Amory, MS) and plots were solid stand grain drilled on Jun 3. Trifluralin (Treflan®, Helena Chemical Co.) was applied at 0.84 kg ai ha^{-1} (0.75 lb ai A^{-1}) immediately after planting and incorporated via rainfall. Plants were hand thinned to the

Table 2 Inter by Intra-Row Spacing Treatments and Respective Plant Populations for Clemson Spineless Okra Planted at Starkville, MS for 2009 and 2010.

Row Spacing Inter x Intra (cm)	Row Spacing Inter x Intra (in)	Population Density Plants ha^{-1}
22.86 x 7.62	9 x 3	574,000
22.86 x 15.24	9 x 6	287,100†
22.86 x 22.86	9 x 9	191,300
22.86 x 30.48	9 x 12	143,600‡
45.72 x 7.62	18 x 3	287,100
45.72 x 15.24	18 x 6	143,600
45.72 x 22.86	18 x 9	95,600
45.72 x 30.48	18 x 12	71,700
68.58 x 7.62	27 x 3	191,300
68.58 x 15.24	27 x 6	95,600
68.58 x 22.86	27 x 9	63,800
68.58 x 30.48	27 x 12	47,900
91.44 x 7.62	36 x 3	143,600
91.44 x 15.24	36 x 6	71,700
91.44 x 22.86	36 x 9	47,900
91.44 x 30.48	36 x 12	35,600

† Population densities followed by circle tailed line occur in pairs of different planting configurations.
‡Population densities followed by weighted black line occurs as a triplet of different planting configurations.

appropriate intra and inter-row spacing. Nitrogen was split applied (56 kg ha^{-1} pre-plant and 56 kg ha^{-1} two weeks after emergence) in the form of ammonium nitrate. Two applications of pyrithiobac sodium (Staple®,DuPont Crop Protection) were applied early postemergence at 0.036 kg ai ha^{-1} (0.0325 lb ai A^{-1}) to control of morningglory (*Ipomoea spp.* L). Sequential applications of sethoxydim (Poast® BASF Ag Products) at 0.42 kg ai ha^{-1} (0.375 lb ai A^{-1}) and fluazifop (Fusilade®, Syngenta Crop Protection Inc.) at 0.21 kg ai ha^{-1} (0.1875 lb ai A^{-1}) were made throughout the season to control grassy weeds. Two applications of permethrin (Hi Yield, Fertilome Inc.) were applied at 0.22 kg ai ha^{-1} (0.2 lb ai A^{-1}) during the season for control of aphids (*Aphis gossypi*) and white flies (*Trialeurodes abutilonea*). Plants were rainfed and allowed to mature until natural senescence occurred. Plots were harvested on November 2 using a plot combine harvester (Massey Ferguson, AGCO® ; Duluth, GA). Seed were bagged and labeled according to their respective plots. Seed were allowed to dry to 10% moisture. Moisture content was determined using a (Farmex®; Streetsboro, OH) hand held moisture testing implement. A belt thresher (Almaco® ; Nevada, IA) was used to further separate and discard foreign material from quality seed. Seed were then weighed and yield was determined. Plot weights were analyzed using SAS® (v.9.2) PROC GLM (SAS Institute, Cary, NC) and mean separation procedures were conducted using Fisher's Protected LSD ($p \leq 0.05$).

The second experiment conducted was a randomized complete block design and consisted of four replications using 18 okra varieties (Table 3). This test was initiated on a Catalpa silty clay loam at the Leveck Animal Research Center (South Farm, Starkville, MS). Objectives of this study were to determine superior plant morphological characteristics (days to flower, percent lodge, percent shatter, height at maturity, and pods per plant) along with seed and oil yields.

The previous crop planted at this location was sweet sorghum. Field conditioning was achieved prior to planting by disking and harrowing smooth. Glyphosate was applied pre-plant burn-down at 1.54 kg ai ha^{-1} (1.375 lb ai A^{-1}). The test was planted on Jun 1. Plots were 3.05m (10ft) long on 96.52cm (38 in) centers and were planted using a John Deere 71 (Kincaid ®; Haven, KS) cone research planter. Eighty seed per row were planted to achieve maximum emergence. Trifluralin (Treflan®) was applied immediately after planting at 0.84 kg ai ha^{-1} (0.75 lb ai A^{-1}) and incorporated through irrigation. Nitrogen was split applied (56 kg ha^{-1} pre-plant and 56 kg ha^{-1} two weeks after emergence) in the form of ammonium nitrate.

After establishment, plants were thinned to one plant per 30.48cm (1ft) of row. Sequential applications of sethoxydim (Poast®) at 0.42 kg ai ha^{-1} (0.375 lb ai A^{-1}) and fluazifop (Fusilade®) at 0.21 kg ai ha^{-1} (0.1875 lb ai A^{-1}) were made throughout the season to control grassy weeds. Two applications of permethrin were applied at 0.22 kg ai ha^{-1} (0.2 lb ai A^{-1}) during the season for control of aphids (*Aphis* gossypi) and white flies (*Trialeurodes abutilonea*). One plant was randomly selected from each row in which each pod was bagged to ascertain percent shatter of mature pods. Plots were hand harvested at various times from Oct through Nov according to cultivar maturity (senescence). Data taken on each variety included: days to first flower, percentage of lodging, percentage of shatter, mean height, seed yield, and percentage seed oil content.

Table 3 Varieties and Seed Source for the 2009 Full Season Okra Variety Trial at Starkville, MS Consisting of 18 Varieties.

Cultivar	Seed Source
Alabama Red	Reimer Seeds Inc. Mount Holly, NC
Annie Oakley II	Reimer Seeds Inc. Mount Holly, NC
Baby Bubba	Reimer Seeds Inc. Mount Holly, NC
Burmese	Reimer Seeds Inc. Mount Holly, NC
Cajun Delight	Reimer Seeds Inc. Mount Holly, NC
Cajun Jewel	Reimer Seeds Inc. Mount Holly, NC
Clemson Spineless	Wax Seed Co. Amory, MS
Cow Horn	Reimer Seeds Inc. Mount Holly, NC
Dwarf Long Green	Reimer Seeds Inc. Mount Holly, NC
Evertender	Reimer Seeds Inc. Mount Holly, NC
Fife Green Cowbean	Reimer Seeds Inc. Mount Holly, NC
Green Velvet	Reimer Seeds Inc. Mount Holly, NC
Hill Country Red	Reimer Seeds Inc. Mount Holly, NC
Lee	Reimer Seeds Inc. Mount Holly, NC
Mammoth Spineless	Reimer Seeds Inc. Mount Holly, NC
Red Velvet	Reimer Seeds Inc. Mount Holly, NC
Star of David	Reimer Seeds Inc. Mount Holly, NC
White Velvet	Reimer Seeds Inc. Mount Holly, NC

2010 Field Studies

In 2010, the row spacing by population density study was replicated according to the previous year's study. Planting occurred on May 11, at the Leveck Animal Research Center, a differing location than in 2009. Location of planting was changed due to the previous year's location being allocated for planting of an arboretum in 2010. Planting style, pesticides, fertility, harvesting, and seed cleaning methods were kept consistent according to the previous year's protocol, with the exception of defoliation before harvesting. Senescence of pods were not as uniform as in 2009 and harvest occurred at an earlier date (Sep 22), therefore, plants were dessicated with paraquat (Gramoxone Inteon®, Syngenta Crop Protection Inc.) at 2.943 kg ai ha^{-1} (2.625 lb ai A^{-1}). Seed yield was taken and analyzed as before.

Full and half-season variety trials were also conducted for this year. Seed availability was limited, eliminating seven cultivars from this study. Two additional cultivars were added to these trials (Green Best and Jambalaya) for a total of 13 cultivars (Table 4). Full-season plots were planted May 11. Field design, pesticide applications, fertility, and harvest methods were consistent with the previous year's study. Initial seeding rate was changed from 80 to 50 seed per row for this year due to limited inventory. Measurement of shatter estimates also differed for this year. Determination of shattering initially was conducted through the bagging method identical to the prior year. However, early during the season it was discovered that the bagging method was protecting pods from weathering, making it difficult to obtain accurate percentage shatter for 2010. Measurements for 2009 are believed to be accurate, due to the fact that very little visual shattering was observed on plants that were not bagged. In 2010, 10 mature pods (5 shattered and 5 unshattered) of similar size were selected in each cultivar for each rep. Total weights were take for shattered pods versus unshattered pods, and differences were determined for percentage shatter. Plots were harvested Oct 1. Pods were threshed, seed weights were taken, and yield data was analyzed identical to the previous year.

Table 4 Varieties and Seed Source for the 2010 Full and Half-Season Okra Variety Trial at Starkville, MS Consisting of 13 Varieties.

Cultivar	Seed Source
Alabama Red	Reimer Seeds Inc. Mount Holly, NC
Annie Oakley II	Reimer Seeds Inc. Mount Holly, NC
Burmese	Reimer Seeds Inc. Mount Holly, NC
Cajun Delight	Reimer Seeds Inc. Mount Holly, NC
Cow Horn	Reimer Seeds Inc. Mount Holly, NC
Dwarf Long Green	Reimer Seeds Inc. Mount Holly, NC
Evertender	Reimer Seeds Inc. Mount Holly, NC
Green Best	Sakata Seed America, Morgan Hill, CA
Hill Country Red	Reimer Seeds Inc. Mount Holly, NC
Jambalaya	Sakata Seed America, Morgan Hill, CA
Lee	Reimer Seeds Inc. Mount Holly, NC
Red Velvet	Reimer Seeds Inc. Mount Holly, NC
Star of David	Reimer Seeds Inc. Mount Holly, NC

Seed Oil Analysis

Fatty acid methyl ester (FAME) analyses were performed on 18 varieties of okraseed in 2009 and 13 varieties in 2010. A modified Dionex-accelerated solvent extraction (ASE®) was performed to extract okraseed oil (Lopez et al., 2009). One gram seed samples were ground fine using a IKA® A11 basic mill. The mill was cleaned with HPCL Optima grade water and ethanol (Fisher Scientific, Pittsburgh, PA) each time a sample was ground to prevent sample contamination. Each sample consisted of two replicates. One gram of each variety of seed was weighed and placed in a Falcon tube. Enough Hydromatrix (Varian Medical Systems Inc., Palo Alto, CA, USA) to fill each extraction cell (ASE) was then added into the Falcon tube. ASE cells were labeled according to each corresponding sample number. ASE cells were then assembled with a Dionex cellulose filter added. ASE cells were filled with the sample/hydromatix mix corresponding to the sample number and ASE® method was performed to extract oil. Solvent extraction was conducted using a 200 series ASE® (Dionex, Sunnyvale, CA, USA). A 100% hexane flush was performed. The system was operated at 120ºC and 10.34 Mpa for three static cycles at 15 minutes each. A 60% purge was conducted after extraction. The hexane solvent was evaporated with nitrogen using a Turbo Vap at 40º C. Oil content was calculated by taking the weight of the extracted oil and dividing it by the weight of the sample and multiplying by 100 to obtain a percentage.

Ten mg of oil were then transesterified. Two mL of 1% H_2SO_4 in MeOH were added to each oil sample and thoroughly vortexed. Samples were placed on heating blocks at 60º C for 2hr and then allowed to cool. Each sample was then neutralized using 10% $NaHCO_3$ 5% NaCl to an approximate pH of 8.0.   The FAMES were extracted twice with two mL toluene. The toluene contained 200 ng/mL dichlorobenzene (internal standard) and 100 ng/mL butylated hydroxytoluene (BHT) (antioxidant).

Extracts for each sample were then transferred to auto-sampler vials and analyzed by gas chromatography mass spectrometry (GC-MS) for FAME composition. A standard fatty acid mix purchased from Supelco was used for GCMS calibration. Standard calibration curves used in this analysis were methyl octanoate (8:0), methyl caprate (10:0), methyl laurate (12:0), methyl myristate (14:0), methyl plamitate (16:0), methyl palmitoleate (16:1), methyl stearate (18:0), cis9oliecmethester (18:1), methyl linoleate (18:2), methyl linolenate (18:3), methyl arachidate (20:0), and methyl behenate (22:0).

All standard calibration curves used had a coefficient of determination (r2) of 0.995 or greater. All analysis was carried out on a Varian 3400 GC Saturn® 2000 MS (Varian Medical Systems Inc., Palo Alto, CA, USA). A Stabilwax® DA column (30m x 0.25mm x 0.05mm) (Restek, Bellfonte, PA) was used with helium as the carrier gas. Initial column temperature was 50ºC with a hold time and thermal stabilization time of two minutes. Final column settings were 250ºC at a rate of 10ºC/min and held for 13 minutes and a total run time of 35 minutes per sample. For MS analysis, electron impact ionization was performed. Ions with an m/z of 50 to 300 were scanned at 0.75 seconds per scan. The library search was conducted using NIST library and analyte identification was confirmed using reference standards. The percentage of separated compounds were calculated from total ion chromatography by computerized integrator. Data was analyzed using SAS® (v.9.2) PROC GLM (SAS Institute, Cary, NC) and mean separation procedures were conducted using Fisher's Protected LSD ($p \leq 0.05$).

CHAPTER IV

RESULTS AND DISCUSSION

Row Spacing and Planting Density

Row spacing and planting density studies were conducted with 'Clemson Spineless' okra in 2009 and 2010. Analysis of variance indicated no treatment by year interaction, data to be pooled across the two years of testing. Yields ranged from 1,438 kg ha^{-1} to 1,856 kg ha^{-1} (Table 5). Yield trends indicated a plastic effect for this crop among treatments. No significant difference in seed yield were found between least and greatest planting densities 91.44 x 30.48 (35,800 seed ha^{-1}) and 22.86 x 7.62cm (574,000 seed ha^{-1}) respectively. Data indicated the treatment combinations of 22.86 x 7.62cm, 22.86 x 22.86, and 45.72 x 30.48cm (9 x 3, 9 x 9, 18 x 12 in, respectively) all resulted in greater seed yields than the treatment of 91.44 x 15.24cm (36x6 in). Variance around the mean of the other row spacing population treatments caused them to fall into both high and low mean separation groups.

Previously mentioned, the parameters of this study included five pair and a triplet of identical plant populations, but at different row spacing configurations (22.86 x15.24 cm, 45.72 x 7.62 cm = 287,100 plts ha^{-1} ; 22.86 x 22.86 cm, 68.58 x 7.62 cm = 191,300 plts ha^{-1} ; 22.86 x 30.48 cm, 45.72 x 15.24 cm, 91.44 x 7.62 cm = 143,600 plts ha^{-1} ;

Table 5 Mean Seed Yield Affected by Row Spacing and Planting Density for Clemson Spineless Okra at Starkville, MS for 2009 and 2010.

Row Spacing Inter x Intra (cm)	Row Spacing Inter x Intra (in)	Population Density Plants ha^{-1}	Seed Yield kg ha^{-1}
22.86 x 7.62	9 x 3	574,000	1856 a †
22.86 x 15.24	9 x 6	287,100	1641 ab
22.86 x 22.86	9 x 9	191,300	1805 a
22.86 x 30.48	9 x 12	143,600	1704 ab
45.72 x 7.62	18 x 3	287,100	1733 ab
45.72 x 15.24	18 x 6	143,600	1768 ab
45.72 x 22.86	18 x 9	95,600	1765 ab
45.72 x 30.48	18 x 12	71,700	1793 a
68.58 x 7.62	27 x 3	191,300	1658 ab
68.58 x 15.24	27 x 6	95,600	1614 ab
68.58 x 22.86	27 x 9	63,800	1782 ab
68.58 x 30.48	27 x 12	47,900	1690 ab
91.44 x 7.62	36 x 3	143,600	1688 ab
91.44 x 15.24	36 x 6	71,700	1438 b
91.44 x 22.86	36 x 9	47,900	1578 ab
91.44 x 30.48	36 x 12	35,600	1618 ab
Coeff. of Var.	----	----	20.7

† Letters differing among means denote significant difference. $\alpha = 0.05$

45.72 x 22.86 cm, 68.58 x 15.24 cm = 95,600 plts ha^{-1} ; 45.72 x 30.48 cm, 91.44 x 15.24 cm = 71,700 plts ha^{-1} ; 68.58 x 30.48 cm x 91.44 x 22.86 cm = 47,900 plts ha^{-1}) (Table 2).

Population comparisons indicated significant differences within the row spacing configurations for this study. Of the five paired populations, the row spacing configuration of 45.72 x 30.48 cm was better than 91.44 x 15.24 cm, although their plant populations were the same (71,700 plts ha^{-1}) (Table 5). There were no other significant differences in yield among the paired row spacings or the triplet, due to configuration. A regression analysis was performed in order to determine if a predictive model could be applied to the parameters of this study (Figure 3). Regression analysis indicated that a predictive model was unable to be determined.

Our findings did not coincide with the study of Amjad et al. (2001) who conducted a study consisting of three okra planting densities (37,000; 55,500; and 111,000 plts ha^{-1}) and three phosphorous treatments (0, 33, and 66 kg ha^{-1}) for effect on seed yield. Their inter-row spacing was held constant at 60 cm and intra-row spacing consisted of 15, 30, and 45 cm, according to planting density. Regardless of phosphorous treatment, the greatest seed yields were obtained at the greatest planting densities (111,000 plts ha^{-1}). Overall top ranking seed yields (3,665.1 and 3,225.1 kg ha^{-1}) were substantially higher for Amjad et al. (2001) than for our study (1856, 1805, and 1793 kg ha^{-1}) . Location of planting and cultivars used are possible reasons for differing results. Tests conducted by Amjad et al. (2001) were planted in Faisalabad, Pakistan using 'Sabz Pari' okra. Although phosphorous was applied in their study, it would not likely have impacted the results for our study. Soil conditions where our study was planted are innately high in phosphate. Phippen (2005) also obtained substantially higher seed yields (3 year avg. of 2800 kg ha^{-1}) with Green Best okra in replicated field trials (Macomb, IL), at a constant inter-row spacing and plant population of 76.2 cm and 90,000 plants ha^{-1},

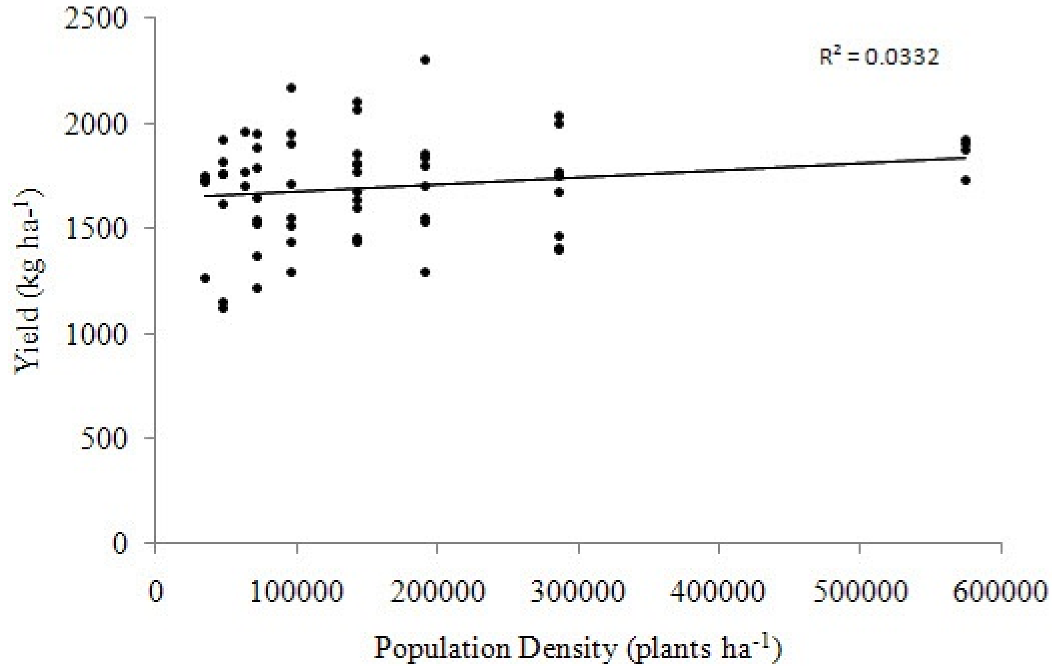

Figure 3 Regression analysis of 'Clemson Spineless' okra seed yield affected by population density.

respectively. Phippen's (2005) row spacing configuration and plant population was similar to one the spacings and configurations in our study (68.58 x 15.24 cm and 95, 600 plants ha^{-1}). A seed yield of 1614 kg ha^{-1} was produced in our study at the previously mentioned spacing and configuration. A study conducted on cotton population density in Georgia, USA proved to have similar results to our row spacing study, stating that varying population densities resulted in different fruiting sites and boll size, but no differences in overall yield were observed (Bednarz et al., 2000). Data related to fruiting size and location in this okra row spacing trial were not recorded, but visual differences in these characteristics were observed at the different population densities.

While there were no significant differences among the least (35,600 plts ha^{-1}) and greatest planting densities (574,000 plts ha^{-1}) in this okra row spacing and planting density study, there would be economic impacts for an okra-for-oil producer. If maximum yields are achievable by planting less seed (35,600 plts ha^{-1}), doing so would offer a competitive economic advantage for the farmer over planting at a higher seeding rate (574,000 plts ha^{-1}) that would give you the same yield. Knowing this, even if a crop failure were to occur early in the season, cost of replanting would remain relatively low if lower populations were recommended.

2009 Full-Season Variety Trial

In 2009, the full-season variety trial consisting of 18 cultivars was planted Jun 1. The objective was to determine distinct morphological characteristics, seed, and oil yields among these cultivars. Planting date was delayed due to excessive spring rainfall. Rainfall events continued to occur frequently throughout the 2009 growing season (May-Oct), resulting in 109 cm, cumulatively (Figure 4).

Flowering of all okra cultivars occurred between 53 and 73 days (Table 6). Flowering of Burmese occurred sooner than 11 cultivars (Baby Bubba, Cajun Delight, Cajun Jewel, Clemson Spineless, Cow Horn, Dwarf Long Green, Fife Green Cowbean, Green Velvet, Lee, Red Velvet, and White Velvet). Red Velvet Flowered later than 12 other cultivars (Alabama Red, Annie Oakley II, Burmese, Cajun Delight, Cajun Jewel, Cow Horn, Dwarf Long Green, Evertender, Green Velvet, Hill Country Red, Lee,

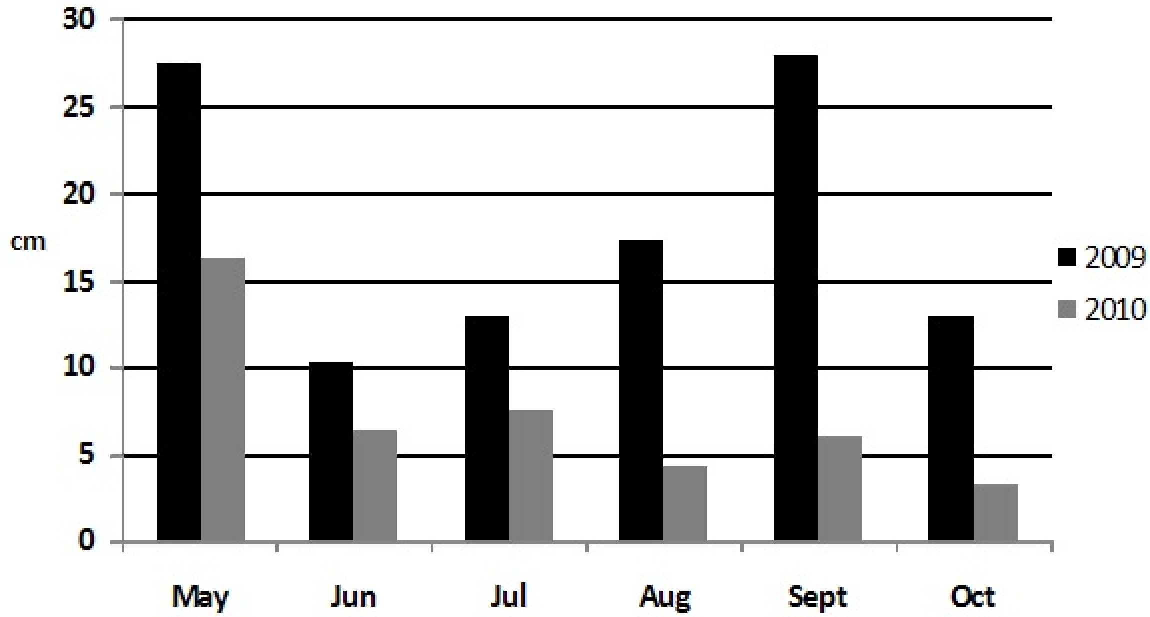

Figure 4 Cumulative rainfall in cm for the 2009-2010 growing season recorded at the R.R Foil Plant Science Research Center.

Mammoth Spineless, and Star of David). Flowering was within the published time frame of 30 to 60 days for eight cultivars (Alabama Red, Annie Oakley II, Burmese, Cajun Delight, Evertender, Hill Country Red, Mammoth Spineless, and Star of David) in this study according to previously stated literature (Yamaguchi, 1983), while the other 10 were later flowering. Differences in photoperiod, ambient temperature, and cultivar can all have and impact on time to flowering (Dhankhar and Singh, 2009). It is believed that some cultivars flowered later in this study than others due to the rainfall and corresponding poorer light quality impact on photosynthate contributing to extended vegetative growth, a phenomena observed in cotton.

Table 6 Morphological Characteristics of 18 Okra Varieties Grown Under Full Season Conditions from Jun-Oct in 2009 at Starkville, MS.

Variety	Mean Days to Flower	Mean Lodging (%)	Mean Shatter (%)	Mean Height (m)	Mean Pods Plant $^{-1}$
Alabama Red	57 ghi †	0 b	0 abc	1.7 cd	16 bcd
Annie Oakley II	59 efghi	0 b	1 abc	1.4 efgh	25 a
Baby Bubba	67 abc	0 b	3 a	0.9 I	9 e
Burmese	53 I	0 b	3 ab	1.2 hi	16 bcd
Cajun Delight	60 defg	0 b	1 abc	1.5 defgh	18 bc
Cajun Jewel	61 defgh	0 b	0 c	1.3 fgh	10 de
Clemson Spineless	70 ab	0 b	0 c	1.8 cd	21 ab
Cow Horn	66 bcd	0 b	0 c	1.5 gh	14 cde
Dwarf Long Green	63 cdef	0 b	1 abc	1.6 def	14 cde
Evertender	58 fghi	4 a	0 c	1.7 cde	19 bc
Fife Green Cowbean	70 ab	0 b	0 c	1.8 cd	14 cde
Green Velvet	66 bcd	0 b	0 c	1.6 def	18 bc
Hill Country Red	57 ghi	0 b	1 abc	1.8 cd	17 bc
Lee	63 cdefg	0 b	0 c	1.2 gh	15 bcd
Mammoth Spineless	59 efghi	0 b	0 bc	1.4 efgh	15 bcd
Red Velvet	73 a	0 b	0 c	2.2 b	13 cde
Star of David	56 hi	0 b	0 bc	1.9 c	13 cde
White Velvet	70 ab	0 b	0 c	2.5 a	25 a
Coeff. of Var.	6.6	762.4	283.5	12.1	25.2

† Letters differing among means denote significance. Differing letters among means with the same values are due to rounding. $\alpha = 0.05$

Morphological characteristics (lodging and shatter) related to seed loss proved to be almost non-existent in 2009. Lodging was observed only in Evertender and seed shatter was less than four percent for all cultivars (Table 6). Shatter was greater for Baby Bubba than for 11 other cultivars (Cajun Jewel, Clemson Spineless, Cow Horn, Evertender, Fife Green Cow bean, Green Velvet, Lee, Mammoth Spineless, Red Velvet, Star of David, and White Velvet). Seed dehiscence (shatter) can be triggered by environmental and genetic factors. It occurs when lignified pod cell walls dehydrate and split open along weak seems, resulting in the abscission and dispersal of seed (Roberts et al., 2002). Lack of drought stress even late into the growing season proved to keep pods closed longer, preventing seed loss and weathering.

Okra plant heights in 2009 ranged between 0.9 and 2.5 m (Table 6). Baby Bubba (0.9 m) was significantly shorter than all other cultivars, except Burmese (1.2 m) (Table 6). White Velvet (2.5 m) was significantly taller than all other cultivars, followed by Red Velvet (2.2 m). Plant height is not always desirable because it causes difficulty in harvestability. Excessive plant heights create difficulty for equipment to reap upper pods. In addition, threshing of seed is made inefficient by the excess vegetative material of tall plants. Plants of comparable heights to traditional row crops ($\leq$ 1 m) with heavy pod yields are desirable for mechanical harvest. Annie Oakley II and White Velvet yielded more pods per plant than all other cultivars, with the exception of Clemson Spineless, but Annie Oakley II was significantly shorter than both of these cultivars, lending itself to easier harvest (Table 6).

Seed and oil yields varied for the 2009 full-season trial. The variety, Hill Country Red, produced a greater seed yield (4050 kg ha^{-1}), than 11 of 18 cultivars, but not greater than Alabama Red (3968 kg ha^{-1}) , Annie Oakley II (3457 kg ha^{-1}) , Clemson Spineless (3368 kg ha^{-1}) , Dwarf Long Green (2995 kg ha^{-1}), Star of David (3284 kg ha^{-1}) , and White Velvet (3322 kg ha^{-1}) (Table 7). Seed yields for several cultivars in study

Table 7 Mean Seed and Oil Yield 18 Okra Varieties Grown Under Full-Season Conditions from Jun-Oct in 2009 at Starkville, MS.

Variety	Mean Seed Yield (kg ha^{-1})	Mean Oil (%)	Mean Oil Yield (L ha^{-1})
Alabama Red	3968 ab †	33 ab	1373 a
Annie Oakley II	3547 abc	37 a	1376 a
Baby Bubba	1204 g	29 abcd	365 ij
Burmese	2628 cdef	31 abc	858 cd
Cajun Delight	2812 cde	34 a	1103 ab
Cajun Jewel	1684 fg	34 a	592 efgh
Clemson Spineless	3368 abc	34 a	1190 a
Cow Horn	2891 cde	24 bcde	728 def
Dwarf Long Green	2995 abcde	22 def	670 def
Evertender	2646 cdef	22 cdef	618 efg
Fife Green Cowbean	2687 cdef	11 g	317 ij
Green Velvet	2106 efg	18 efg	398 ghij
Hill Country Red	4050 a	18 e	765 de
Lee	2091 efg	14 fg	296 j
Mammoth Spineless	2725 cde	14 fg	387 hij
Red Velvet	2303 def	17 efg	396 ghij
Star of David	3284 abcd	20 efg	672 def
White Velvet	3322 abc	15 efg	527 fghi
Coeff. of Var.	24.8	18.4	22.7

† Letters differing among means denote significance. Differing letters among means with the same values are due to rounding. $\alpha = 0.05$

(Alabama Red, Annie Oakley II, Hill Country Red) were similar to those observed by Phippen (2005), who obtained 3800 kg ha^{-1} with Green Best at Macomb, IL.

Okraseed oil content ranged from 11% (Fife Green Cowbean) to 37% (Annie Oakley II) (Table 7). These results are on par with a previously mentioned study which found okraseed to contain 15% to 34% seed oil (Nagel, 1987). Commercial oil yield is a function of seed yield area^{-1} combined with percent seed oil content. Although Hill Country Red produced a seed yield greater than those studies previously cited (Amjad, 2001; Phippen, 2005), it was not among the top four cultivars in oil yield for this study (Table 7). Alabama Red, Annie Oakley II, Cajun Delight, and Clemson Spineless, had greater oil yields area^{-1} than all other cultivars for 2009. Oil yields comparable to these cultivars are competative with soybean (2800 kg ha^{-1} seed yield and 590 L ha^{-1} oil yield), the primary biodiesel feedstock in the U.S. (USDA, 2008; ASA, 2009).

2009 Full-Season Okraseed Fatty Acid Profiles

Okraseed oil fatty acid profiles were analyzed in 2009 for specific compounds (Table 8). The mean palmitic acid (16:0) content of all cultivars was 54.7% (of total fatty acids tested). The varieties Baby Bubba and Lee were found to have greater amounts of palmitic acid at 64.5% and 66.5%, respectively; than Star of David (44.5%). No other differences noted for palmitic acid content among cultivars. Mean palmitoleic acid was 14.5% (of total fatty acids tested; Table 8). Palmitoleic acid (16:1) content was greater for seven cultivars (Baby Bubba, 22.5%; Burmese, 23.0%; Cajun Delight, 18.0%; Cajun Jewel, 20.0%; Green Velvet, 18.0%; Red Velvet, 21.5%; White Velvet 20.%) than for Clemson Spineless, which contained none (Table 8). Mean stearic acid (18:0) percentages ranged from 3.5% to 6.0%, with a mean of 4.9% (of total fatty acids tested) (Table 8). The only differences were observed between the extremes, Cajun Jewel (3.5%) and Lee (6.0%), respectively. Mean oleic acid (18:1) content was relatively low with a mean of 0.5% (of total fatty acids tested) (Table 8). Oleic acid and ranged from 0.0%

Table 8 Mean Target Fatty Acid †Composition of 18 Okra Varieties Grown Under Full Season Conditions from Jun-Oct in 2009 at Starkville, MS.

Variety	16:0 ¶	16:1	18:0	18:1	18:2	18:3
Alabama Red	62.0 ab‡	13.0 ab	5.5 ab	0.0 b	14.5 ab	2.5 bc
Annie Oakley II	50.0 ab	18.5 ab	4.5 ab	2.5 a	21.0 ab	1.5 c
Baby Bubba	64.5 a	22.5 a	5.5 ab	0.0 b	1.0 b	3.5 abc
Burmese	56.5 ab	23.0 a	4.5 ab	0.0 b	10.5 ab	3.5 abc
Cajun Delight	50.5 ab	18.0 a	4.5 ab	1.0 ab	21.0 ab	3.0 bc
Cajun Jewel	49.5 ab	20.0 a	3.5 b	0.5 b	22.5 ab	2.5 bc
Clemson Spineless	58.5 ab	0.0 b	5.0 ab	0.0 b	30.0 a	5.0 ab
Cow Horn	53.5 ab	11.0 ab	4.5 ab	2.5 a	23.0 ab	3.5 abc
Dwarf Long Green	51.0 ab	10.5 ab	5.5 ab	0.0 b	28.0 a	3.0 bc
Evertender	63.0 ab	11.0 ab	5.5 ab	0.0 b	12.0 ab	4.0 abc
Fife Green Cowbean	51.0 ab	12.5 ab	4.5 ab	0.0 b	25.5 ab	3.5 abc
Green Velvet	47.0 ab	18.0 a	4.5 ab	0.0 b	25.5 ab	3.5 abc
Hill Country Red	51.0 ab	6.5 ab	5.0 ab	1.5 ab	30.0 ab	3.5 abc
Lee	66.5 a	6.0 ab	6.0 a	0.0 b	16.5 ab	2.5 bc
Mammoth Spineless	58.0 ab	12.0 ab	5.5 ab	0.0 b	13.5 ab	6.0 a
Red Velvet	56.0 ab	21.5 a	4.5 ab	1.0 ab	11.0 ab	4.0 abc
Star of David	44.5 b	17.5 ab	4.0 ab	0.0 b	28.5 a	2.5 bc
White Velvet	52.0 ab	20.0 a	5.5 ab	0.0 b	16.5 ab	4.0 abc
Cottonseed (*hirsutum*)	60.6	13.8	4.6	0.0	18.6	0.7
Means	54.7	14.5	4.9	0.5	19.5	3.4
Coeff. of Var.	16.9	58.1	20.0	147.7	60.5	37.6

† Percentages are of the target fatty acids tested by gas chromatograph.
‡ Differing letters differing among cultivars denote significant differences. Differing letters among means with the same values are due to rounding. α =0.05. Means and significant differences do not include cottonseed oil values.
¶ C16:0, methyl palmitate; C16:1, methyl palmitoleate; C18:0, methyl stearate; C18:1, methyl oleate; C18:2, methyl linoleate; C18:3, methyl linoleneate.

(Alabama Red, Baby Bubba, Burmese, Clemson Spineless, Dwarf Long Green, Evertender, Fife Green Cowbean, Green Velvet, Lee, Mammoth Spineless, Star of David, White Velvet) to 2.5% (Annie Oakley II and Cow Horn). Mean linoleic acid (18:2) content was 19.5% (of total fatty acids tested) (Table 8). Baby Bubba (1.0%), was the only cultivar to contain less than 10.0% oleic acid. Clemson Spineless (30.0%), Dwarf Long Green (28.0%), and Star of David (28.5%) all contained greater amounts of linoleic acid than Baby Bubba. Linolenic acid (18:3) constituents ranged between 1.5% and 6%, with a mean of 3.4% (of total fatty acids tested). Mammoth Spineless produced a greater percentage of linolenic acid than six other cultivars (Alabama Red, 2.5%; Annie Oakley II, 1.5%; Cajun Delight, 3.0%; Cajun Jewel, 2.5%; Dwarf Long Green, 3.0%; Lee, 2.5%; Star of David, 2.5%).

Oil analysis for 2009 indicated that palmitic acid (16:0) accounted for a mean of 55% of target compounds tested for all cultivars (Table 8). Palmitoleatic (16:1) and linoleic acid (18:2) were found to comprise the rest of the target compounds at 15% and 20%, respectively. Stearic, oleic, and linolenic acid comprised minute amounts of the fatty acid profile, barely registering when tested. Variation for percentage of each fatty acid among the cultivars is affected by area of origin. Oilseed cultivars grown farther south with increased temperatures during seed development, result in greater accumulations of saturated fatty acids (Martinez-Force et al., 1998). Recently, there have been breeding programs in specific crops (soy, canola, sunflower) to specifically overide nature, and alter fatty acid profile (Dolde et al., 1999).

An analysis on cottonseed oil was also performed for the 2009 full-season variety trial. Cottonseed (*hirsutum* L.) collected in 2009 from the R.R. Foil Plant Science Research Center (North Farm) at Starkville, MS was used. Data indicated cottonseed oil to have a mean fatty acid profile of: palmitic (16:1) 60.6%, palmitoleic (16:1) 13.8%, stearic (18:0) 4.6%, oleic (18:1) 0.0%, linoleic (18:2) 18.6%, and linolenic (18:3) 0.7%. This data closely mimics our results for okraseed oil fatty acid means for the same year (Table 8). This deviates from the previous profile given for cottonseed oil: palmitic (16:1) 23.0%, palmitoleic (16:1) 0.0%, stearic (18:0) 2.0%, oleic (18:1) 17.0%, linoleic (18:2) 56.0%, and linolenic (18:3) 0.0% (Table1).

Results in 2009 were for target compounds tested and were not representative of the whole, therefore, true percentages would be slightly lower. Regardless of this fact, the results of this study were contradictory to preponderance of literature stating that okraseed oil is comprised primarily of unsaturated fatty acids (Clopton et al., 1948; Crossly and Hilditch, 1951; Martin, 1982). Our observations indicated that palmitic acid (16:0), a saturated fat was the primary constituent of fatty acids tested (Table 8). The two saturated fats (palmitic 16:0 and stearic 18:0) accounted for 60% of the fatty acids tested. Knowing that the oils tested in this study are comprised of approximately half saturated fatty acids and half unsaturated, properties for biodiesel should be inherently functional based on fatty acid structural features effect on fuel properties (Moser, 2009). A study using transesterified cottonseed oil found only a slight reduction in thermal efficiency for engine properties, while improving carbon monoxide, particulate matter, and smoke emissions (Nabi et al., 2009). Based on some similarities between cottonseed oil and okraseed oil, like results may be expected.

2010 Full-Season Variety Trial

Planting of the 2010 full-season variety trial occurred 20 days earlier (May 11) than the previous year due to drier conditions**.** Limited seed availability resulted in the deletion of six cultivars (Baby Bubba, Cajun Jewel,Fife Green Cowbean, Green Velvet, Mammoth Spineless, and White Velvet), and two additional cultivars were included in this trial (Green Best and Jambalaya). As mentioned, statistical analysis indicated an effect due to year among cultivars common to both years. Weather conditions between 2009 and 2010 differed greatly. While 2009 had well distributed rains, 2010 conditions remained droughty throughout the season. Total rainfall for May-October was only 44 cm (Figure 4). This had a substantial impact on morphological characteristics and yield components.

Days to flower were reduced for all okra cultivars common to both years in 2010 (Table 9) versus 2009 (Table 6). In 2010, flowering occurred within 12 days of one another for all cultivars, ranging from 40 (Green Best) to 52 (Hill Country Red) days. Alabama Red (51), Hill Country Red (52), and Red Velvet (51) took longer to flower than all other varieties, with the exception of Cow Horn (50) (Table 9). Many okra cultivars have a short photoperiod requirement for maximum flowering to occur (Tenga and Ormrod, 1985). Earlier planting coupled with reduction in rainfall one month after planting, is likely responsible for expedited conversion to reproductive growth in 2010. Reduced time to flower also subjected early matured pods to prolonged periods of weathering in the field, contributing to deleterious effects related to seed retention , oil

Table 9 Morphological Characteristics of 13 Okra Varieties Grown Under Full Season Conditions from May-Sep in 2010 at Starkville, MS.

Variety	Mean Days to Flower	Mean Lodging (%)	Mean Shatter (%)	Mean Height (m)	Mean Pods Plant $^{-1}$
Alabama Red	51 a †	9 de	20 abcd	1.3 abc	9 bcd
Annie Oakley II	41 d	17 bcde	8 d	1.1 efg	12 abc
Burmese	43 cd	14 bcde	26 abc	1.1 defg	10 bcd
Cajun Delight	41 d	29 ab	35 a	1.1 efg	13 ab
Cow Horn	50 ab	12 cde	36 a	1.5 a	14 a
Dwarf Long Green	42 cd	17 bcde	21 abcd	1.2 cdef	10 bcd
Evertender	43 cd	35 a	33 ab	1.3 bcd	12 abc
Green Best	40 d	16 bcde	33 ab	1.1 efg	10 bcd
Hill Country Red	52 a	11 cde	26 abc	1.2 cde	9 bcd
Jambalaya	45 c	5 e	16 bcd	1.0 fg	9 bcd
Lee	46 bc	26 abc	16 bcd	1.0 g	9 cd
Red Velvet	51 a	20 bcd	26 abcd	1.2 cdef	10 abcd
Star of David	44 cd	7 de	14 cd	1.4 ab	7 d
Coeff. of Var.	6.9	62.7	54.8	12.1	24.3

† Letters differing among means denote significance. Differing letters among means with the same values are due to rounding. $\alpha = 0.05$

content, and oil composition (to be explained later in greater detail) (Hepperly and Sinclair, 1978).

Lodging and shatter percentages significantly increased in the 2010 full-season trial compared to the full-season study for 2009. Lodging ranged from zero to four percent in the 2009 full-season study (Table 6). In 2010, lodging ranged from five percent (Jambalaya) to 35% (Evertender) (Table 9). Increases are likely related to drought stress and weathering of senesced okra stalks. Okra plants lodged due to stalk breakage and not root lodging. Lodging was significantly greater for Evertender (35%), than for other varieties, except Cajun Delight (29%) and Lee (26%). Shatter percentage for the 2009 full-season study ranged from zero to three (Table 6). Seed shatter in 2010 ranged from 7% (Annie Oakley II) to 36% (Cow Horn) (Table9). Shatter was observed to be greater for Cajun Delight 35% and Cow Horn 36%, than for; Annie Oakley II 8%, Jambalaya 16%, Lee 13%, and Star of David 14%, but not greater than the other cultivars in this study. Okra pods underwent dessication and shattered prematurely in this full-season assessment as the growing season progressed. Drought stress can contribute to expedited lignification of cell walls. Low moisture, again due to drought, would also result in premature dehiscence (Roberts et al., 2002). Seed losses for the greater ranges observed in 2010 related to lodging and shatter would be unacceptable for most traditional crops.

Plant heights for the 2010 okra full-season variety trial were reduced numerically for all cultivars common to the full-season study for 2009, except Cow Horn (Table 9, Table 6). Plant heights for the 2009 full-season trial ranged from 1.2 to 2.2 m for cultivars common to both trials (Table 6). Plant heights were between 1.0 m (Jambalaya and Lee) and 1.5 m (Cow Horn) for the 2010 full-season study (Table 9). Cow Horn was taller than all cultivars, with the exception of Alabama Red (1.3 m) and Star of David (1.4 m). It is believed that drought stress contributed to slowed vegetative growth and triggered early reproductive growth in 2010, resulting in reduced plant heights. Lack of water is known to decrease inter-node elongation, making plants shorter. Shorter plants can be advantageous with respect to ease of harvest, allowing for all pod material to be collected by harvesting equipment.

Mean pods per plant were reduced for all cultivars in this study from 3 (Red Velvet) to 13 (Annie Oakley II) (Table 9) common to the 2009 full-season study (Table 6), with the exception of Cow Horn (0). Within the 2010 full-season test, no significant differences in pods per plant were observed between Annie Oakley II (12), Cajun Delight (13), Cow Horn (14), Evertender (12), and Red Velvet (10) (Table 9). Cow Horn produced more pods per plant than Alabama Red (9), Burmese (10), Dwarf Long Green (10), Green Best (10), Hill Country Red (9), Jambalaya (9), Lee (9), and Star of David (7). Reduction in rainfall and increase in temperature for the 2010 growing season is believed to have played a significant role in the reduction of pod production (Figure 4, Figure 5). Drought stress, especially at the time of flowering can have significant impacts on pod yield and result in as much as a 70% reduction due to abscission (Mbagwu and Adespie, 1987). Temperatures of 36ºC can halt flower development, and temperatures greater than 42ºC can initiate flower abortion (Tenga and Ormrod, 1985). Mean number of stalks per row were greater in 2010 than 2009 because plants were not thinned after planting. This also likely contributed to less pods per plant because of the plastic growth response observed as part of the row spacing and population density study (Table 5). Other studies have reported that pods per plant were greater in reduced populations (Amjad et al. 2001; Shreshtha, 1983).

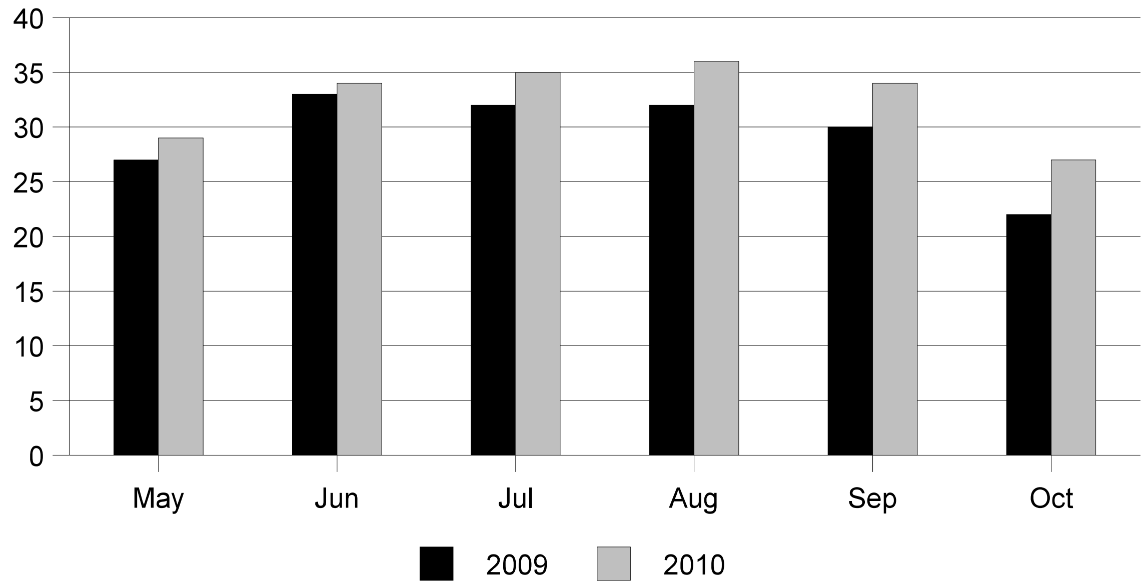

Figure 5 Mean monthly daytime high temperature (degrees Celsius) for the 2009 and 2010 growing season (May-Oct) recorded at the R.R Foil Plant Science Research Center.

Seed yields in the 2010 full-season test (Table 10) remained comparable for many, but not all cultivars when compared to the 2009 full-season test (Table 7). Seed yields decreased substantially for Alabama Red and Hill Country Red (1194 and 1644 kg ha^{-1} reduction, respectively), while Cow Horn and Dwarf Long Green increased (667 and 768 kg ha^{-1} increase, respectively) when comparing the 2009 and 2010 full season trials. Seed yields for the 2010 full-season study ranged between 1819 kg ha^{-1} (Lee) and 3799 kg ha^{-1} (Annie Oakley II) (Table 7). Significant differences were not observed in seed yields for: Annie Oakley II, Cow Horn (3558 kg ha^{-1}), Dwarf Long Green (3763 kg ha^{-1}), Green Best (3039 kg ha^{-1}) , and Star of David (3182 kg ha^{-1}) (Table 10). Annie Oakley II produced a greater seed yield than eight cultivars in this study (Burmese 2482 kg ha^{-1}, Cajun Delight 2868 kg ha^{-1}, Evertender 2037 kg ha^{-1}, Hill Country Red 2406 kg ha^{-1},

Table 10 Mean Seed and Oil Yield 13 Okra Varieties Grown Under Full-Season Conditions from May-Sep in 2010 at Starkville, MS.

Variety	Mean Seed Yield kg ha^{-1}	Mean Oil (%)	Mean Oil Yield L ha^{-1}
Alabama Red	2774 cde †	15 a	433 ab
Annie Oakley II	3799 a	12 abc	474 a
Burmese	2482 def	15 a	388 abc
Cajun Delight	2868 cdef	14 ab	418 ab
Cow Horn	3558 abc	10 c	370 abc
Dwarf Long Green	3763 ab	13 abc	490 a
Evertender	2037 ef	13 abc	276 cde
Green Best	3039 abcd	10 c	316 bcde
Hill Country Red	2406 def	13 abc	326 bcd
Jambalaya	2796 cde	13 abc	378 abc
Lee	1819 f	11 bc	199 e
Red Velvet	1822 f	11 bc	209 de
Star of David	3182 abcd	12 abc	397 abc
Coeff. of Var.	22.9	13.2	24.1

† Letters differing among means denote significance. Differing letters among means with the same values are due to rounding. $\alpha = 0.05$

Jambalaya 2796 kg ha^{-1}, Lee 1819 kg ha^{-1}, and Red Velvet 1822 kg ha^{-1}). Green Best, one of the two cultivars added in 2010, proved to have a relatively high seed yield (3,039 kg ha^{-1}) in comparison to all other cultivars. Green Best produced a seed yield statistically the same as Annie Okley II , which produced the greatest seed yield. Seed yield produced by Green Best for the

2010 full-season study was not as substantial as Phippen's (2005) (3,800 kg ha^{-1}) for the same cultivar.

In the 2010 full-season study, mean percent seed oil content ranged from 10% (Cow Horn and Green Best) to 15% (Alabama Red) (Table 10). This is substantially lower than the ranges for the cultivars common to the 2009 full-season study, (14%) Lee to (37 %) Annie Oakley II (Table 7). Alabama Red and Burmese (15%, respectively) had significantly greater seed oil content than Cow Horn (10%), Green Best (10%), Lee (11%), and Red Velvet (11%) (Table 10).

Seed oil content and oil yields were substantially reduced in the 2010 full-season test (Table 10) for previously top ranking common cultivars (Alabama Red 1373 L ha^{-1} and Annie Oakley II 1376 L ha^{-1}) in the 2009 full-season test (Table 7). Annie Oakley II, one of the top ranking cultivars with respect to oil yield in the 2009 full-season test, had a reduction in seed oil content and oil yield of 25% and 902 L ha^{-1}, respectively for the 2010 full-season test (Table 10).

Mean oil yields ranged from 199 L (Lee) to 490 L ha^{-1} (Dwarf Long Green) for the 2010 full-season study (Table 10). Annie Oakley II (474 L ha^{-1}) and Dwarf Long Green produced more oil per $area^{-1}$ than five cultivars (Evertender 276 L ha^{-1}, Green Best 316 L ha^{-1}, Hill Country Red 326 L ha^{-1}, Lee 199 L ha^{-1}, Red Velvet 209 L ha^{-1}), but no differences were observed for the remaining six cultivars (Alabama Red 433 L ha^{-1}, Burmese 388 L ha^{-1}, Cajun Delight 418 L ha^{-1}, Cow Horn 370 L ha^{-1}, Star of David 397 L ha^{-1}).

Yield reductions observed for the 2010 full-season study could seriously impact okra as an alternative feedstock for biodiesel. Drought stress effects during seed fill have been well studied in soybean (Dornbos and Mullen, 1992). Soybean plants were subjected to different ranges of drought stress and temperatures. Soybeans grown under greater drought stress and higher air temperatures resulted in reduced seed oil percentages, but greater protein concentrations, while the inverse proved to be true for non-stressed plants and lower air temperatures. Premature pod shatter allowed for prolonged periods of seed dessication due to weathering. The characteristics described by Dornbos and Mullen (1992) are the same as those occurred in the 2010 full-season variety trial, resulting in same outcomes in okraseed oil percentages, attributed to weathering.

2010 Full-Season Okraseed Fatty Acid Profiles

Temperature is known to play a critical role in fatty acid accumulation (Dornbos and Mullen, 1992). Mean temperatures were greater for the 2010 growing season compared to the 2009 growing season (Figure 5). Unsaturated fatty acids in rapeseed (18:1 and 18:2) were reported to increase in proportion to saturated fatty acids (16:0) as temperature increased (Deng and Scarth, 1998). We observed this to be true for the mean fatty acid percentages in our 2010 full-season study (Table 11). Mean palmitic (16:0, 37.9%) and palmitoleic (16:1, 4.4%) acids were lower, while oleic (18:1, 25.3%) and linoleic (18:2, 27.1%) acids increased (Table 11) when compared to the 2009 full-season trial (palmitic 54.7%, palmitoleic 14.5%, oleic 0.5%, linoleic 19.5%) (Table 8). Stearic acid (18:0) and linolenic acid decreased by 1.9% (Table 11),

Table 11 Mean Target Fatty Acid [†]Composition of 13 Okra Varieties Grown Under Full Season Conditions from May-Sep in 2010 at Starkville, MS.

Variety	16:0 [¶]	16:1	18:0	18:1	18:2	18:3
Alabama Red	40.0 ab[‡]	5.5 ab	3.0 a	25.0 ab	25.5 ab	0.5 b
Annie Oakley II	40.5 ab	0.0 b	3.0 a	26.5 ab	29.0 ab	1.5 ab
Burmese	39.5 ab	1.5 ab	3.5 a	24.5 ab	28.5 ab	2.0 ab
Cajun Delight	36.0 ab	0.0 b	3.0 a	26.0 ab	32.0 ab	2.0 ab
Cow Horn	46.0 a	7.5 ab	3.5 a	29.0 a	12.0 c	1.5 ab
Dwarf Long Green	41.5 ab	0.0 b	3.5 a	25.5 ab	25.5 ab	1.0 ab
Evertender	37.0 ab	9.5 ab	2.5 a	22.0 b	26.5 ab	1.5 ab
Green Best	38.5 ab	0.0 b	3.0 a	24.5 ab	32.5 ab	0.5 b
Hill Country Red	32.0 b	14.0 a	2.5 a	23.0 ab	25.5 ab	2.0 ab
Jambalaya	35.0 b	7.5 ab	3.0 a	25.0 ab	27.0 ab	1.5 ab
Lee	32.0 b	0.0 b	3.5 a	25.5 ab	36.0 a	3.0 a
Red Velvet	35.0 b	6.0 ab	2.5 a	27.0 ab	27.5 b	1.5 ab
Star of David	39.5 ab	6.0 ab	2.5 a	25.5 ab	24.5 b	1.5 ab
Means	37.9	4.4	3.0	25.3	27.1	1.5
Coeff. of Var.	23.8	139.9	27.8	24.5	31.3	64.2

[†] Percentages are of the target fatty acids tested by gas chromatograph.
[‡] Differing letters differing among cultivars denote significant differences. Differing letters among means with the same values are due to rounding. α =0.05
[¶] C16:0, methyl palmitate; C16:1, methyl palmitoleate; C18:0, methyl stearate; C18:1, methyl oleate; C18:2, methyl linoleate; C18:3, methyl linoleneate.

respectively, when compared to the 2009 full-season test (stearic 3.0% and linolenic1.5%) (Table 8).

Within the 2010 full-season test, palmitic acid (16:0) ranged from 32% (Lee) to 46% (Cow Horn)(Table 8). Cow Horn samples had significantly more palmitic acid present than Hill Country Red (32.0%), Jambalaya (35.0%), Lee (32.0%), and Red Velvet (35.0%). Palmitoleic acid (16:1) was lower for most, but not all cultivars common to those in the 2009 full-season study. The only significant differences in palmitoleic acid content were between Hill Country Red (14%) and those cultivars that contained no measurable palmitoleic acid (Annie Oakley II, Cajun Delight, Green Best, Lee) (Table 11). Mean stearic acid (18:0) content was uniform for all cultivars with a mean of three percent (no significant differences present) (Table 11). Mean percentages of oleic acid (18:1) ranged from 22% to 29%, with the only significance observed between Evertender and Cow Horn, respectively. Mean percentages of linoleic (18:2) acid ranged from 12 % (Cow Horn) to 36 % (Lee) in the 2010 full-season study. Lee (36%) produced greater amounts of linoleic acid than Cow Horn (12%), Red Velvet (27.5%), and Star of David (24.5%), but not more than all other cultivars. Differences were found for linolenic acid (18:3) content between Lee (3%) and two other cultivars, Alabama Red and Green Best, 0.5%, respectively.

Mean percentage palmitic acid (16:0) again indicated that it was the greatest single fatty acid, accounting for 38% of those tested (Table 11). This percentage was 17% less than that of the 2009 full-season trial (Table 8). Palmitoleic acid (16:1) for the 2010 full-season test comprised very little of the fatty acid composition (5%), a decrease of 10.1% from the previous full-season test. Mean stearic acid (18:0) content was relatively unchanged for the 2010 full-season (3%) test compared to the 2009 full-season test (4.9%) Unsaturated fatty acids comprised more of the total in 2010 than did saturated fatty acids. Oleic acid (18:1) increased by 24.8 % compared to the 2009 full-season study and was responsible for 25% of the total tested. Linoleic acid (18:2) represented 27% of the total for all cultivars, a 7% increase from the previous year's full-season test. Linolenic acid (18:3) was observed at a mean of 1.5 % for the 2010 full-season trial, a decrease of 1.9 % when compared to the 2009 full-season trial. These results are more comparable to the literature findings previously stated for okraseed fatty acid profile (Clopton et al., 1948; Crossly and Hilditch, 1951; Martin, 1982). Once again, a fatty acid profile such as this would possess desirable properties for biodiesel according to the literature of Moser, (2009).

2010 Half-Season Variety Trial

This half-season study was conducted to determine if okra could produce substantial seed and oil yields when planted after winter oilseeds or other winter crops that are harvested in early summer months. This study was planted Jul 2010, which saw only eight cm of rainfall for the month (Figure 4). Cumulative total rainfall of 21 cm was seen for Jul through Oct (Figure 4). Morphological characteristics for some cultivars were impacted by the drought suffered during the 2010 growing season, while others were not.

Flowering for all cultivars occurred between 41 (Green Best) and 57 (Cow Horn and Jambalaya) days for this half-season study (Table 12), and were similar to flowering ranges for the 2010 full-season study, [41 (Annie Oakley II and Cajun Delight) to 52 days (Hill Country Red)] (Table 9). Although flowering ranges were similar for these two studies, all cultivars took a greater number of days to flower in the 2010 half-season trial, but not all differences were substantial. Varieties; Cajun Delight (43 d) and Green Best (41 d) flowered before all others in this half-season test (Table 12). Cow Horn and Jambalaya (both 57 d) took longer to flower than the

Table 12 Morphological Characteristics of 13 Okra Varieties Grown Under Half Season Conditions from Jul-Sep in 2010 at Starkville, MS.

Variety	Mean Days to Flower	Mean Lodging (%)	Mean Shatter (%)	Mean Height (m)	Mean Pods Plant $^{-1}$
Alabama Red	54 abc †	1 bc	25 abc	1.2 de	7 ab
Annie Oakley II	51 cd	1 bc	20 bcde	1.0 f	7 ab
Burmese	52 bcd	0 c	31 ab	1.0 f	7 ab
Cajun Delight	43 e	3 bc	9 de	1.0 f	8 a
Cow Horn	57 a	5 abc	17 bcde	1.6 a	5 ab
Dwarf Long Green	54 abc	1 bc	25 abcd	1.0 ef	8 a
Evertender	50 cd	8 a	35 a	1.4 ab	8 a
Green Best	41 e	2 bc	16 bcde	1.0 ef	6 ab
Hill Country Red	54 abc	1 bc	7 e	1.3 cd	6 ab
Jambalaya	57 a	2 bc	15 cde	0.9 f	7 ab
Lee	54 abc	2 bc	21 abcde	0.9 f	7 ab
Red Velvet	55 ab	6 ab	20 abcde	1.0 ef	7ab
Star of David	49 d	6 ab	23 abcd	1.3 bc	4 b
Coeff. of Var.	5.2	127.7	53.8	10.0	24.3

† Letters differing among means denote significance. Differing letters among means with the same values are due to rounding. $\alpha = 0.05$

previously two mentioned cultivars, as well as: Annie Oakley II (51 d), Burmese (52 d), and Evertender (50 d).

Percentage of lodged stalks (Table 12) were reduced for this study for all cultivars, compared to the full-season trial for the same year (Table 9). Reductions were substantial for most, but not all cultivars. Lodging ranges for the 2010 full-season study were between 5% (Jambalaya) and 35% (Evertender) (Table 9). Mean lodging percentages ranged from zero (Burmese) to eight percent (Evertender) for the half-season study (Table 12). Lodging of Evertender (8%) occurred more frequently than all other varieties in the half-season study, with the exception of; Cow Horn (5%), Red Velvet (6%), and Star of David (6%). Loding due to stalk breakage was likely reduced for this study since stalks received limited exposure to weathering in the field, a result of less overall time in the field.

Differences were observed for seed shatter when comparing the full (Table 9) and half-season (Table 12) studies for 2010. Seed shatter percentage increased in number for seven cultivars (Alabama Red +5%, Annie Oakley II +12%, Burmese +5%, Dwarf Long Green +4%, Evertender +2%, Lee +5%, Star of David +9%) and decreased for six (Cajun Delight -26%, Cow Horn -19%, Green Best -17%, Hill Country Red -19%, Jambalaya -1%, Red Velvet -6%), although, not all changes were substantial. Evertender (35%) shattered at a rate greater than six other cultivars (Annie Oakley II, Cajun Delight, Cow Horn, Green Best, Hill Country Red, Jambalaya) for the 2010 half-season test (Table 12). Similar to results of the full-season study for this year, seed loss was believed to be due to lack of rainfall received in the months after planting this study (Figure 2), in spite of the later planting date.

Okra plant heights for the half-season study were comparable to the 2010 full-season study. Plant heights ranged from 1.0 m (Jambalaya and Lee) to 1.5 m (Cow Horn) for the 2010 full season test (Table 9), and from 0.9 m (Jambalaya and Lee) to1.6 m (Cow Horn), for the half-season test in the same year (Table 12). Within the half-season test, Cow Horn (1.6 m) produced significantly taller plant heights than all others for the half-season test, except Evertender (1.4 m).

Although little change occurred for heights, mean pods per plant were less in number for all cultivars in the 2010 half-season study (Table 12), when compared to the full-season study (Table 9) for the same year. Significant differences among cultivars with respect to pods per plant for the half season test were present between Cajun Delight, Dwarf Long Green, Evertender (8), respectively and Star of David (4). Later planting, during a reduction in photoperiod and less total number of days to mature are likely the reason for reduced number of pods per plant.

The most substantial impacts for the 2010 half-season trial compared to the full-season trial for the same year, were seen in seed and oil yields. Seed yields for the 2010 full-season test ranged from 1819 kg ha^{-1} (Lee) to 3799 kg ha^{-1} (Annie Oakley II) (Table 10). Seed yields substantially decreased for all cultivars in the half-season study and ranged from 788 kg ha^{-1} (Evertender) to1661 kg ha^{-1} (Annie Oakley II) (Table 13). Reductions in seed yield of 2000 kg ha^{-1} or greater were observed in four cultivars (Annie Oakley II, Cow Horn, Dwarf Long Green, and Star of David). Annie Oakley II produced a greater seed yield than seven other cultivars (Burmese 1021 kg ha^{-1}, Dwarf Long Green 1055 kg ha^{-1}, Evertender 788 kg ha^{-1}, Jambalaya 1166 kg ha^{-1}, Lee 852 kg ha^{-1}, Red Velvet 867 kg ha^{-1}, and Star of David 1181 kg ha^{-1}) for the half-season test (Table 13). Evertender produced less seed than Alabama Red (1306 kg ha^{-1}), Annie Oakley II (1661 kg ha^{-1}), Cajun Delight (1341 kg ha^{-1}), Cow Horn (1404 kg ha^{-1}), Green Best (1329 kg

Table 13 Mean Seed and Oil Yield 13 Okra Varieties Grown Under Half Season Conditions from Jul-Sep in 2010 at Starkville, MS.

Variety	Seed Yield kg ha^{-1}	Oil (%)	Oil Yield L ha^{-1}
Alabama Red	1306 abcd †	18 a	238 ab
Annie Oakley II	1661 a	15 ab	259 a
Burmese	1021 bcde	18 a	191 abcde
Cajun Delight	1341 ab	14 abc	188 abcde
Cow Horn	1404 ab	10 c	146 def
Dwarf Long Green	1055 bcde	15 abc	159 cdef
Evertender	788 e	17 ab	135 ef
Green Best	1329 abc	17 ab	228 abc
Hill Country Red	1344 ab	14 abc	196 abcde
Jambalaya	1166 bcde	15 abc	176 bcde
Lee	852 de	12 bc	106 f
Red Velvet	867 cde	14 abc	122 ef
Star of David	1181 bcde	18 a	215 abcd
Coeff. of Var.	27.9	14.2	28.9

† Letters differing among means denote significance. Differing letters among means with the same values are due to rounding. $\alpha = 0.05$

ha^{-1}), and Hill Country Red (1344 kg ha^{-1}) for the 2010 half-season study. No cultivar registered seed yields of 2800 kg ha^{-1} for this study, which are current U.S. soybean seed yield averages (USDA, 2008). Soybean is the primary feedstock for U.S. biodiesel production. Limited time to mature and drought stress are likely responsible for decreases in seed yield.

Percent seed oil content increased numerically for all cultivars in the half-season trial (Table 13), with the exception of Cajun Delight and Cow Horn (no change) when compared to the 2010 full-season trial (Table 10), and therefore, caused a statistical interaction. Alabama Red, Burmese, and Star of David (all 18%) had significantly more seed oil than Cow Horn (10%) and Lee (12%), but did not differ significantly from other cultivars for the half-season study. Increases in seed oil content for the 2010 half-season study had no effect in offsetting the reduction in seed yields. Subsequently, half-season oil yields area^{-1} were substantially reduced from the full-season test. Half-season mean oil yields area^{-1} were between 106 (Lee) and 259 L ha^{-1} (Annie Oakley II) (Table 13). Previous ranges for the 2010 full-season test were 199 to 490 L ha^{-1} (Table 10). In the half-season test, Annie Oakely II (259 L ha^{-1}) produced significantly more oil than six cultivars (Cow Horn 146 L ha^{-1}, Dwarf Long Green 159 L ha^{-1}, Evertender 135 L ha^{-1}, Jambalaya 176 L ha^{-1}, Lee 106 L ha^{-1}, Red Velvet 122 L ha^{-1}) (Table 13). Annie Oakley II and Dwarf Long Green, the two higher ranking oil producers for the 2010 full-season test, suffered reductions in oil yield area^{-1} of 215 L ha^{-1} and 331 L ha^{-1}, respectively. Reductions in seed, seed oil percentage, and oil yields rendered half-season okra production ineffective for 2010.

<u>2010 Half-Season Okraseed Fatty Acid Profiles</u>

Trends for the half-season oil analysis (Table 14) were similar to those of the full-season analysis for the same year (Table 11). Palmitic acid (16:0) prevailed as the dominant triglyceride with a mean of 40.0% for all cultivars tested, a small increase of

Table 14 Mean Target Fatty Acid †Composition of 13 Okra Varieties Grown Under Half Season Conditions from Jul-Sep in 2010 at Starkville, MS

Variety	16:0 ¶	16:1	18:0	18:1	18:2	18:3
Alabama Red	50.0 a ‡	19.5 a	3.0 ab	25.0 a	1.0 b	1.5 a
Annie Oakley II	39.0 a	0.0 b	3.0 ab	25.5 a	29.5 a	2.0 a
Burmese	46.5 a	0.5 b	4.0 a	22.0 a	23.0 a	2.0 a
Cajun Delight	40.0 a	7.5 ab	3.5 ab	23.5 a	23.5 a	2.5 a
Cow Horn	38.5 a	9.0 ab	2.5 b	22.0 a	23.5 a	3.0 a
Dwarf Long Green	37.5 a	10.5 ab	3.5 ab	22.5 a	25.0 a	1.0 a
Evertender	44.5 a	0.0 b	3.5 ab	22.5 a	27.5 a	1.5 a
Green Best	38.5 a	6.5 ab	3.5 ab	22.0 a	27.0 a	2.0 a
Hill Country Red	39.0 a	11.0 ab	3.0 ab	23.0 a	22.5 a	1.5 a
Jambalaya	36.5 a	7.5 ab	3.0 ab	24.0 a	27.0 a	1.0 a
Lee	42.5 a	7.5 ab	3.5 ab	23.5 a	20.5 ab	2.0 a
Red Velvet	40.0 a	5.0 ab	3.0 ab	24.5 a	25.5 a	2.0 a
Star of David	36.5 a	7.5 ab	3.5 ab	23.5 a	26.5 a	1.5 a
Means	40.7	7.1	3.3	23.3	23.2	1.8
Coeff. of Var.	15.6	110.7	17.0	11.1	42.0	73.0

†Percentages are of the target fatty acids tested by gas chromatograph.
‡ Differing letters differing among cultivars denote significant differences. Differing letters among means with the same values are due to rounding. α =0.05
¶ C16:0, methyl palmitate; C16:1, methyl palmitoleate; C18:0, methyl stearate; C18:1, methyl oleate; C18:2, methyl linoleate; C18:3, methyl linoleneate.

(2.8%) compared to the 2010 full-season test oil analysis. Mean palmitoleic acid (16:1) content was 7.1% for all cultivars tested, a 2.8% increase from the 2010 full-season analysis. Mean stearic acid (18:0) content remained consistent with no appreciable increase (0.3%) comparable to the half-season study (Table 14). Unsaturated fatty acids oleic (18:1) and linoleic (18:2) accounted for 23.3% and 23.2% of the fatty acids tested, respectively (Table 14). These percentages are slightly lower in value than for the full-season trial of the same year (-2.0% and -3.9%, respectively) (Table 11). Similar to the 2010 full-season study, linolenic acid (18:3) was low (1.8%) for the half-season test.

Within the half-season test, oil analyses for 2010 indicated no significant differences among cultivars for palmitic acid (16:0) content (Table 14). Percentages ranged from 50% (Alabama Red) to 36.5% (Jambalaya and Star of David). For palmitioleic acid (16:1), Alabama Red contained significantly more (19.5%) than three other cultivars (Annie Oakley II 0.0%, Burmese 0.5%, Evertender 0.0%). Stearic acid (18:0) constituents for the 2010 full-season test ranged between 2.5% and 3.5% (Table 11) Values for the 2010 half-season test were very similar and ranged from 2.5% (Cow Horn) and 4.0% (Burmese) (Table 14). The only significant differences observed for stearic acid content for the half-season analysis were between the afore mentioned Cow Horn and Burmese. Analysis of variance indicated no differences among cultivars for oleic acid (18:1) content (Table 14). Mean ranges for oleic acid were from 22.0% (Cow Horn and Green Best) to 25.5% (Annie Oakley II). All cultivars contained 20.0%, or greater, composition of linoleic acid (18:2) for the half-season test, with the exception of Alabama Red (1.0%) (Table 14). All varieties contained significantly greater amounts of linoleic acid than Alabama Red (1.0%), with the exception of Lee (20.5%) (Table 14). Significant differences were not observed among cultivars for linolenic acid (18:3) composition. Fatty acid profiles for the 2010 half-season test were similar in nature to those of the 2010 full-season test. Being grown in the same year and location, removes most differences due to year.

CHAPTER V

CONCLUSIONS

Seed yield data for row spacing and population density appeared to be plastic. Row spacing by population density results indicated no optimal plant population for maximum seed yield within the parameters of this study. Row spacing configuration proved to be significant for the plant population of 71,700 plants ha^{-1}. The configuration of 45.72 x 30.48 cm had a significantly greater seed yield than 91.44 x 15.24 cm, although they were the same plant population. Significantly greater yields obtained through varying plant populations indicated that it would be economically better to plant at lower populations. Although yields for this particular study were not comparable to soybean yield averages, Clemson Spineless' yield remained consistent over two growing. Consistency in yield could prove useful for breeding efforts in future studies.

Variety trials in 2009 indicated okra might be a candidate for biodiesel feedstocks based on seed yield and oil content. Seed and oil yields $area^{-1}$ for some cultivars (Alabama Red, Annie Oakley II, Cow Horn, Dwarf Long Green, Hill Country Red, Star of David, and White Velvet) exceeded that of U.S. soybean averages. These were small plot tests, and since quality candidates were identified, larger field trials are recommended. It is also proposed that additional okra lines from the USDA GRIN be screened for desirable traits. Although seed oil content and oil yields were significantly impacted in 2010 by drought, Annie Oakley II showed great consistency over the course of three variety trials as a leading seed and oil producer. These results were obtained without any prior breeding efforts for improvement. Given the significance of cumulative rainfall totals between years, and subsequent yield impacts, it may be necessary to add supplemental irrigation to achieve maximum yields. Feasability of okra as a half-season crop could be possible with adequate rainfall.

Okraseed oil analysis indicated high concentration of saturated fatty acids in the form of palmitic (16:0) and stearic (18:0) (X = 42.8%) for the three variety trials. Unsaturated fatty acid concentration did increase substantially in 2010 (X = 24.0%) compared to 2009 tests (X =0.5%). Linoleic (18:3) fatty acid remained consistent over years (X = 23.3%, range 19.5-27.1%). Linolenic fatty acid ranged from 1.5% (2010) to 3.4% (2009). Differences in fatty acid profiles between years indicates that environmental conditions can have a strong effect on seed oil and fatty acid composition. Such effects would require blending of okraseed oil with other oils to achieve desired fatty acid profiles for biofuel.

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