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Lemongrass Productivity, Oil Content, and Composition as a Function of Nitrogen, Sulfur, and Harvest Time

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Biofuels

ABSTRACT

Lemongrass [*Cymbopogon flexuosus* (Steud.) Wats, (syn. *Andropogon nardus* var. *flexuosus* Hack; *A. flexuosus* Nees)] is one of the most widely grown essential oil plants in the world. Field experiments were conducted at Verona and Poplarville, MS, to evaluate the effects of N (0, 40, 80, and 160 kg N/ha) and S (0, 30, 60, and 90 kg S/ha) on lemongrass biomass productivity, essential oil content, yield, and oil composition. Overall, the essential oil content varied within 0.35 to 0.6% of the dried biomass. The major constituents were geranial (25–53%), neral (20–45%), caryophyllene oxide (1.3–7.2%), and *t*-caryophyllene (0.3–2.2%). Biomass yields at Verona ranged from 9486 to 19,375 kg/ha, while oil yields ranged from 30 to 139 kg/ha. Overall, dry weight yields increased with the application of 80 kg N/ha relative to the 0 kg N/ha and with 160 kg of N/ha relative to the 0 and 40 kg N/ha treatments. At Poplarville, biomass yields varied from 8036 to 12,593 kg/ha, while oil yields ranged from 23.5 to 89.5 kg/ha. The application of N at 160 kg/ha at Poplarville increased dry weight yields relative to the N at 0 or 40 kg/ha rates, irrespective of the rate used for S. At Verona, within each S application rate, biomass yields were highest in Harvest 2, lower in Harvest 1, and the lowest in Harvest 3 (regrowth). The combined biomass yields of Harvest 1 and Harvest 3 would be lower, but oil yields would be higher compared to Harvest 2 (single-harvest system). Lemongrass can be grown as an annual essential oil crop in the southeastern United States, with a potential for dual utilization: essential oil and lignocellulosic material for ethanol production.

Lemongrass is one of the most widely grown essential oil plants in the tropics and subtropics of India, Indonesia, Madagascar, and countries in Africa and South America (Anaruma et al., 2010; Ganjewala and Luthra, 2010; Weiss, 1997). Lemongrass is grown as a perennial crop under either irrigated or nonirrigated conditions, but a large area is harvested from wild natural habitats such as in mixed forests or along banks of canals and rivers (Singh and Sharma, 2001; Weiss, 1997). Lemongrass biomass is steam distilled for the extraction of essential oil, a natural product with wide application in the food and pharmaceutical industries, perfumery and cosmetics, and eco-friendly pesticides (Ganjewala and Luthra, 2010; Weiss, 1997). Lemongrass oil has a pleasant and refreshing aroma and antifungal and antibacterial properties (Anaruma et al., 2010; Guynot et al., 2003; Pandey et al., 2003; Kumar et al., 2007, 2009; Inouye et al., 2001; Pattnaik et al., 1996). However, lemongrass essential oil production is currently limited to the tropics and subtropics. The United States and European countries are major importers of the essential oil.

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Recently, the interest of southern growers towards the establishment of essential oil crops production in the Southeast inspired several studies on peppermint (Zheljazkov et al., 2010a, 2010b), spearmints (Zheljazkov et al., 2010b, 2010c), Japanese mint (Zheljazkov et al., 2010d, 2010e), and basil (Zheljazkov et al., 2008a, 2008b, 2008c, 2008d). As a result, the first commercial peppermint production at a grower's field in Mississippi will be established in the spring of 2011. The essential oil from lemongrass, peppermint, spearmint, Japanese mint, and basil is extracted using the same method and equipment, whereas these crops reach technical maturity at different time. That may allow for better use of distillation equipment in time (a major investment in essential oil industry) and subsequent improved efficiency and economic sustainability of essential oil production in the southeastern United States.

Lemongrass is a high-biomass crop that may have applications for biofuel production. Because of the content of its high-value essential oil, the cost for production of biomass for biofuel may be low, since the biomass would be a by-product of essential oil production. Lemongrass may prove to be a new high-value specialty crop and a good source for biofuel in the southeastern United States, a region known for its hot, humid climate, abundant and cheap water, and well-established irrigation infrastructure.

Nitrogen is known to be the major modifier of crop productivity (Marschner, 1999). Sulfur can increase N uptake, improve N use efficiency (Marschner, 1999; Scherer, 2001), and improve overall yields by mitigating hidden soil S deficiency (Scherer, 2001). Growers usually do not apply S fertilizers because S has been traditionally found as an impurity in chemical fertilizers and agricultural soils receive S with rainfall from atmospheric sulfate or diluted sulfuric acid (Scherer, 2001). However, in the past few decades, chemical fertilizers have become more pure and do not contain S. In addition, SO_2 industrial emissions in the United States were reduced by 61% for the period 1990 to 2009 (USEPA, 2010). These events have reduced S input from the atmosphere to agricultural soils. Since crops continue to take up S, soils in many regions may exhibit various degrees of S deficiency.

The objective of the field experiments was to evaluate lemongrass productivity, essential oil content, yield, and composition as a function of N and S.

MATERIALS AND METHODS

Field Experiments

Field experiments were conducted in 2006 at the Mississippi locations of Verona (34°43'22" N, −88°43'22" W) and Poplarville (30°50'11" N, −89°32'44" W). The experiment was repeated during the 2007 cropping season at Verona but not in Poplarville due to insufficient resources.

Lemongrass transplants for the two locations were produced in a controlled-environment greenhouse in March–April in Verona, using certified seeds purchased from Richters (ON, Canada). Transplants were produced in plastic trays filled with growth medium Metromix 300 (Scotts Co., Marysville, OH), with one plant per cell. Lemongrass (12-cm height) was transplanted in the field by hand in spring 2006 at the two locations and again in 2007 at Verona. In this experiment, lemongrass was grown as an annual crop because a preliminary study indicated that it did not perenniate very well. Under the environmental conditions of northern Mississippi, there was approximately 30% winter survival at the southern location, Poplarville, and below 1% survival at Verona, the northern location. Lemongrass plantlets were transplanted in previously prepared raised beds. The beds (12 cm in height and 80 cm wide at the top) were formed by a bed-shaping machine that also placed black plastic mulch on the top, and an irrigation drip tape at 5-cm soil depth and under the plastic. The same beds were used for two cropping seasons at Verona. The experimental units were 1.4 by 6 m plots with 24 lemongrass plants transplanted in two rows, in an offset pattern spaced 60 cm apart.

The design was a 4 × 4 factorial in three blocks, with 0, 40, 80, and 160 kg/ha N treatments, and 0, 30, 60, and 90 kg/ha S treatments. The 16 combinations of N and S were randomized within each block. The N fertilizers (as NH_4NO_3) and S fertilizers (as sulfur bentonite, 90% S) were applied approximately 2 wk after transplanting, by opening the plastic in the middle of the bed and resealing it with duct tape after fertilizer application.

The soil type at Verona was a Quitman sandy loam (fine-loamy, ciliceous, semiactive, therm Aquic Paleudults); at Poplarville, the soil was a Ruston loamy sand (fine-loamy, siliceous, semiactive, thermic Typic Paleudults). Soil texture analyses showed that the soil at Verona had 7.5% clay, 24% silt, and 68.5% sand, whereas the soil at Poplarville had 2.5% clay, 8.5% silt, and 89% sand. The concentration of soil-available nutrients was estimated following the Lankaster soil test method, used in Mississippi (Cox, 2001) and measured on an inductively coupled argon plasma spectrometer (Thermo Jarrell Ash, Franklin, MA). The necessary amounts of P and K fertilizers (rates according to the soil test results and fertilizers free of S) were applied and incorporated before bed formation.

Half of each plot was harvested in the first week of September (Harvest 1), and the other half was harvested in the first week of November (Harvest 2). The half plots harvested in the first week of September had regrowth, which was harvested again in the first week of November (Harvest 3). Since the three harvests were harvested repeatedly from the same plot (experimental unit), repeated measures analysis of the data was done. Whole aboveground plant parts, cut 20 cm above the soil surface using a gas powered hedge trimmer with oscillating teeth were harvested from each plot. Fresh and dried weights were taken, and essential oil extracted from every treatment combination in each of the three blocks. Representative samples of 300 g dried material from each experimental unit were steam distilled for 60 min in 2 L steam distillation units, in a manner similar to that described previously (Zheljazkov et al., 2008, 2010). At the end of each distillation, the essential oil was separated and weighed on an analytical scale. The essential oil content was determined as grams of oil per weight of dry lemongrass tissue. The oil was kept at −5°C until chemical analyses for oil composition could be performed.

Gas Chromatography–Mass Spectroscopy Analysis of the Essential Oil

The qualitative and quantitative analyses of oil samples were performed using gas chromatography–mass spectroscopy (GC–MS) at the National Center for Natural Products Research in Oxford, MS, using the GC–MS methods described in both Zheljazkov et al. (2008) for basil oils and in Zheljazkov et al. (2010) for mint oils.

Quantitative Analysis

Commercial standards (R)-(+)-limonene and (+)-δ-cadinene were purchased from Fluka (Switzerland); citral, (-)-*trans*-caryophyllene, and caryophyllene oxide were purchased from Sigma-Aldrich (St. Louis, MO). With five concentration points, an external standard least squares regression for quantification was used. Each specific analyte was used to formulate a separate calibration curve using MS total ion chromatogram (TIC) data. Linearity was imposed by using response factors and regression coefficients independently. Response factors were calculated using the equation $RF = DR/C$, where DR was the detector response in peak area (PA) and C was the analyte concentration. Since citral was only available as a mixture of *E* and *Z* isomers, the TIC area from both isomers (Fig. 3) was added together to generate the response factor used for the two individual isomers which were quantified separately.

The chromatograms of each of the essential oil samples from the field experiments were compared to the chromatograms from standards. Target analytes were confirmed by retention time and mass spectra. Confirmed integrated peaks were used to determine percentage of each chemical constituent in the essential oil itself. The RF of the target chemical constituent was used to determine the percentage of oil for each sample using the equation $PA/RF/C \times 100$ = % analyte in the oil on a wt/wt basis.

Statistical Methods

Repeated measures analysis was completed separately for the data collected from the experiments conducted at Verona and Poplarville. At the Verona location, the combinations of the 2 yr (2006, 2007) and the three blocks in the field were used as six blocks to filter out differences due to the years and the spots in the field. However, for the Poplarville location, where the experiment was conducted in 2006 only, the three blocks in the field were used as blocks. The three harvests at Verona and

Table 1. *P* values showing effects of N, S, and harvest (H) on essential oil (EO) content, dry weight yield, EO yield, and composition and yeild of *t*-caryophyllene, neral, and geranial at Verona, MS.

Source	EO content	Dry weight yield	EO yield	*t*-Caryo-phyllene	Neral	Gera nial	Caryophyllene oxide	*t*-Caryophyllene yield	Neral yield	Geranial yield	Caryophyllene oxide yield
	%					%					
						P value					
Block	0.058	0.322	0.516	0.001	0.453	0.001	0.446	0.002	0.249	0.035	0.212
N	0.311	0.001	0.001	0.503	0.190	0.042	0.481	0.001	0.001	0.001	*0.001*
S	0.694	0.873	0.333	0.353	0.832	0.625	0.761	0.017	0.644	0.493	0.197
N × S	0.782	*0.058*	0.172	0.576	*0.023*	0.033	0.779	*0.010*	*0.017*	*0.016*	0.106
H	0.001	0.001	0.001	0.009	0.001	0.001	*0.001*	0.014	0.001	0.001	*0.001*
N × H	*0.032*†	0.426	*0.049*	*0.049*	*0.022*	0.025	0.896	*0.010*	*0.043*	*0.030*	0.896
S × H	0.236	*0.040*	0.103	0.443	*0.008*	0.018	0.541	0.361	*0.012*	*0.006*	0.349
N × S × H	0.580	0.877	0.943	0.518	0.107	*0.032*	0.710	0.261	0.982	0.965	0.820

† *P* values that suggest significance of the effects requiring multiple means comparison are in italics.

the two harvests at Poplarville were used as time points for the repeated measures analysis that included identifying the most appropriate covariance structure using the AIC and BIC values and incorporating it in the model using the Mixed Procedure of SAS (SAS Institute, 2008). For each response, the validity of model assumptions was verified by examining the residuals, as described in Montgomery (2009). Some of the responses required cubic root transformation to achieve normality of the error terms; however, the means shown in the tables and figures are back-transformed to the original scale. For significant ($p < 0.05$) and marginally significant ($0.05 < p < 0.1$) effects, further multiple means comparison was completed by comparing the least squares means of the corresponding treatment combinations. Letter groupings were generated using a 1% level of significance for two-factor and three-factor interaction effects and using a 5% level of significance for main effects.

RESULTS

Lemongrass Responses at Verona Location

Both N and S treatments affected plant dry weight and essential oil components (Table 1). However, the effect of one depended on the rate used for the other, and this effect was not uniform at all harvests. This was reflected in the significant (*p* values <0.05 or *p* values between 0.05 and 0.1 shown in italics in Table 1) interaction effects. Specifically, N × S interaction affected dry weight yield, % neral and yields of *t*-caryophyllene, neral and geranial; N × harvest interaction affected percent and yield of essential oils, % *t*-caryophyllene and neral, and yields of *t*-caryophyllene, neral and geranial; and S × harvest interaction affected dry weight yield, % neral, and the yields of neral, and geranial. The N × S × harvest interaction effect on % geranial was also significant ($p < 0.05$, Table 1). Also, at the Verona location, the concentration of *t*-caryophyllene oxide was affected by harvest alone regardless of the amount of N and S, while only the main effects of N and harvest were significant ($p < 0.05$) on the yield of *t*-caryophyllene (Table 1).

The average essential oil content at Verona varied from 0.34 to 0.55% depending on the N × harvest combination, with the highest content obtained from the third harvest (regrowth) at either 40 or 80 kg N/ha (Table 2). These results indicate that essential oil content in lemongrass may decrease when plants get older. Within each of the N application rates (with the exception at 40 kg/ha of N), essential oil yields were higher in Harvest 2, lower in Harvest 1, and the lowest in Harvest 3 (regrowth) (Table 2). The regrowth had shorter time to produce enough biomass, and despite the higher oil content, essential oil yields from Harvest 3 (regrowth) were the lowest. However, if the essential oil yields from Harvest 1 and Harvest 3 are combined, the oil yield would be (numerically) greater than the yield from Harvest 2. Compared to a single-cut cropping system, a double-cut cropping system may provide greater oil yields. However, the economics

Table 2. Lemongrass mean essential oil (EO) content, EO yield, concentration and yield of *t*-caryophyllene and neral, and geranial yield from the combinations of N application rate and harvest (H) at Verona, MS.

N rate	Harvest	EO content	EO yield	*t*-Caryophyllene conc	Neral conc	*t*-Caryophylleneyield	Neral yield	Geranial yield
kg/ha		%	kg/ha	%		kg/ha		
0	1	0.516a†	56.8de	0.768abc	35.9abc	0.471abc	20.2de	25.0de
0	2	0.394cd	73.4bc	0.599cd	35.7abc	0.409bc	25.6bcd	33.4bc
0	3	0.471ab	30.1g	1.035a	35.3abc	0.357c	10.1g	12.0g
40	1	0.495ab	65.2cd	0.536d	36.6ab	0.345c	23.6cd	29.0cd
40	2	0.387cd	81.9bc	0.732bcd	32.6c	0.588abc	26.5bc	34.2bc
40	3	0.549a	36.6fg	0.910ab	38.1a	0.346c	13.6fg	15.7fg
80	1	0.436bc	67.3cd	0.577cd	38.9a	0.396bc	26.0bc	31.4bc
80	2	0.344d	89.1b	0.604cd	33.1bc	0.608ab	29.4bc	37.9b
80	3	0.523a	46.8ef	1.002a	36.8ab	0.642ab	16.9ef	20.0ef
160	1	0.477ab	86.2b	0.702bcd	36.6ab	0.582abc	31.3b	37.4b
160	2	0.432bc	138.7a	0.611cd	33.1bc	0.811a	45.7a	57.8a
160	3	0.504ab	53.1d	0.720bcd	32.2c	0.549abc	16.9ef	19.9ef

† Means followed by the same letter within a column are not significantly different at the 1% level.

Table 3. Lemongrass mean dry weight (DW) yield, neral concentration, and yield of neral and geranial from the combinations of S application rate and Harvest at the Verona, MS location.

S rate	Harvest	DW yield	Neral conc	Neral yield	Geranial yield
kg/ha		kg/ha	%	kg/ha	
0	1	13,305c†	34.8bcd	21.1c	26.1c
0	2	23,729a	35.0abcd	29.5ab	37.4ab
0	3	8,721d	38.0ab	16.2d	19.2d
30	1	15,077b	36.8abc	26.0abc	32.1bc
30	2	24,633a	33.3cd	29.9ab	37.2ab
30	3	7,567e	36.5abc	14.3de	16.3d
60	1	14,686bc	38.7a	27.6ab	32.4bc
60	2	25,975a	32.8d	31.3ab	41.0a
60	3	7,665de	33.3cd	12.1e	14.5d
90	1	15,316b	37.6ab	25.8bc	31.7bc
90	2	2,5037a	33.4cd	34.1a	45.1a
90	3	7,956de	34.6bcd	14.3de	16.9d

† Means followed by the same letter within a column are not significantly different at the 1% level.

of double-cuts needs to be evaluated to ascertain profitability. Although the interaction of N application rate and harvest modified the concentration of neral in the oil and neral yield, the trend was not clear. The interaction of N application rate and harvest also affected geranial yield, with Harvest 2, with an N application rate of 160 kg/ha giving the largest yield (Table 2).

Within each S application rate, dry weight yields were highest in Harvest 2, lower in Harvest 1, and the lowest in Harvest 3 (regrowth) (Table 3). Even if yields from Harvest 1 and Harvest 3 are combined, the result would still be numerically lower than yields at Harvest 2. However, due to the greater essential oil content in Harvest 3, the total amount of essential oil yield from Harvest 1 and Harvest 3 (regrowth) would be greater than the yield from the single harvest system, Harvest 2.

Table 4. Lemongrass mean dry weight (DW) yield, neral concentration, and yield of *t*-caryophyllene, neral, and geranial from the combinations of N and S application rates at the Verona, MS location.

N	S	DW yield	Neral conc	Yield: t-Caryophyllene	Yield: Neral	Yield: Geranial
kg/ha			%	kg/ha		
0	0	9,486e†	35.9ab	0.288e	13.9f	17.5e
0	30	12,356cd	38.4a	0.551bc	21.0cde	25.4cd
0	60	12,093de	33.7ab	0.401cde	16.9ef	20.9de
0	90	12,358cd	34.4ab	0.433bcd	19.8def	25.5cd
40	0	14,521bcd	36.2ab	0.478bcd	22.7bcde	28.0bcd
40	30	11,770de	33.7ab	0.338de	17.0ef	20.6de
40	60	11,565de	35.8ab	0.435bcd	20.6cde	25.5cd
40	90	13,864cd	37.4a	0.426bcd	22.8bcde	27.9bcd
80	0	15,521abc	34.3ab	0.401cde	20.2cde	24.8cd
80	30	15,891abc	35.0ab	0.624ab	23.2bcde	28.4bcd
80	60	18,195ab	38.7a	0.587abc	27.0abcd	33.2abc
80	90	14,944bcd	37.1a	0.571bc	24.6abcd	30.5abc
160	0	19,218a	37.3a	0.599abc	33.3a	40.4a
160	30	19,251a	35.1ab	0.841a	30.8ab	36.9ab
160	60	18,508ab	31.5b	0.520bc	26.3abcd	32.2abc
160	90	19,375a	32.0b	0.631ab	28.4abc	35.0ab

† Means followed by the same letter within a column are not significantly different at the 1% level.

Generally, the lowest dry weight yields were obtained from the 0 kg/ha N and S treatment combination, and higher from the 80 or 160 kg/ha of N (Table 4). Overall, dry weight yields increased with application of 80 kg N/ha relative to the 0 kg N/ha treatment and with 160 kg of N/ha relative to the 0 and 40 kg N/ha treatments. The application of 80 kg/ha N increased caryophyllene oxide yield relative to the N at 0 and 40 kg/ha treatments, at 160 kg/ha of N yield was further increased relative to 80 kg/ha of N (data not shown). The concentration of caryophyllene oxide in the oil was greater in Harvest 1 relative to harvests 2 or 3, while the yield of caryophyllene oxide was greater in harvests 1 and 2 and lower in Harvest 3 (data not shown).

The three-way interaction effect of N, S, and harvest influenced the concentration of geranial in the oil (Fig. 1). At Harvest 1 and at 80 kg/ha of N, the concentration of geranial was low at 0 kg/ha of S but much higher at higher S application rates. At Harvest 2 and 0 kg/ha of N, the geranial concentration was higher at 0 and lower at 90 kg/ha of S. At Harvest 3 and N at 160 kg/ha, geranial concentration was higher at S at 0 and 30 kg/ha and lower at S at 60 and 90 kg/ha rates. The lack of clear trend in Fig. 1 indicates the presence of factors other than N, S, and harvest, that affect the concentration of geranial in lemongrass essential oil at Verona.

Lemongrass Responses at Poplarville

At the Poplarville location, the main effect of harvest on dry weight and the concentration of *trans*-caryophyllene in the oil was significant ($p < 0.05$); the interaction of N and S was marginally significant ($0.05 < p < 0.1$) on dry weight yield; and the interaction of N and harvest was marginally significant ($0.05 < p < 0.1$) on essential oil yield (Table 5). Also at Poplarville, the interaction effect of N and S was significant ($p < 0.05$) on the yields of neral, geranial, and cariophyllene oxide (data not shown). The interaction of N and S modified dry weight yields, (with S at 90 kg/ha, productivity was generally lower), and yields of neral, geranial, and caryophyllene oxide (Table 6). The application of 160 kg/ha of N increased dry weight yields relative to 0 or 40 kg/ha N rates, irrespective of S rate.

Within each N application rate, essential oil yields were greater from Harvest 2 relative to Harvest 1, indicating that in a single-harvest system, lemongrass should be harvested at harvest time 2 (Table 7). Within each S application rate, the concentration of caryophyllene oxide was higher in Harvest 1 than in Harvest 2 but lower from 90 kg/ha of S (Table 7).

The interaction of N, S, and harvest was significant on the concentration of neral and geranial in the oil (Table 5, Fig. 2). Within Harvest 1, and 40 and 160 kg/ha of N, geranial concentration was lower at 0 kg/ha of S and increased as S application rate increased. In most instances, interaction effects of N, S, and harvest on concentrations of neral and geranial did not follow a clear trend, indicating existence of other factors affecting the chemical profile of lemongrass oil at Poplarville, MS.

DISCUSSION

Effect of Nitrogen and Sulfur on Biomass Yields

This is the first report on the effect of S and the interaction effect of S and N on lemongrass productivity, oil content, and

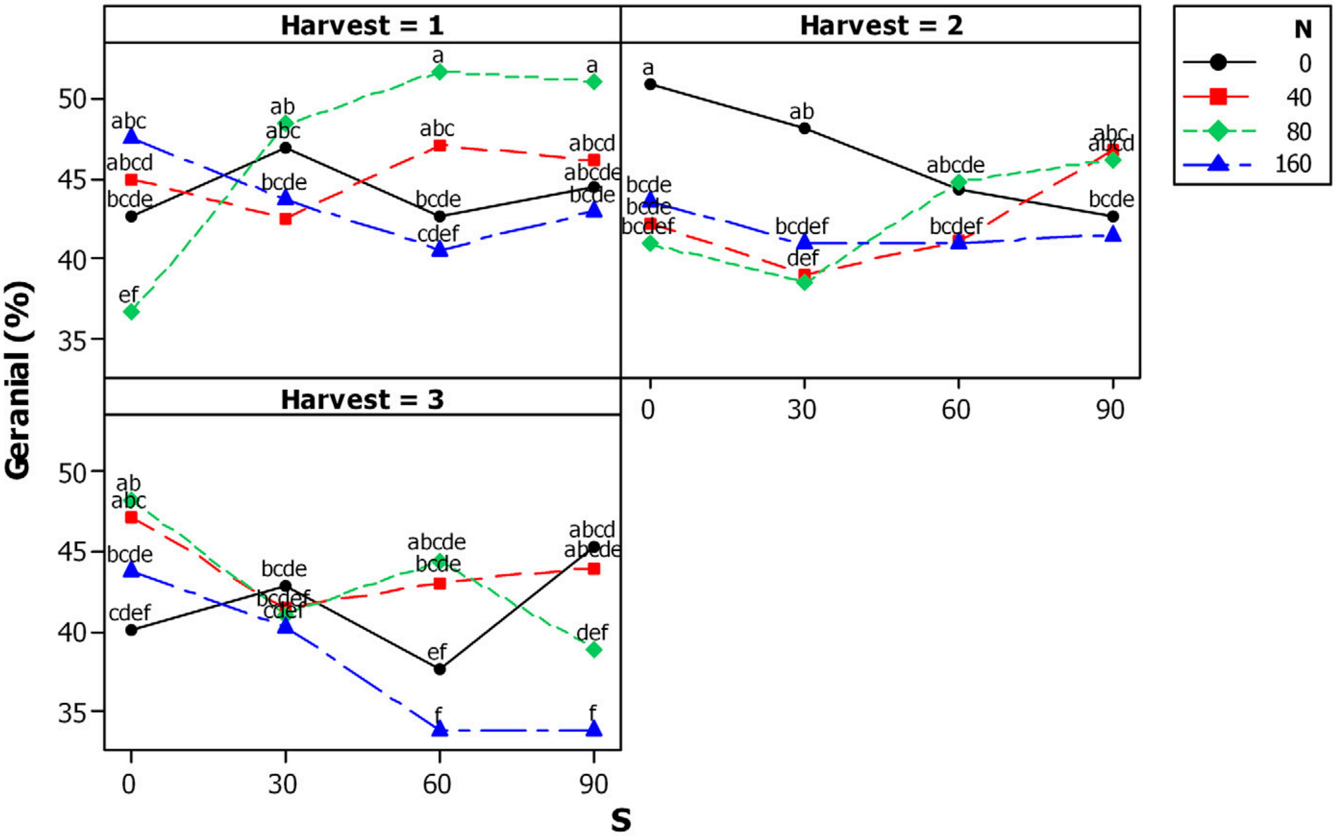

Fig. 1. Interaction effect of N, S, and Harvest on geranial (%) at Verona, MS. Means sharing the same letter are not significantly different at the 1% level.

composition. It is also the first report on lemongrass productivity and oil composition in the southeastern United States. Results demonstrate that lemongrass yields, oil content, and composition may respond differently in different locations. There has been a report on similar aromatic grass in the region (Igbokwe and Asumeng, 2007), but this report was on *Cymbopogon citratus*, a different species. Our study demonstrated that lemongrass could be grown as an annual crop in the southeastern United States and may provide biomass yields similar to literature reports (Rao et al., 1998; Singh, 2001; Singh et al., 1997, 2009). The latter authors also found that lemongrass biomass yields increased linearly in response to increasing N rates (Singh, 2001; Singh et al., 1996) or reached optimal levels at 100 kg/ha of N (Singh et al., 1997; Rao et al., 1998). Generally, biomass yields in our study were highest at 160 kg/ha of N, but yields at 80 kg/ha of N were not different.

Effects on Oil Content and Composition

Oil content varied from 0.35 to 0.6%, which is typical for lemongrass in India (Sarma and Sarma, 2005). The essential oil composition of lemongrass grown in Mississippi was similar to the composition of lemongrass oil from traditional producing regions (Akbar and Saxena, 2009; Chandrashekar and Joshi, 2006; Chowdhury et al., 1998; Kulkarni et al., 1997; Pandey et al., 2003; Sarma and Sarma, 2005). Lemongrass essential oil produced in the southeastern United States is similar to those marketed from traditional production regions of the world. The overall means of chemical constituents in this study were 0.78%

Table 5. *P* values showing the effect of N, S, and harvest (H) on lemongrass essential oil (EO) content, dry weight yield, EO yield, and concentrations of *t*-caryophyllene, neral, geranial, and caryophyllene oxide in the oil at Poplarville, MS.

Source	EO content	Dry weight yield	EO yield	*t*-Caryophyllene	Neral	Geranial	Caryophyllene oxide
	%			%	%	%	%
	P value						
Block	0.023	0.018	0.056	0.544	0.446	0.246	0.038
N	0.106	0.001	0.001	0.742	0.732	0.636	0.298
S	0.248	0.015	0.830	0.535	0.017	0.050	0.584
N × S	0.162	*0.083*†	0.258	0.594	0.011	0.030	0.229
H	0.838	*0.001*	0.001	*0.001*	0.001	0.001	0.001
N × H	0.137	0.562	*0.084*	0.846	0.168	0.183	0.609
S × H	0.583	0.445	0.874	0.933	0.470	0.386	*0.095*
N × S × H	0.433	0.683	0.222	0.610	*0.004*	*0.028*	0.778

† *P* values that suggest significance of the effects requiring multiple means comparison are in italics.

Table 6. Lemongrass mean dry weight (DW) biomass yield, and yields of Neral, Geranial, and Caryophyllene oxide from the combinations of N and S application rates at Poplarville, MS.

N	S	DW	Neral	Geranial	Caryophyllene oxide
rates, kg/ha		yield, kg/ha			
0	0	10,160bcd†	12.35a	14.16a	1.35ab
0	30	9,109de	8.52abc	10.02abc	1.01abc
0	60	8,732de	10.85ab	12.66ab	1.06abc
0	90	8,036e	8.32abc	9.63abc	0.86c
40	0	9,393cde	5.67bc	6.66c	1.02abc
40	30	9,158cde	7.12abc	8.18bc	0.87bc
40	60	9,042de	10.69ab	11.98ab	1.40a
40	90	8,593de	9.05abc	9.73abc	0.83c
80	0	10,925abc	8.49abc	9.99abc	0.95abc
80	30	9,872bcd	10.48ab	11.93ab	1.42a
80	60	9,635cde	4.66c	5.15c	0.95abc
80	90	8,335e	8.24abc	9.62abc	0.73c
160	0	12,593a	7.33abc	8.59bc	1.17abc
160	30	11,770ab	12.05a	13.58a	1.36ab
160	60	11,592ab	9.98ab	11.54ab	1.01abc
160	90	11,396ab	11.35a	13.12ab	1.41a

†Means followed by the same letter within a column are not significantly different at the 1% level.

Table 7. Mean lemongrass essential oil (EO) yield from the combinations of N application rates and harvest; and mean caryophyllene oxide concentration from the combinations of S application rates and harvest at Poplarville, MS.

N rate	Harvest	EO yield	S rate	Harvest	Caryophyllene oxide concentration
kg/ha		— kg/ha —			%
0	1	29.1cd†	0	1	4.12ab
0	2	62.9b	0	2	1.67c
40	1	24.6d	30	1	4.15ab
40	2	66.5b	30	2	1.81c
80	1	23.5d	60	1	4.30a
80	2	65.7b	60	2	1.73c
160	1	32.4c	90	1	3.44b
160	2	89.5a	90	2	1.99c

† Means followed by the same letter within a column are not significantly different at the 1% level.

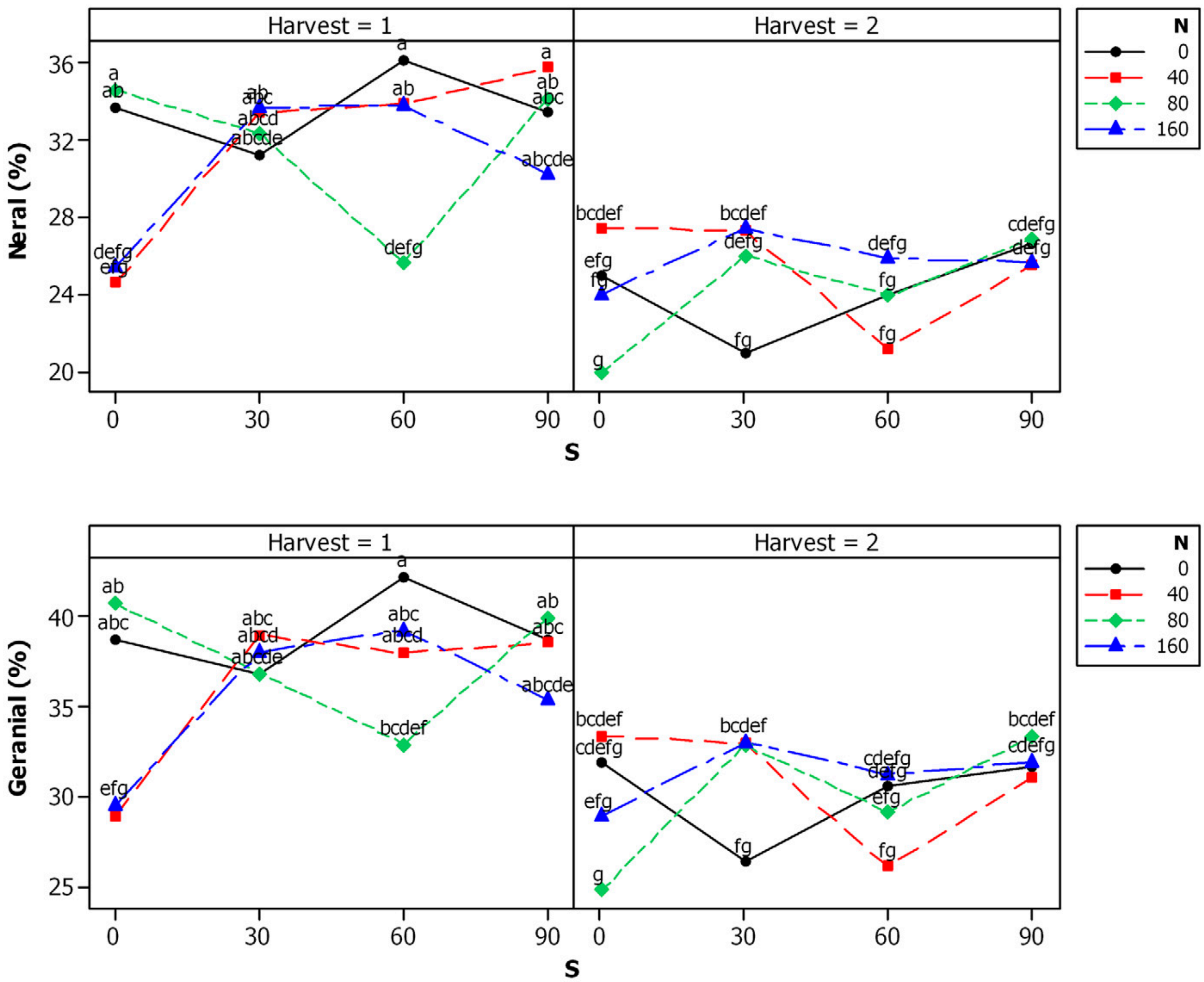

Fig. 2. Interaction effect of N, S, and Harvest on neral (%) and geranial (%) at Poplarville, MS. For each response, means sharing the same letter are not significantly different at the 1% level.

t-caryophyllene, 33.9% neral, 44.7% geranial, and 2.8% caryophyllene oxide. Literature reports vary considerably with respect to lemongrass chemical composition. Weiss (1997) reported 30.06, 51.19, and 1.95% of neral, geranial, and geranyl acetate, respectively. Pandey et al. (2003) reported 43.8, 18.9, 5.27, and 3.66% for (*E*)-citral (geranial), (*Z*)-citral (neral), geranyl acetate, and trans-geraniol, respectively. Kulkarni et al. (1997) found 13.1% geraniol, 11.2% citronellyl acetate, and 25.9% geranyl acetate; Nath et al. (1994) reported 30.5% geraniol, 24.1% citronellol, 10.3% neral, and 13.6% geranial; while Chandrashekar and Joshi (2006) reported 61% citral (neral), 13% geraniol, 8% geranyl acetate, and 6% limonene. Lemongrass essential oil composition in this experiment varied as a function of harvest, N, and S application and location, with geranial varying from 25 to 53%, neral from 20 to 45%, caryophyllene oxide from 1.3 to 7.2%, and *t*-caryophyllene from 0.3 to 2.2%.

Potential for Biofuel Production

Owing to its high biomass, lemongrass may have a potential for biofuel production in the southeastern United States. The advantage of lemongrass over other lignocellulosicspecies currently under investigation in the United States is the production of a high-value natural product (essential oil), which may offset production costs. Double utilization of biofuel crops may be more promising than crops grown only for biomass. Lemongrass yields in this study ranged from 7500 to 25,900 kg/ha at Verona and from 8036 to 12,593 kg/ha at Poplarville. A recent review of switchgrass (*Panicum virgatum* L.) production potential in the United States from 39 trials reported mean yields of 8700 ± 4200 kg/ha for upland ecotypes and 12,900 ± 5900 kg/ha for lowland ecotypes (Wullschleger et al., 2010). The lemongrass in this study was grown under irrigation, while switchgrass for ethanol production has been tested mostly as a rain-fed crop. Lemongrass will likely not have the same wide adaptability to environmental conditions as switchgrass. However, lemongrass's advantages for the southeastern United States are (i) the production of a high-value natural product and (ii) that the lemongrass biomass is essentially cooked during the extraction of the essential oil. Steam distillation of lemongrass essential oil (basically a 2-h treatment of the biomass with hot steam) may overcome biomass recalcitrance, as described by Himmel et al. (2007). Lemongrass biomass may be more readily convertible to ethanol compared to biomass from other species that have not received pretreatment with hot steam. The essential oil from the lemongrass may rectify current ongoing discussions of the economic, agronomic, and environmental effects of the large production of biofuel crops. Lemongrass biomass is consistent because plants form rosettes with long leaves. Lemongrass did not form stems and inflorescences in the southeastern United States. The lemongrass biomass produced in the southeastern United States would be homogeneous, consisting only of leaves, which may facilitate bioethanol production from the biomass.

There is no current production of lemongrass essential oil in the United States; the demand is being met by imports from India. The development of lemongrass as an essential oil and biofuel crop in the southeastern United States would provide a new cash crop for growers. Lemongrass oil production may also encourage local value-added processing of the essential oil, a spin-off effect that may improve the economic sustainability of the region.

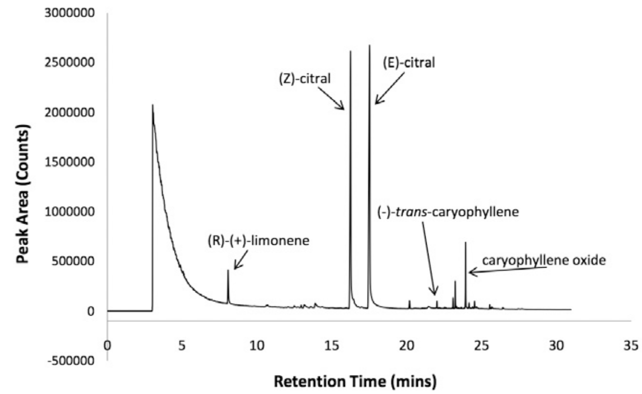

Fig. 3. Representative Chromatogram of *Cymbopogon flexuosus*.

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