HortScience 43(5):1415–1422. 2008.

Productivity, Oil Content, and Oil Composition of Sweet Basil as a Function of Nitrogen and Sulfur Fertilization

Valtcho D. Zheljazkov[3]
Mississippi State University, North Mississippi Research and Extension Center, 5421 Highway 145 South, Verona, MS 38879

Charles L. Cantrell
Natural Products Utilization Research Unit, Agricultural Research Service, U.S. Department of Agriculture, P.O. Box 8048, University, MS 38677

M. Wayne Ebelhar[1]
Delta Research and Extension Center, P.O. Box 197, Stoneville, MS 38776

Dennis E. Rowe
Mississippi State University, Experimental Statistics, Mississippi State, MS 39762

Christine Coker[2]
Mississippi State University, Coastal Research and Extension Center, 1815 Popps Ferry Road, Biloxi, MS 39532

Additional index words. Ocimum basilicum, essential oil, (–)-linalool, eugenol, (–)-bornyl acetate, eucalyptol

Abstract. **Nitrogen (N) and sulfur (S) were believed to be important nutrient management tools for the production of sweet basil (*Ocimum basilicum* L. 'German') with desirable oil content and composition and acceptable herbage yields. A multilocation research study was initiated to evaluate the effect of N (0, 60, 120, and 180 kg·ha^{-1} N) and S (0, 20, 40, and 80 kg·ha^{-1} S) rates on biomass production, oil content, and oil composition for sweet basil. The three locations in Mississippi (Stoneville, Poplarville, and Verona) were selected based on soil type, geographic, and climatic variation. Location, N rate, and their interaction were significant on basil dry herbage yields. The herbage yield means were 4967 kg·ha^{-1}, 2907 kg·ha^{-1}, and 2122 kg·ha^{-1} for Poplarville, Verona, and Stoneville, respectively. Oil content was significantly affected by location with means of 0.69%, 0.80%, and 0.64% for Stoneville, Poplarville, and Verona, respectively. Location, N, and S had significant effects on oil yields with means of 14.7, 38.7, and 18.5 kg·ha^{-1} for Stoneville, Poplarville, and Verona, respectively. The significant quadratic response of essential oil yields to N fertilization rates showed oil yields were maximized for fertilization between 50 and 60 kg·ha^{-1} N. In contrast, the response to S fertilization appeared to continue to increase beyond the maximum fertilization rate evaluated of 80 kg·ha^{-1} S. Location and N application rates had a significant effect on the yields of the major basil oil constituents (–)-linalool, eugenol, (–)-bornyl acetate, and eucalyptol, whereas S had a significant effect on eucalyptol yield only. Eucalyptol concentration was positively correlated to the concentration of (–)-bornyl acetate. This is the first study to quantify (in real concentration) the response of the major sweet basil oil constituents (–)-linalool, eugenol, (–)-bornyl acetate, and eucalyptol as a function of N and S application rates. Also, it is the first study to demonstrate a strong response of basil oil yield to S. The results from this study demonstrated that N and S applications can be used as management tools with respect to sweet basil production, oil content, and oil composition.**

Sweet basil (*Ocimum basilicum* L.) is the most widely grown species of the genus *Ocimum* L. (Javanmardi et al., 2002; Simon et al., 1990, 1999). Recent research demonstrated that sweet basil could be a feasible essential oil crop for Mississippi and possibly the southeastern United States (Zheljazkov et al., 2008a, 2008b); however, basil plant nutrition has not been investigated in the southeastern United States. Prior research in other parts of the world demonstrated a significant effect of plant nutrition in general (Golcz et al., 2006; Sifola and Barbieri, 2006; Singh et al., 2004a; Topalov, 1962; Zheljazkov, 1998) and N fertilization (Arabaci and Bayram, 2004; Golcz et al., 2006; Sifola and Barbieri, 2006; Singh et al., 2004a, 2004b; Yassen et al., 2003) on sweet basil productivity and oil composition.

Sulfur is another macronutrient with important functions in plants. It is a part of plant proteins as a component of the amino acids cysteine and methionine and coenzymes (R-SH) (Marschner, 1999). One of the plant nutrient deficiencies identified in both Europe and North America was found to be from a lack of S in the plants. Traditionally, growers have not applied S because: 1) a large S deposition as sulfate (SO_4) or mild solution of sulfuric acid (H_2SO_4) was available from the atmosphere as a result of the significant SO_2 emissions; 2) S was found as "impurities" in mineral fertilizers; and 3) S-SO_4 was a byproduct in the formulation of other nutrient fertilizers such as ordinary phosphate. Hence, soils have been receiving S with the rain or with the application of N, P, or K mineral fertilizers for many years. As a result of the use of high purity fertilizers in recent decades and changes in processing, continuous cropping with high-yielding cultivars and a significant reduction of anthropogenic SO_2 emission (MacGrath et al., 1996; Riley et al., 2002), these S input modes have been diminished or eliminated, whereas the S removal from agricultural land continued. As a result, many soils have been depleted of available S (Hedge and Babu, 2007; Morris, 1994; Srinivasarao et al., 2008). In addition, S-SO_4 can be leached from the surface soil, especially in regions with high precipitation (above 1200 mm/year) such as in Mississippi. According to Stats Canada and the U.S. Environmental Protection Agency (Statistics Canada, 2003; USEPA, 2006), SO_2 emissions in North America (Canada and the United States) have been reduced by ≈50% for the period 1980 to 2000. These factors have resulted in a significant reduction of wet S deposition on agricultural land.

Sulfur deficiency could reduce N uptake, potential crop yield, and the quality of plants (Marschner, 1999). Previous research has found that SO_4 additions increased yields in alfalfa (*Medicago sativa*), wheat (*Triticum aestivum*), corn (*Zea mays*), canola or oilseed rape (*Brassica napus*), and potato (*Solanum tuberosum*) (Millins and Mitchell, 1989; Pavlista, 2005; Rehm, 2005;

Received for publication 12 Feb. 2008. Accepted for publication 6 Apr. 2008.
This research was funded by ARS Specific Coop. Agreement 58-6402-4-026 with CRIS MIS-172050. Specific project, "Field Establishment of Medicinal Herbs and Potential for Commercial Production", awarded to Dr. Jeliazkov (Zheljazkov).
Contribution of the Mississippi Agricultural and Forestry Exp. Sta. journal article No J-11301.
We thank Ganisher Abbasov, Thomas Horgan, and Marie Rogers for their help in the field and laboratory and Amber Callahan for her help with quantitative analysis. We thank Dr. F. Matta, Dr. C. Sloan, Dr. J. Kushla, and Dr. N. Buehring for reviewing the manuscript and suggesting improvements.

[1] Research Professor.

[2] Associate Research Professor.

[3] To whom reprint requests should be addressed; e-mail vj40@pss.msstate.edu

Stewart and Porter, 1969; Weil and Mughogho, 2000). Agricultural crops vary widely with respect to their tolerance to low solution S and different crops may have different critical tissue S concentration (Hitsuda et al., 2005). Sweet basil requirement for S are unknown or have not been reported in the literature.

The hypothesis to be evaluated in this study was that N and S rates would have a significant effect on sweet basil productivity and oil content and would significantly impact the concentration and yield of (–)-linalool, eugenol, (–)-bornyl acetate, and eucalyptol. Furthermore, N and S fertilization of sweet basil could be used as a tool to target the production of basil with increased essential oil yields or oil with a desirable composition (an elevated concentration of a compound of interest). The chemical composition of basil is of a great interest to various industries that are using basil essential oil. Hence, in systems in which basil is grown for essential oil production, it is the oil composition and yield that are more important than herbage (shoot) biomass. The objective of this study was to evaluate the effects of N and S application rates on total basil production, oil content, dry herbage yield, essential oil composition, and yield of major oil constituents. The research has focused on the quantification of (–)-linalool, eugenol, (–)-bornyl acetate, and eucalyptol, because these are the major important basil oil constituents and previous research indicated that these constituents were relatively conservative (stable) traits. The minor constituents fluctuate depending on chemotype or environmental factors (Kruger et al., 2002; Topalov, 1962; Zheljazkov et al., 2008a). Herewith, the actual (real) concentrations of the four major constituents in basil oil as a function of N and S fertilization are reported.

Table 1. Selected initial soil characteristics (0 to 15 cm), the concentration of extractable nutrients in the soil, and the average daily minimum and maximum temperatures of the three locations in Mississippi.

Location	Soil type	OM^z	pH	P	K	Ca	Mg	Zn	S	Na
		kg·ha^{-1}								
Poplarville	Ruston fine sandy loam, Typic Paleudults	1.03	6.8	135	187	1,758	413	—	166	78
Stoneville	Dundee silty clay loam, Typic Endoaqualfs	0.80	7.1	105	349	2,560	440	4.3	127	359
Verona	Quitman sandy loam, Aquic Paleudults	1.05	6.5	50	62	1,935	97	1.3	164	141

^{z}OM = organic matter.

Table 2. Standard curve data of the commercial standards run on Varian CP-3800 GC coupled to a Varian Saturn 2000 mass spectrometry/mass spectrometry.

Standard^z	R^2 from standard curve	Retention index^y	Range (mg·mL)^x	Limit of detection (mg·mL)
(–)-Linalool	0.997	1,099	10.00:0.500	0.0500
Eucalyptol	0.999	1,035	1.00:0.001	0.0001
Eugenol	0.990	1,356	1.00:0.250	0.0250
(–)-Bornyl acetate	0.991	1,285	1.00:0.001	0.0001

^{z}All standards were commercially available.

^{y}Retention index computed as described previously by Kovats (Kovats, 1965).

^{x}Range used for determination of R^2 and for quantitative analysis.

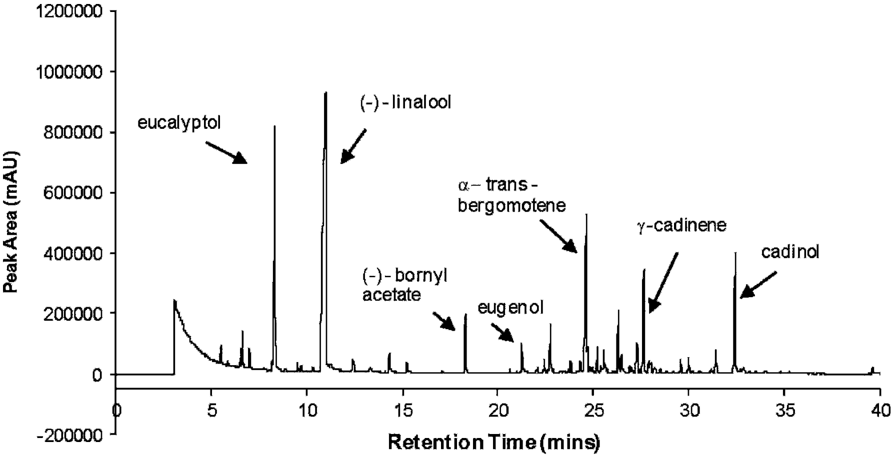

Fig. 1. Representative basil chromatogram (Verona).

Table 3. Commercially unavailable analytes identified by retention time and Kovats Index.

Analyte	Cited retention time	Observed retention time	Cited Kovats Index^z	Calculated Kovats Index
α-Trans bergamotene	25.99	24.871	1,435	1,436.95
γ–Cadinene	29.35	27.956	1,514	1,512.39
Cadinol	34.38	32.736	1,640	1,639.71

^{z}Retention index computed as described previously by Kovats (Kovats, 1965).

Material and Methods

Plant material and experimental design. The experiment was conducted in 2006 at three locations in Mississippi: the North Mississippi Research and Extension Center in Verona (lat. 34°43′22′′ N, long. –88°43′22′′ W), the Delta Research and Extension Center in Stoneville (lat. 33°25′18″ N, long. –90°54′28′′ W), and at the South Mississippi Branch Experiment Station in Poplarville (lat. 30°50′11″ N, long. –89°32′44″ W) using sweet basil (*O. basilicum* L. 'German'). Basil seedlings were produced from certified basil seeds (donated by the Research Institute for Roses and Medicinal Plants, Kazanluk, Bulgaria). All the basil seedling transplants for the three locations were produced in a greenhouse at the Verona location during May through June. Seeds were placed in 48-cell plastic trays filled with Metromix 300 (The Scotts Co., Marysville, OH) growth medium. Transplants were grown in the greenhouse for 40 d with a day/night temperature regime of 22 to 25 °C and 18 °C, respectively. Seedlings were fertilized weekly with 1.8 g of 20N–20P_2O_5–20K_2O dissolved in 300 mL of water and irrigated daily as needed. This particular fertilizer did not contain S. Basil seedlings (≈12 cm in height) were transplanted into the field at the three locations in 19 June 2006. To follow the common horticultural practices in Mississippi and the southeastern United States, basil plants were transplanted in previously prepared raised beds. The irrigation was provided by a drip tape irrigation system (1.29 L·min^{-1}/30 m with emitter spacing of 0.3 m) placed 3 to 4 cm into the soil in the middle of the bed at two locations and as overhead sprinkler irrigation at the Stoneville location.

Before soil preparation, soil samples were collected from the basil experimental sites and analyzed for extractable nutrients following the Mississippi State University Soil Testing Laboratory analytical procedures, which included the Lancaster extraction method (Cox, 2001). The three locations had different soil types and concentration of available plant nutrients (Table 1).

The experiment at each location was a randomized complete block design with four

Table 4. Analysis of variance *P* values for the main and interaction effects of location, N rate, and S rate on basil dry herbage yields (DHY), oil content, oil yields, (–)-linalool, eugenol, (–)-bornyl acetate (Bornyl A), and eucalyptol[z].

Effect	DHY (kg·ha^{-1})	Oil content (%)	Oil yield (kg·ha^{-1})	Major essential oil constituents in percent of oil: (–)-Linalool	Eugenol	(–)-Bornyl A.	Eucalyptol
Location	**<0.001**	**0.007**	**<0.001**	**<0.001**	**0.003**	**<0.001**	**<0.001**
N rate	**<0.001**	0.45	**<0.001**	**0.02**	0.34	0.08	**0.04**
S rate	0.06	0.99	**0.03**	0.33	0.07	0.08	0.78
N rate*loc	**<0.001**	0.19	0.25	**<0.001**	0.56	0.08	**<0.001**
S rate*loc	**0.03**	**0.03**	**0.003**	0.70	0.054	0.17	0.13
N rate*N rate	**<0.001**	0.19	**<0.001**	**0.002**	0.145	0.58	**0.02**
S rate*S rate	0.84	0.12	0.49	**0.002**	0.38	0.06	0.46
N rate*N rate*loc	0.15	0.72	0.37	0.49	0.13	0.07	0.006
S rate*S rate*loc	0.93	0.92	0.95	0.58	0.092	0.06	0.53
N rate*S rate	0.17	0.06	0.43	0.23	0.62	0.44	0.09

[z]Significant effects are shown in bold.

replicates. The experimental units were single-bed plots (2.1 m × 6 m) with 40 plants per plot. Basil was planted in an offset manner with two rows on each bed 30 cm on centers. Phosphorus and K fertilizers (free of S and at the same rate across the experimental site at every location) were added, if needed, based on the soil tests results before the land preparation and subsequently incorporated. Both N (as ammonium nitrate) and S (as sulfur bentonite, 90% S) were applied 1 to 3 d before transplanting. Single N and S fertilization events were applied to this relatively short-season crop (Zheljazkov, 1998; Zheljazkov et al., 2008a). Basil plants at the three locations were harvested at the same time, on 10 Aug. (55 d after transplanting), at full bloom (the optimal commercial stage for oil production), when the content and the composition of the essential oil would be optimal (Simon et al., 1990; Topalov, 1962; Zheljazkov, 1998). Plants were harvested by hand cutting the herbage at ≈12 cm above the soil surface. Herbage fresh weight was recorded at the time of harvest. Because of the large number of samples and the fact that it was impossible to extract the fresh samples from all plots at the same time, the plants from all plots were dried at 40 °C for 72 h and the dry weight recorded.

Essential oil extraction and analyses. The essential oils from all basil samples were extracted with steam distillation using a modified Clevenger collector apparatus (Furnis et al., 1989) (Scientific Glass, CA) using representative subsamples of 150 g of dry herbage (stems, leaves, and flowers) from each plot with a distillation time of 120 min. Basil essential oil yield was calculated as the weight (g) of oil per weight (g) of dry basil tissue.

Gas chromatography–mass spectrophotometry analyses. Chemical standards and basil oil from the field experiment were analyzed by gas chromatography–mass spectrophotometry (GC-MS) on a Varian CP-3800 GC (Palo Alto, CA) coupled to a Varian Saturn 2000 MS/MS. The GC was equipped with a DB-5 fused silica capillary column (30 m × 0.25 mm, with film thickness of 0.25 µm) operated using the following conditions: injector temperature, 240 °C, column temperature, 60 to 240 °C at 3 °C/min then held at 240 °C for 5 min; carrier gas,

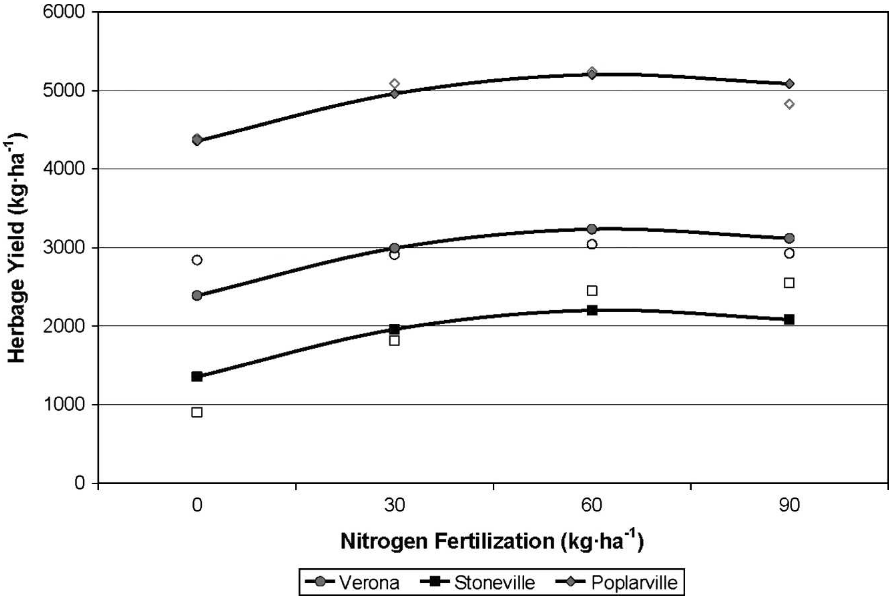

Fig. 2. Herbage production as affected by nitrogen fertilization at three locations (fitted curves have $R^2 = 0.72$).

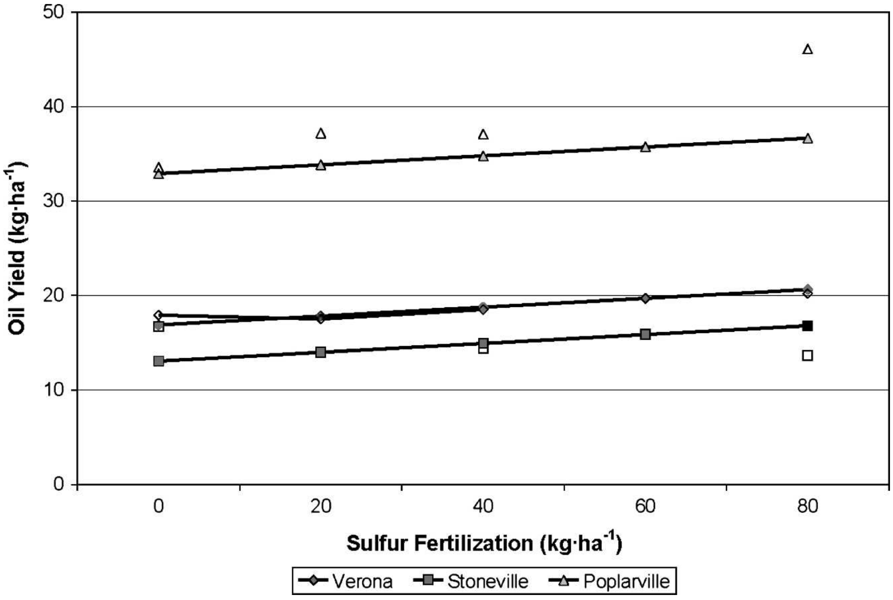

Fig. 3. Oil production as a function of sulfur fertilization at three locations (fitted curves have $R^2 = 0.62$).

He; injection volume, 1 μL (splitless). The MS mass ranged from 40 to 650 *m/z*, filament delay of 3 min, target total ion chromatogram (TIC) of 20,000, a prescan ionization time of 100 μsec, an ion trap temperature of 150 °C, manifold temperature of 60 °C, and a transfer line temperature of 170 °C.

Quantitative analysis. Commercial standard eugenol was purchased from Aldrich (St. Louis, MO), whereas (–)-linalool, eucalyptol, and (–)-bornyl acetate were purchased from Fluka (Buchs, Switzerland). With five concentration points, an external standard least squares regression for quantification was used. All four analytes were used to formulate separate calibration curves. All calculations were performed by generation of standard curves within Varian's Saturn GC/MS Workstation software package Version 6.40 (Table 2). The chromatograms of each of the oils from the field experiments (Fig. 1 shows a sample chromatogram) were compared with the standard injections. The target peaks (Fig. 1) were confirmed by both retention time and mass spectral data. Confirmed integrated peaks were then used to determine the percentage of each chemical constituent in the essential oil based on the equation for the line (Table 3). There were three commercially unavailable analytes that were constituents in sweet basil samples (Table 3). Identification of these components was obtained using retention times, Kovats Indices, and mass spectra. Kovats Indices were calculated using the equation: $\mathrm{KI}(x) = 100[(\log \mathrm{RT}(x) - \log P_z)/(\log \mathrm{RT}(P_{z+1}) - \log \mathrm{RT}(P_z)]$, where: $\mathrm{RT}(P_z) \leq \mathrm{RT}(x) \leq \mathrm{RT}(P_{z+1})$, and $P_4 \ldots P_{25}$ are n-paraffins.

Statistical analysis. Covariate analysis (Proc Mixed; SAS Institute, 2003) was executed on the 11 response variables to determine which response variables were significantly ($P = 0.05$) affected by location, S fertilization, N fertilization, and by their interactions with class variables location and replication. When response variables were affected by fertilization, polynomial regression parameters for S fertilization and N fertilization rates were estimated using Proc GLM (SAS Institute, 2003).

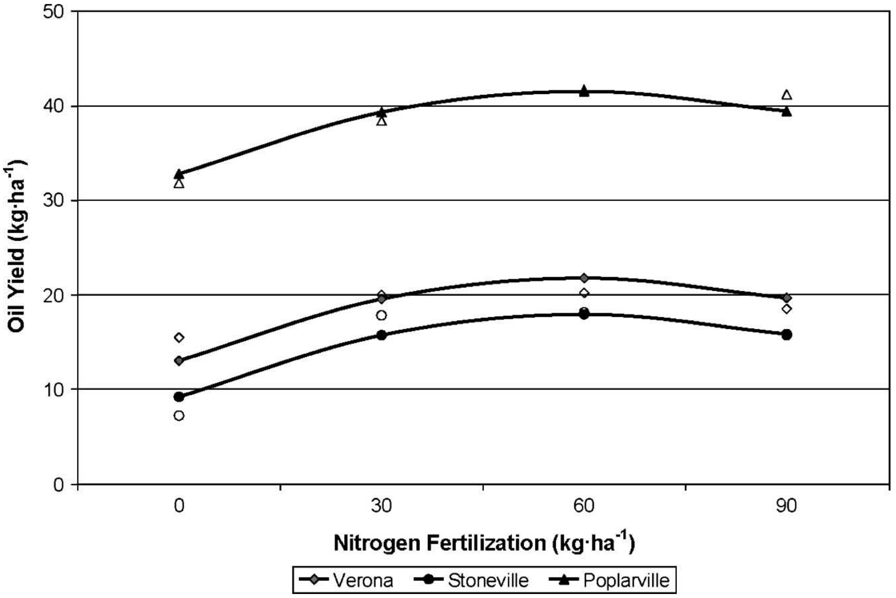

Fig. 4. Oil production as a function of nitrogen fertilization and location (fitted curves have $R^2 = 0.54$).

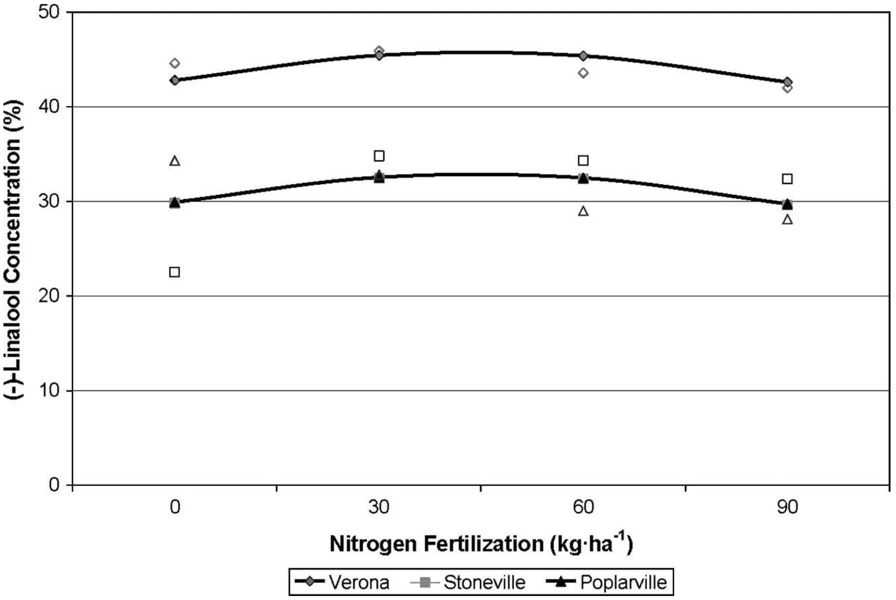

Fig. 5. Concentrations of (–)-linalool as a function of nitrogen fertilization and location (fitted curves have $R^2 = 0.52$ and lines for Stoneville and Poplarville appear as one line).

Results

Dry herbage yields. Location, N rate, and their interaction were significant on basil dry herbage yields (DHY) (Table 4). Mean yields were 4967, 2907, and 2122 kg·ha^{-1} for Poplarville, Verona, and Stoneville, respectively, and were all significantly different from each other. Herbage yields at Poplarville were approximately twice the yields at the other two locations, indicating that location (environment) could be a major modifier of basil herbage yields. Nitrogen application rates greatly affected herbage yields (Fig. 2). In general, N application rates of up to 60 kg·ha^{-1} increased yields at each location. Further increases of N rates did not bring a corresponding significant yield increase. Sulfur application rates had no effect on herbage yields.

Oil content and yields. Oil content (as percent in dry herbage) was affected by location (Table 4) with means of 0.69%, 0.80%, and 0.64% for Stoneville, Poplarville, and Verona, respectively. These three means were significantly different from each other. Analysis of variance found an S fertilization by location interaction effect on basil oil content (Table 4). Regression on S was statistically significant but biologically insignificant with $R^2 = 0.09$. Thus, the oil content was affected by unknown location effects but was little affected by the fertilization. Overall, basil essential oil content at all locations varied from 0.39% to 0.97%, a typical range for oil content in sweet basil (Topalov, 1962; Zheljazkov et al., 2008a).

Basil essential oil yield was a function of herbage yields and essential oil content and was calculated as the weight (g) of oil per weight (g) of dried herbage for each treatment. Oil yields ranged from 5.5 to 56 kg·ha^{-1}. Location, N fertilization, and S fertilization had significant effects on basil essential oil yields (Table 4). For the reason that the oil content was little affected by N fertilization but herbage yields (DHY) were, the oil yields showed similar (to herbage yield) responses to N fertilization. The average oil yields (means) for Stoneville, Poplarville, and Verona were 14.7, 38.7, and 18.5 kg·ha^{-1}, respectively, and were different among locations. The high oil yields at Poplarville resulted from the overall greater average herbage yields and greater oil content relative to other locations. Sulfur fertilization had a significant effect on oil yields ($R^2 = 0.62$) with yields increasing after increased S application rates (Fig. 3). Overall, essential oil yields were maximized at an intermediate level of N application rates (at ≈50 to 60 kg·ha^{-1}) (Fig. 4), whereas maximum response to S fertilization appeared to be beyond 80 kg·ha^{-1} S (Fig. 3).

Concentrations of (–)-linalool, eugenol, (–)-bornyl acetate, and eucalyptol. The concentration of (–)-linalool in basil oil ranged from 15% to 50% of the total oil. The means of (–)-linalool concentrations at Poplarville and Stoneville (31.0% and 31.1%), respectively, were almost equal, whereas the mean of (–)-linalool concentration at Verona was significantly greater at 44%. A regression of (–)-linalool concentration on S application rate was not significant. Regression on N application rate was significant but caused little change in (–)-linalool concentrations (Fig. 5), indicating N fertilization was of little biological significance to (–)-linalool concentration of basil essential oil.

In general, eugenol concentration in basil oil ranged from 2.2% to 5.4%, whereas (–)-bornyl acetate concentration ranged from 0.3% to 1.8% of the oil. Only location had a significant effect on eugenol or (–)-bornyl acetate concentrations, suggesting that the concentrations of these constituents might be relatively stable traits and could be influenced by the environment and climate but not by N or S fertilization. All location averages were statistically different (P = 0.05). The means of eugenol concentrations at the three locations were 2.5%, 4.1%, and 3.2%, whereas the means of (–)-bornyl acetate concentrations were 0.7%, 0.5%, and 1.3% for Stoneville, Poplarville, and Verona, respectively. The concentration of eucalyptol in basil oil varied from 2.8% to 9.2% and was significantly affected by location and by N fertilization. Locations were significantly different from each other with mean eucalyptol concentrations of 5.7%, 4.3%, and 7.7% for Stoneville, Poplarville, and Verona, respectively. Regression on N rate was statistically significant but caused a relatively small change in eucalyptol concentration (Fig. 6).

Effect on yields per area basis (kg·ha^{-1}) of the four major basil oil constituents. Location and N application rates had significant effect on the yields (per area basis) of (–)-linalool, eugenol, (–)-bornyl acetate, and eucalyptol (Table 5). Sulfur application rate had an effect on eucalyptol yield, but not on the yield of the other constituents. The relation of eucalyptol yield to S fertilization was quadratic.

Yields of (–)-linalool were significantly affected by location and N fertilization (Table 5) with intermediate N rates (50 to 60 kg·ha^{-1}) maximizing (–)-linalool yields per area basis (Fig. 7). Overall, (–)-linalool yield means were 4.91, 11.57, and 8.23 kg·ha^{-1} for Stoneville, Poplarville, and Verona, respectively. Eugenol yields were much greater at Poplarville than in the other two locations (Fig. 8) with yield means of 0.37, 1.54, and 0.61 kg·ha^{-1} for Stoneville, Poplarville, and Verona, respectively. Intermediate N fertilization rates (50 to 60 kg·ha^{-1}) maximized eugenol yields per area

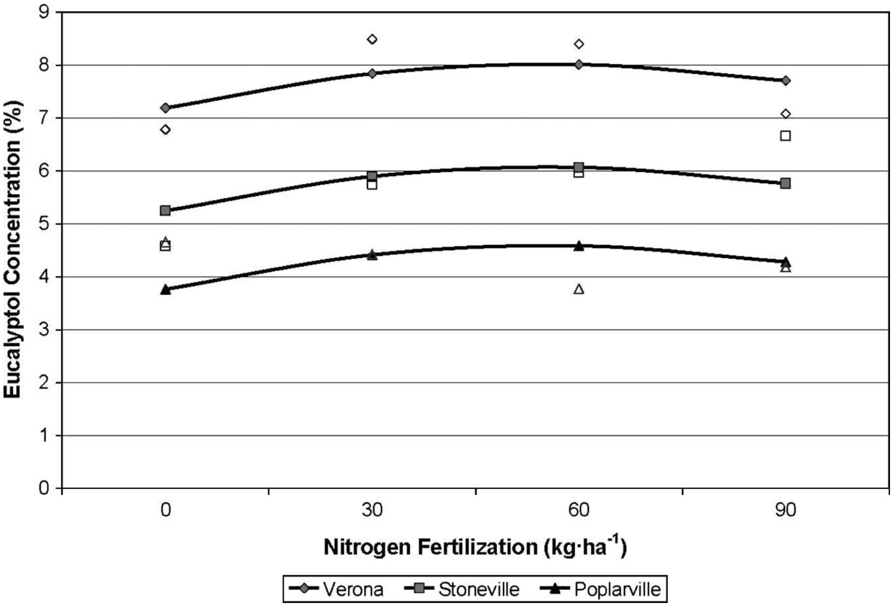

Fig. 6. Concentrations of eucalyptol as affected by location and by nitrogen fertilization (fitted curves have an $R^2 = 0.57$).

Table 5. Tests for significance of linear and nonlinear effects of N fertilization rate and S fertilization rate at three locations and their interactions on yield of the four major constituents in basil essential oil^z.

	--------------Yield of major essential oil constituents in kg·ha^{-1}	-------------		
Effect	(–)-Linalool	Eugenol	(–)-Bornyl acetate	Eucalyptol
Location (loc)	**<0.001**	**<0.001**	**<0.001**	**<0.001**
N fertilization (N rate)	**0.03**	**0.02**	0.06	**0.02**
S fertilization (S rate)	0.17	0.37	0.94	0.33
N rate*loc	0.30	0.56	0.06	**<0.001**
S rate*loc	0.17	0.40	0.45	0.70
N rate*N rate	**<0.001**	**0.007**	**0.01**	**0.002**
S rate*S rate	0.56	0.33	0.11	**0.002**
N rate*N rate*loc	0.24	0.66	0.52	0.49
S rate*S rate*loc	0.80	0.63	0.13	0.58
N rate*S rate	0.35	0.37	0.15	0.23

^{z}Significant effects (P < 0.05) are shown in bold.

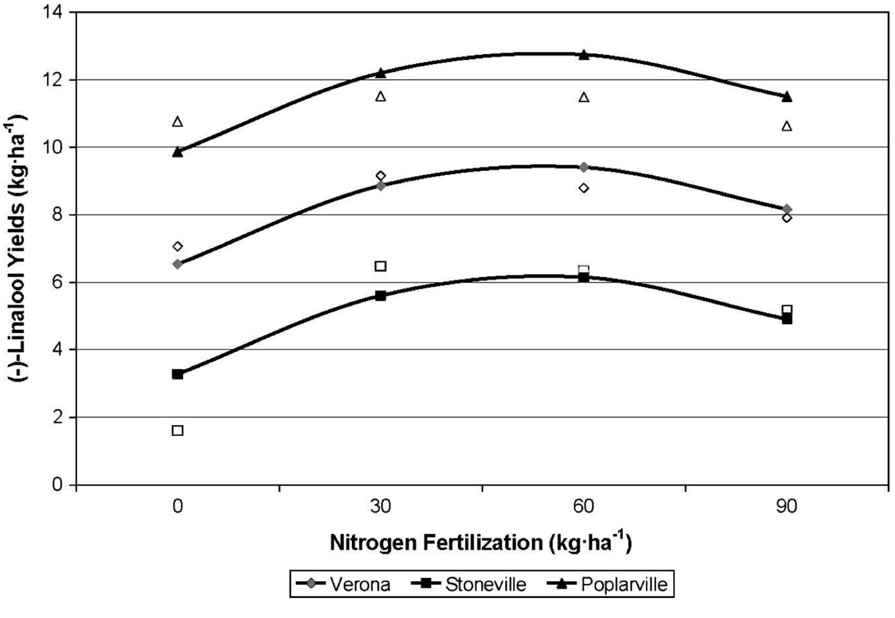

Fig. 7. Yields of (–)-linalool as a function of location and nitrogen fertilization (fitted curves have $R^2 = 0.65$).

basis (Fig. 8). The yields of (–)-bornyl acetate at the three locations were 0.10, 0.18, and 0.23 kg·ha^{-1} for Stoneville, Poplarville, and Verona, respectively. Like with the other constituents, intermediate N application rates near 50 to 60 kg·ha^{-1} resulted in maximum yields of (–)-bornyl acetate per area basis (Fig. 9). Eucalyptol yields were 0.83, 1.60, and 1.46 kg·ha^{-1} for Stoneville, Poplarville, and Verona, respectively. Like with the other two constituents, N application rates at ≈60 kg·ha^{-1} resulted in maximum yields of eucalyptol per area basis (Fig. 10). Although the effect of S rates on eucalyptol was significant, this resulted in relatively small yield change (Fig. 11). Interestingly, the maximum response to S seemed to be beyond the highest S rate of 80 kg·ha^{-1} (Fig. 11).

Correlations between the measured responses. The yields of the four oil constituents [(–)-linalool, eugenol, (–)-bornyl acetate, and eucalyptol] are a function of the percentage oil, oil yield, and herbage yield to the extent that they were calculated from those values. Therefore, those correlations (marked in bold) are expected to be significant (Table 6). There were some significant correlations among the measured responses (Table 6). The concentration of (–)-bornyl acetate in basil oil was negatively correlated to basil herbage yields, which might be an indication that greater amounts of this compound are synthesized and accumulated when the plant growth conditions are less favorable (resulting in lower herbage yields). The concentration of eucalyptol was correlated to (–)-linalool concentration and to oil content, i.e., the greater the oil content or (–)-linalool content, the greater the concentration of eucalyptol. Also, eucalyptol concentration was positively correlated to oil yield, presumably because it was positively correlated to oil content to which oil yields were a function. As expected, oil yields were positively correlated to oil content. The yields (per area basis) of the four major basil oil constituents [(–)-linalool, eugenol, (–)-bornyl acetate, and eucalyptol] were positively correlated to dry herbage yields, to the oil content and oil yields, which should be self-explanatory. (–)-Linalool yields were positively correlated to eucalyptol concentration, hence confirming the positive correlation (indicated previously) between the concentrations of these two constituents. Also, the yields of eugenol, (–)-bornyl acetate, and eucalyptol were positively correlated to (–)-linalool yields. Furthermore, yields of (–)-bornyl acetate and eucalyptol were positively correlated to the eugenol yields, and eucalyptol yields were correlated to the (–)-bornyl acetate yields.

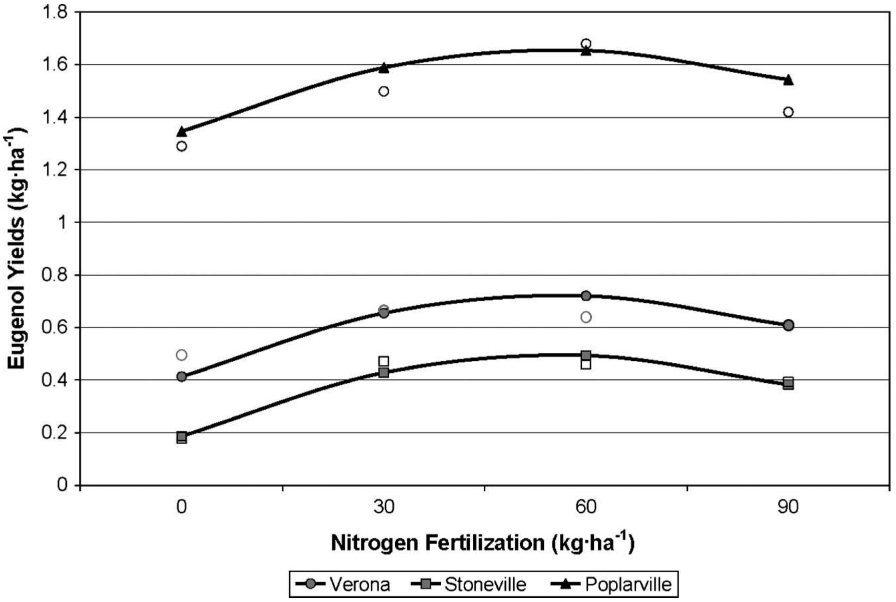

Fig. 8. Yield of eugenol as a function of location and nitrogen fertilization (fitted curves have an $R^2 = 0.43$).

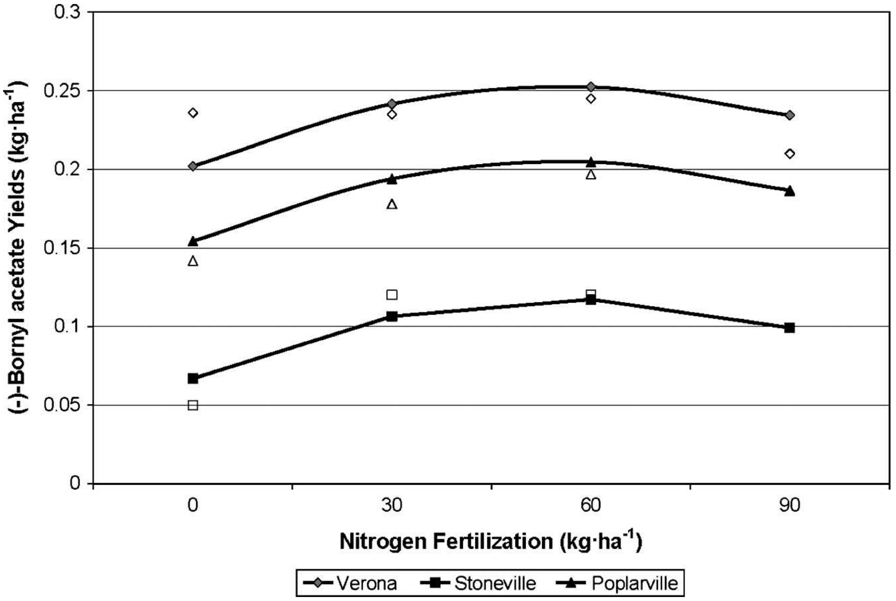

Fig. 9. Yield of (–)-Bornyl acetate as a function of location and nitrogen fertilization. (Fitted curves have $R^2 = 0.43$).

Discussion

Overall, basil herbage yields in this study were relatively high and were similar to sweet basil yields reported in the literature or reported recently for Mississippi (Topalov, 1962; Zheljazkov et al., 2008a, 2008b). Basil essential oil content and yield in this study were within the typical range for basil reported in the literature for other countries (Anwar et al., 2005; Bowes and Zheljazkov, 2004; Juliani and Simon, 2002) and comparable to the results from recent research in Mississippi (Zheljazkov et al., 2008a, 2008b). The concentration of major oil constituents (–)-linalool, eugenol, (–)-bornyl acetate, and eucalyptol were within the range of these constituents in European sweet basil chemotypes (Marotti et al., 1996; Simon et al., 1990; Topalov, 1962; Zheljazkov et al., 2008a).

Although the concentration of the four major oil constituents is important with relation to the oil quality, the actual yield of each constituent may significantly affect the overall economic importance of the basil crops. It may be possible to use N and S fertilization as a tool to target the production of basil essential oil with a specific oil profile, i.e., constituent-specific oil. Some industries require (–)-linalool; others make use of eugenol or eucalyptol or (–)-bornyl acetate. For example, (–)-linalool (produced by fractional distillation of basil oil) is widely used as an aromatic agent in soaps, shampoos, detergents, as a biochemical pesticide, and even for the production of vitamin E. Furthermore, (–)-linalool can be used in the production of linalyl esters with different aromas. Hence, different industries may require special profiles of sweet basil oil, which could render premium prices for primary producers.

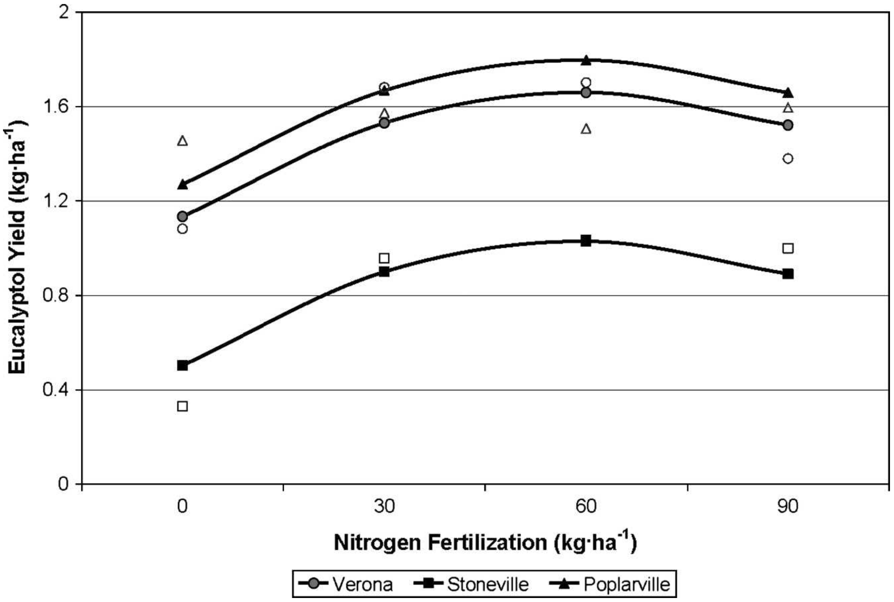

Fig. 10. Yield of eucalyptol as a function of location and nitrogen fertilization (fitted curves have and $R^2 = 0.39$).

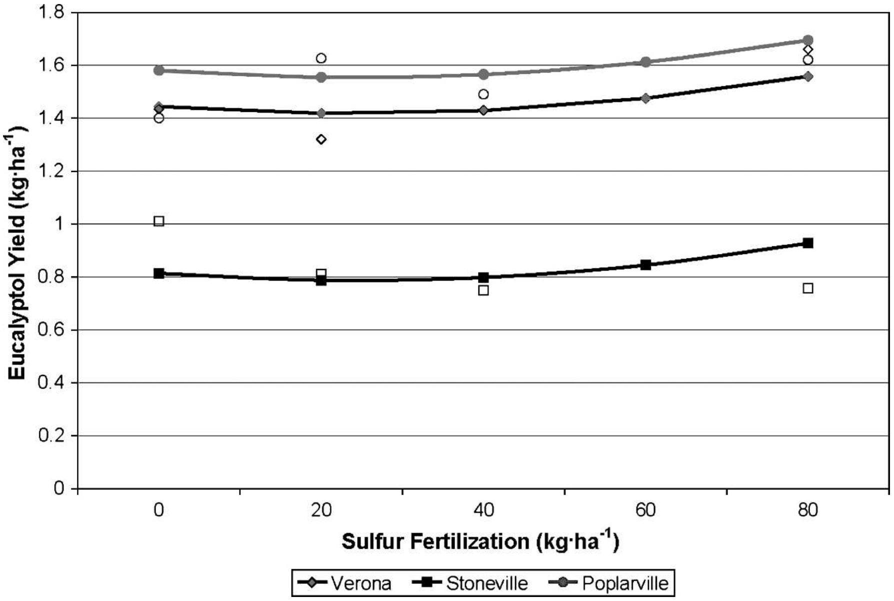

Fig. 11. Yield of eucalyptol as a function of sulfur fertilization and location (fitted curves have an $R^2 = 0.30$).

This is the first report to find such a strong response of basil essential oil yields to S fertilization, confirming part of our hypothesis for a possible significant effect of S fertilization on basil essential oil yields. In addition, to our knowledge, this is the first study to quantify (in real concentration) the response of the major sweet basil oil constituents (–)-linalool, eugenol, (–)-bornyl acetate, and eucalyptol as a function of N and S application rates in a variety of environments. In most cases, N rate, S rate, or N by S interaction resulted in altered oil composition of sweet basil but not in oil content. These results suggest that sweet basil reacts differently to the environmental conditions in different parts of Mississippi with respect to productivity and oil composition in general. In general, this and previous studies (Zheljazkov et al., 2008a, 2008b) demonstrated sweet basil could be grown as a viable essential oil crop in Mississippi and possibly other areas or regions in the southeastern United States.

This study partially confirmed the hypothesis that N and S rates, and probably their interaction, would have a significant effect on sweet basil productivity, essential oil content, and composition. However, the assumption that S addition may improve N use efficiency, which may have a direct effect on herbage yields, was not supported and this part of the hypothesis should be rejected. The lack of a strong yield response to N may be the result of: 1) relatively late transplanting time and the relatively short vegetation period of sweet basil in Mississippi; 2) relatively high fertility soils; 3) frequent heavy rainfalls and high temperatures; and 4) relatively shallow roots system of this plant. The relatively short period from transplanting to harvest of basil may not be sufficient for the complete N or S utilization from the applied fertilizer, especially for S, which is less soluble compared with most N sources. Previous research has demonstrated that sweet basil may require relatively low N application rates, as low as even 5 kg·ha^{-1} N, to provide good yields (Arabaci and Bayram, 2004). However, other studies found increased yield response with increasing N application rates of up to 80 kg·ha^{-1} N (Singh et al., 2004a), 80 to 120 kg·ha^{-1} N (Yassen et al., 2003), 200 kg·ha^{-1} N, or even up to 300 kg·ha^{-1} N (Sifola and Barbieri, 2006). These differences in sweet basil response to N application rates could be the result of differences in soil organic matter, soil organic N, and its mineralization rates among various soils. Hence, this and previous studies suggested that sweet basil yield response to N application rates depends largely on environmental and management conditions. Basil is a frost-sensitive species and is transplanted relatively late, when larger pools of inorganic N would be readily available as a result of mineralization of organic residues. On the other hand, with the extreme rain events that occur with some regularity in Mississippi, water may transport necessary nutrients beyond reach of the basil roots and may contribute to denitrification nullifying a potential yield response to N application rates.

Conclusions

From this work at three locations it has been demonstrated that location, N, and S had significant effects on sweet basil oil yields. Basil essential oil yields were maximized at N fertilization of ≈50 to 60 kg·ha^{-1} N, whereas maximum response to S fertilization appeared to be beyond 80 kg·ha^{-1} S. Location and N had significant effects on the yields of the major basil oil constituents (–)-linalool, eugenol, (–)-bornyl acetate, and eucalyptol, whereas S had a significant effect on eucalyptol yield only. We found that eucalyptol concentration was positively correlated to the concentration of (–)-bornyl acetate. This is the first study to quantify the response of the major sweet basil oil constituents (–)-linalool, eugenol, (–)-bornyl acetate, and eucalyptol as a function of N and S application rates. In addition, it is the first study to demonstrate a strong response of basil oil yield to S. Further research with higher S application rates and intermediate N background may be needed to estimate the optimal sweet basil yield and compositional response to N and S. In addition, further research is needed to explain the strong effect of location on basil productivity.

Table 6. Correlations and their *P* value statistical significances for all measurements.

	Oil (%)	Oil Y^z	Linalool (%)	Eugenol (%)	Bornyl (ac.%)	Eucalyptol (%)	Linalool Y^z	Eugenol Y	Bornyl ac.Y	Eucalyptol Y
Dry weight	0.213	0.854	0.085	–0.237	–0.547	0.328	0.728	0.653	0.556	0.755
P values	0.43	<0.0001	0.75	0.38	0.028	0.21	0.001	0.006	0.025	0.0007
Oil %		0.621	0.422	0.267	–0.283	0.534	0.705	0.691	0.581	0.668
P values		0.010	0.10	0.32	0.29	0.03	0.002	0.003	0.018	0.0047
Oil yield			0.242	0.001	–0.495	0.535	0.934	0.896	0.798	0.948
P values			0.37	0.99	0.051	0.03	<0.0001	<0.0001	0.0002	<0.0001
Linalool %				0.286	0.061	0.526	0.451	0.268	0.218	0.253
P values				0.28	0.82	0.036	0.08	0.31	0.42	0.34
Eugenol %					0.370	0.189	0.030	0.394	0.241	–0.019
P values					0.16	0.48	0.91	0.13	0.37	0.94
Bornyl acetate%						0.029	–0.487	–0.352	–0.064	–0.387
P values						0.92	0.055	0.18	0.81	0.14
Eucalyptol %							0.508	0.521	0.424	0.679
P values							0.04	0.04	0.10	0.003
Linalool yield								0.861	0.814	0.912
P values								<0.0001	0.0001	<0.0001
Eugenol yield									0.814	0.840
P values									0.0001	<0.0001
Bornyl acetate yield										0.829
P values										<0.0001

^{z}Y = yield per area basis.

^{y}Percent of the compound in the total essential oil.

Literature Cited

Anwar, M., D.D. Patra, S. Chand, A. Kumar, A.A. Naqvi, and S.P.S. Khanuja. 2005. Effect of organic manures and inorganic fertilizer on growth, herb and oil yield, nutrient accumulation, and oil quality of French basil. Commun. Soil Sci. Plant Anal. 36:1737–1746.

Arabaci, O. and E. Bayram. 2004. The effect of nitrogen fertilization and different plant densities on some agronomic and technologic characteristic of *Ocimum basilicum* L. (basil). J. Agron. 3:255–262.

Bowes, K. and V. Zheljazkov. 2004. *Ocimum sanctum* L. and *Ocimum basilicum* L. grown in Nova Scotia, Canada show potential as essential oil crops. J. Amer. Soc. Hort. Sci. 129:789–794.

Cox, M.S. 2001. The Lancaster soil test method as an alternative to the Mehlich 3 soil test method. Soil Sci. 166:484–489.

Furnis, B.S., A.J. Hannaford, P.W.G. Smith, and A.R. Tatchell. 1989. Vogel's textbook of practical chemistry. 5th Ed. Longman Scientific & Technical, New York, NY. p. 171–175.

Golcz, A., B. Politycka, and B. Seidler-Lozykowska. 2006. The effect of nitrogen fertilization and stage of plant development on the mass and quality of sweet basil leaves (*Ocimum basilicum* L.). Herba Polonica. 52:22–30.

Hedge, D.M. and S.N.S. Babu. 2007. Correcting sulfur deficiencies in soils and crops. Ind. J. Fertilizers. 3:65–79.

Hitsuda, K., M. Yamada, and D. Klepker. 2005. Sulfur requirement of eight crops at early stages of growth. Agron. J. 97:155–159.

Javanmardi, J., A. Khalighi, A. Kashi, H.P. Bais, and J.M. Vivanco. 2002. Chemical characterization of basil (*Ocimum basilicum* L.) found in 'Local' accessions and used in traditional medicines in Iran. J. Agr. Food Chem. 50:5878–5883.

Juliani, H.R. and J.E. Simon. 2002. Antioxidant activity of basil, p. 575–579. In: Janick, J. and A. Whipkey (eds.). Trends in new crops and new uses. ASHS Press, Alexandria, VA.

Kovats, E. 1965. Gas chromatographic characterization of organic substances in the retention index system. In: Giddings, J.C. and R.A. Keller (eds.). Advances in chromatography. Vol. I. Marcel Dekker, Inc., New York, NY. p. 229–247.

Kruger, H., S.B. Wetzel, and B. Zeiger. 2002. The chemical variability of *Ocimum* species. J. Herbs Spices Med. Plants 9:335–344.

MacGrath, S.P., F.J. Zhao, and P.J.A. Withers. 1996. Development of sulphur deficiency in crops and its treatment. Proc. Fertilizer Soc. No. 379. The Fertilizer Society, London, UK.

Marotti, M., R. Piccaglia, and E. Giovanelli. 1996. Differences in essential oil composition of basil (*Ocimum basilicum* L.) Italian cultivars related to morphological characteristics. J. Agr. Food Chem. 44:3926–3929.

Marschner, H. 1999. Mineral nutrition of higher plants. Academic Press, London, UK.

Millins, G.L. and C.C. Mitchell, Jr. 1989. Wheat varietal response to sulfur. J. Plant Nutr. 12:909–922.

Morris, R.J. 1994. Sulfur: Deficiencies spawn demand. Farm Chem. Int. 8:20–21.

Pavlista, A.D. 2005. Early-season applications of sulfur fertilizers increase potato yield and reduce tuber defects. Agron. J. 97:599–603.

Rehm, G.W. 2005. Sulfur management for corn growth with conservation tillage. Soil Sci. Soc. Amer. J. 69:709–717.

Riley, N.G., F.J. Zhao, and S.P. McGrath. 2002. Leaching losses of sulphur from different forms of sulfphur fertilizers: A field lysimeter study. Soil Use Manage. 18:120–126.

SAS Institute. 2003. SAS for Windows, v 9.1.3. SAS Inst., Cary, NC.

Sifola, M.I. and G. Barbieri. 2006. Growth, yield and essential oil content of three cultivars of basil grown under different levels of nitrogen in the field. Sci. Hort. 108:408–413.

Simon, J.E., M.R. Morales, W.B. Phippen, R.F. Vieira, and Z. Hao. 1999. Basil: A source of aroma compounds and popular culinary and ornamental herb, p. 499–505. In: Janick, J. and J.E. Simon (eds.). Perspectives on new crops and new uses. ASHS Press, Alexandria, VA.

Simon, J.E., J. Quinn, and R.G. Murray. 1990. Basil: A source of essential oils, p. 484–489. In: Janick, J. and J.E. Simon (eds.). Advances in new crops. Timber Press, Portland, OR.

Singh, K., P.P. Singh, S.U. Beg, D. Kumar, and D.D. Patra. 2004a. Effect of harvesting time and nitrogen application on essential oil yield and quality of a methyl chavicol rich cultivar of Indian basil (*Ocimum basilicum* L.). J. Medic. Arom. Plant Sci. 26:293–296.

Singh, K., P.P. Singh, S.U. Beg, D. Kumar, and D.D. Patra. 2004b. Effect of NPK fertilizers on growth, oil yield and quality of French basil (*Ocimum basilicum* L.). J. Spices Arom. Crops. 13:52–54.

Srinivasarao, C., S.P. Wani, K.L. Sahrawat, T.J. Rego, and G. Pardhasarandhi. 2008. Zinc, boron, and sulfur deficiencies are holding back the potential of rainfed crops in semi-arid India: Experiences from participatory watershed management. Int. J. Plant Prod. 2:89–99.

Statistics Canada. 2003. Agriculture Census 2003: Canadian agriculture at a glance. Feb. 2006. <http://www.statcan.ca/english/Pgdb/agricul.htm>.

Stewart, B.A. and L.K. Porter. 1969. Nitrogen – sulfur relationships in wheat (*Triticum aestivum* L.) and corn (*Zea mays*) and beans, (*Phaseolus vulgaris*). Agron. J. 61:267–271.

Topalov, V.D. 1962. Essential oil and medicinal plants. Hr. G. Danov Press, Plovdiv, Bulgaria.

USEPA. 2006. The acid rain challenge: Achieving progress through committed actions. SO2 emission reduction requirements. Sept. 2006. <http://www.epa.gov/aimarkets/usca/brochure/acid_chart1htm>.

Weil, R.R. and S.K. Mughogho. 2000. Sulfur nutrition of maize in four regions of Malawi. Agron. J. 92:649–656.

Yassen, M., P. Ram, A. Yadav, and K. Singh. 2003. Response of Indian basil (*Ocimum basilicum*) to irrigation and nitrogen schedule in Central Uttar Pradesh. Annals Plant Phys. 17:177–181.

Zheljazkov, V.D. 1998. Basil, p. 317–320. In: Phehlivanor, M., G. Moskov, B. Jankov, J. Terziev, V. Zheljazkov, and H. Yantcheva (eds.). Plant production edition 1. Academic edition of Higher Institute of Agriculture, Plovdiv, Bulgaria.

Zheljazkov, V.D., A. Callahan, and C.L. Cantrell. 2008a. Yield and oil composition of thirty-eight basil (*Ocimum basilicum* L.) accessions grown in Mississippi. J. Agr. Food Chem. 56:241–245.

Zheljazkov, V.D., C.L. Cantrell, B. Tekwani, and S. Khan. 2008b. Content, composition, and bioactivity of the essential oil of three basil genotypes as a function of harvesting. J. Agr. Food Chem. 56:380–385.