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EFFECT OF PLANTING DATE, NITROGEN RATE, AND HYBRID ON SUNFLOWER

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□ Sunflower is a potential crop for the southeastern United States for production of cooking oil or biodiesel. In 2007, we evaluated the effect of planting date (PD, 20 April, 20 May, and 20 June), nitrogen (N) application rate (0, 67, 134, and 202 kg ha⁻¹), and hybrid ('DKF3875', 'DKF2990', 'DKF3510', and 'DKF3901') on sunflower productivity and oil profile in four Mississippi locations, Newton, Starkville, and two sites in Verona. There was a trend of increased oleic acid concentration with earlier planting dates, especially in hybrids with lower oleic acid concentration. Earlier planting dates of 'DKF3901' and 'DKF2990' (the hybrid with the lowest oleic acid) actually had 200 to 300 g kg⁻¹ higher concentration of oleic acid when grown in Mississippi vs. the original seed of the same hybrids used for planting and produced at a more northern latitude. This and a recent study in Mississippi suggest that modern hybrids could provide ample yields even when the N fertilization is relatively low.

Keywords: fatty acid composition, oil content, oil yield, biodiesel

INTRODUCTION

The major sunflower (*Helianthus annuus* L.) production areas in North America include North Dakota, South Dakota, Colorado, Kansas, Minnesota, Nebraska, and Texas (Johnston et al., 2002; National Agricultural Statistics

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Service, 2006) in the United States, and Manitoba and Saskatchewan in Canada (Angadi and Entz, 2002; Johnston et al., 2002). The fatty acid composition of sunflower oil is genotype dependent (Miller and Vick, 1999; Burton et al., 2004; Izquierdo and Aguirrezábal, 2008); however, it can also be significantly altered by the environment (Robertson et al., 1978; Lajara et al., 1990; Sobrino et al., 2003; Izquierdo and Aguirrezábal, 2008; Zheljazkov et al., 2008). As with other vegetable oils, the fatty acid profile of sunflower oil determines its nutritional properties and specific uses (Warner et al., 2003; Burton et al., 2004). Consumers prefer sunflower hybrids with mid-oleic or high-oleic acid content because of the health benefits associated with oleic acid (Jing et al., 1997; Krajcovicova-Kudlackova et al., 1997; Hu et al., 2001). There is limited amount of information on sunflower as a potential crop for the southeastern United States. The objective of this study was to evaluate the effects of hybrid, planting date, and nitrogen (N) rate and the interactions of these factors on sunflower yield and seed oil oleic acid concentration under the hot humid environment of the southeastern United States. This work extends previous research on the effect of hybrid and N rate on sunflower yields, oil content, and fatty acid profile (Zheljazkov et al., 2008).

MATERIALS AND METHODS

Field Experiments

A field experiment was conducted during the 2007 cropping season at four locations in Mississippi: Newton (33.43° N lat), Starkville (33.47° N lat), and two sites in Verona (34.16° N lat). Treatments included planting date (20 April, 20 May, and 20 June), hybrid ('DKF3875', 'DKF2990', 'DKF3510', and 'DKF3901'), and N rate (0, 67, 134, and 202 kg ha⁻¹). The certified sunflower seed of the four hybrids were obtained from Monsanto Co. (St. Louis, MO, USA) and produced in Woodland, CA (38.41° N lat.). 'DKF3875' is a traditional type; 'DKF2990' is a traditional type and downy mildew resistant; 'DKF3510' is a mid-oleic acid type (NuSun) and downy mildew resistant; and 'DKF3901' is a traditional type and downy mildew resistant. The seed of the original four hybrids (used for planting) had different fatty acid concentrations (Table 1). Based on oleic acid concentration, 'DKF3510' is a mid-oleic sunflower, 'DKF3875' and 'DKF2990' are the traditional class of sunflower with high polyunsaturated fatty acid (linoleic), whereas 'DKF3901' is a traditional acid sunflower, intermediate in both oleic and linoleic acid compared to the high and low values of other hybrids (Canadian Food Inspection Agency, 2007; Codex Alimentarius Committee, 2005).

The four locations for the experiment were chosen to represent growing areas from south to north Mississippi and different soil types. The soil at Newton was a Prentiss fine sandy loam (coarse-loamy, siliceous,

TABLE 1 Mean fatty acid concentrations of the original certified seed of the four sunflower cultivars. The analysis of variance and subsequent results in the following tables are based on the response measurements minus these values to correct for the differences among the cultivars

Hybrid	Palmitic	Stearic	Oleic	Linoleic	Arachidic	Gondoic	Behenic	Lignoceric
-----g kg ⁻¹ -----								
'DKF2990'	67.8	53.9	262.0	561.0	3.1	1.7	7.0	1.8
'DKF3510'	41.4	43.1	862.0	31.0	4.0	2.4	10.5	3.6
'DKF3875'	65.3	40.3	402.0	459.0	3.3	1.4	8.3	2.6
'DKF3901'	62.0	64.6	328.0	509.0	4.7	1.3	10.3	2.5

semiactive, thermic, Glossic Fragiudult) with pH 6.9, the soil in Starkville was a Marietta fine sandy loam (find-loamy, siliceous, active, thermic Fluvaquentic Eutrudept) with pH 5.2, the soil at the Verona 1 location was a Catalpa silty loam (fine, montmorrillonite, thermic, Fluvaquentic Halludoll) with pH 7.1, and the soil at Verona 2 location was a Quitman sandy loam (fine-loamy, siliceous, semiactive, thermic, Aquic Paleudult) with pH 5.9.

Soil Nutrient Analyses

Prior to planting, representative soil samples were taken at the four experimental sites, air-dried, ground to pass through a 1-mm sieve, and extracted using the Lancaster soil test method (Cox, 2001). The concentration of phytoavailable nutrients was determined by an inductively coupled argon plasma spectrometer (Perkin Elmer, Norwalk, CT, USA). Following the soil analysis, phosphorus (P) and potassium (K) fertilizers were broadcast and incorporated at each location. Nitrogen (as urea-ammonium nitrate solution 32% N) was knifed in on either side of the row (20–25 cm from row centers) and applied in 67 kg ha⁻¹ increments: the first 67 kg ha⁻¹ increment was applied at planting, the side dressing was applied a month later at the V-4 growth stage, and the last one at V-6 (Schneider and Miller, 1981).

Plant Growth Conditions

Land preparation at the four locations was conducted following the procedure for row crops soil preparation in the region: disking and formation of raised beds at 97 to 102 cm center to center at the beginning of April. The herbicide Treflan [trifluralin; 2,6-dinitro-*N,N*-dipropyl-4-(trifluoromethyl) benzenamine] at 4.5 to 5.6 kg ha⁻¹ (preplant incorporated, right after the formation of raised beds) was applied to improve weed control at the four locations. Individual experimental units (plots) were 6 m by 4 m. Sunflower seed was planted at the four locations using a cone planter at 3.8-cm depth, at 97- to 102-cm interrow space, and a seeding rate of 6.4 seeds m⁻¹ of linear row. Different seed sizes of the four hybrids (and different 1000-seed

weight) resulted in different seeding rates (by weight) for the hybrids, but seeding rate was the same for a particular hybrid at every location. Seeding rates were 3.47, 5.12, 4.37, and 4.13 kg ha⁻¹ for the hybrids 'DKF2990', 'DKF3510', 'DKF3875', and 'DKF3901', respectively.

When all plants in a given treatment reached maturity (after physiological stage R-9) (Schneider and Miller, 1981), the two inner rows in each plot were harvested. Sunflower seed was threshed on a stationary thresher (Almaco, LPR-E, Nevada, IA, USA), and seed moisture was determined by a stationary electronic grain-moisture tester (Model GAC2000, Dickey-John, Auburn, IL, USA) in a 500-g sample from each plot. Sunflower seed subsamples (100 g from each plot) were further cleaned by hand to remove any broken seed or impurities, placed overnight in a freezer at -20°C to prevent disease development and eliminate living insects, and then stored in a refrigerator at 4°C awaiting further analysis.

Analysis of Sunflower Fatty Acid Composition and Seed Oil Content

The sunflower subsamples from every plot in a location (cleaned as described above) were ground in a coffee grinder and sub-subsamples were extracted and analyzed on a Hewlett-Packard Model (Palo Alto, CA, USA) 5890 gas chromatograph (GC), fitted with a DB-23 capillary column (30 m by 0.25 mm; J&W Scientific, Folsom, CA, USA) as described previously (Zheljazkov et al., 2008). Seed oil content was estimated on a 40-mL sample from each plot of cleaned, weighed seed using a Maran Ultra Resonance nuclear magnetic resonance (NMR) instrument (Resonance Instruments Ltd., Witney, UK), following the American Oil Chemists Society Official Methods and Recommended Practices, AK4-95 (American Oil Chemists Society, 1994). At harvest, seeds from different treatments had various moisture contents. To make the results comparable, oil contents were adjusted to 100 g kg⁻¹ moisture content.

Statistical Methods

Within each of the four locations (Newton, Starkville, Verona 1, and Verona 2), the experimental field had four blocks; each block was partitioned into three parts, which were randomly assigned to one of the three planting dates (1, 2, and 3). Within each planting date the 16 combinations of hybrid ('DKF2990', 'DKF3510', 'DKF3875', and 'DKF3901') and N rate (0, 67, 134, and 202 kg ha⁻¹) were completely randomized. For each location, this layout made the design a split-plot factorial with planting dates (PDs) as the whole-plot treatments and the factorial of hybrid and N rate as the subplot treatments. The three factors of interest (PD, hybrid, and N rate) were considered as fixed, and block was considered as random. Seed

yield response was collected from all four blocks, but oil yield and oleic acid concentration responses were measured from three blocks. Analysis of variance was completed for each location using the Mixed Procedure of SAS (SAS Institute, Cary, NC, USA), and further multiple means comparison was completed for significant ($P < 0.05$) and marginally significant (P value between 0.05 and 0.1) by comparing the least squares means of the corresponding treatment combinations. Letter groupings were generated using a 1% level of significance for two- or three-factor interaction effects and using a 5% level of significance for main effects. For each response, the validity of model assumptions on the error terms was verified by examining the residuals as described in Montgomery (2005) and appropriate transformations were applied on responses that violated assumptions.

RESULTS AND DISCUSSION

Seed Yields

The ANOVA P values of the main and interaction effects on seed yield (Table 2) indicated that there was a significant interaction effect of planting date and hybrid at the Newton and two Verona locations, whereas the main effect of N rate was significant only at the Verona 1 location. Also, there was a significant interaction effect of hybrid and N rate at the Verona 2 location, while the three-way interaction effect between planting date, hybrid, and N rate was significant at Starkville. At Verona 1, N rates at 67 and 202 kg ha⁻¹ increased seed yields relative to the untreated control. Seed yields at Newton (the southernmost location) were higher in the first planting date, reduced in the second planting date, and lowest at the third planting date (Figure 1). The third planting date at Verona 1 resulted in lower seed yields from ‘DKF3510’ and ‘DKF3875’ relative to the first and second planting date (Figure 1). The second planting date for ‘DKF2990’ was higher than the first or the third. The second planting date for ‘DKF3901’ resulted in

TABLE 2 ANOVA P values for the main and interaction effects of planting date (PD), hybrid, and N rate on seed yield (kg ha⁻¹) at four locations in Mississippi

SV [†]	Newton	Starkville	Verona 1	Verona 2
PD	0.001	0.023	0.002	0.000
Hybrid	0.001	0.001	0.001	0.001
PD × hybrid	<u>0.001</u> ^{††}	0.672	<u>0.001</u>	<u>0.015</u>
N rate	0.311	0.021	<u>0.001</u>	0.594
PD × N rate	0.774	0.101	<u>0.413</u>	0.123
Hybrid × N rate	0.651	0.831	0.183	<u>0.052</u>
PD × hybrid × N rate	0.273	<u>0.098</u>	0.764	0.206

[†]SV – Source of variation.

^{††}Significant effects that needed multiple means comparison are underlined.

FIGURE 1 Interaction plot of seed yield (kg ha^{-1}) versus planting date for the four hybrids at the Newton, Verona 1, and Verona 2 locations. Means sharing the same letter are not significantly different at the 1% level of significance.

higher seed yields than the first but was not different from the third planting date (Figure 1). Overall, in the first and the second planting date 'DKF3510' provided higher yields than 'DKF2990'. The third planting date resulted in lower seed yields at Verona 2 relative to the second planting (Figure 1). The

Panel variable: Planting Date

FIGURE 2 Interaction plot of seed yield (kg ha^{-1}) versus N rate for the four hybrids and two planting dates at the Starkville location. Means sharing the same letter are not significantly different at the 1% level of significance.

second planting at Verona 2 had higher seed yields of ‘DKF3510’, ‘DKF3901’, and ‘DKF2990’ than either the first or the third planting, while seed yields of ‘DKF3875’ were higher from the second planting relative to the third, but not significantly different from the first planting. Generally, ‘DKF3510’ and ‘DKF3901’ at Verona 2 provided higher yields than ‘DKF2990’. With the exception of ‘DKF3510’, seed yields in Starkville from the first planting date were not affected by N rate (Figure 2).

Oil Yields

There was a significant interaction effect of planting date and hybrid at the Newton and two Verona locations, planting date and N rate at the Starkville location, and hybrid and N rate at the Verona 1 location (Table 3). The main effect of hybrid was significant at Starkville, and N rate was significant at Newton. In general, oil yields at Newton (the most southern location) were higher from the first planting date, lower from the second planting, and lowest in the third planting date (Figure 3). However, at the Verona 2 location, oil yields were generally higher from the first and the second planting date and lower from the third planting (Figure 3).

At Starkville, the fertility applications increased oil yields relative to the untreated control from the first planting date (data not shown). However, the fertility treatments had no significant effect on oil yields from the second

TABLE 3 ANOVA *P* values for the main and interaction effects of planting date (PD), hybrid, and N rate on oil yield (kg ha^{-1}) at four locations in Mississippi

SV [†]	Newton	Starkville	Verona 1	Verona 2
PD	0.001	0.004	0.012	0.001
Hybrid	0.001	<u>0.001</u>	0.001	0.001
PD $\times$ hybrid	<u>0.001</u> ^{††}	0.776	<u>0.001</u>	<u>0.079</u>
N rate	<u>0.008</u>	0.107	0.001	0.201
PD $\times$ N rate	0.317	<u>0.023</u>	0.514	0.273
Hybrid $\times$ N rate	0.307	0.944	<u>0.024</u>	0.120
PD $\times$ hybrid $\times$ N rate	0.128	0.731	0.170	0.296

[†]SV – Source of variation.^{††}Significant effects that needed multiple means comparison are underlined.

planting date. Also, at the Verona 1 location, fertility treatments did not generally affect oil yields from the three planting dates. Oil yields from the first and especially from the second planting date at Verona 1 were greater than the oil yields from the third planting date.

Oleic Acid Concentration

There was a significant planting date $\times$ hybrid interaction at the Starkville and Verona 2 locations, and hybrid $\times$ N rate interaction at the Starkville and

Panel variable: Location

FIGURE 3 Interaction plot of oil yield versus planting date for the four hybrids at the Newton and Verona 2 locations. Within each location, means sharing the same letter are not significantly different at the 1% level of significance.

TABLE 4 ANOVA *P* values for the main and interaction effects of planting date (PD), hybrid, and N rate on adjusted oleic FA at four locations in Mississippi

SV [†]	Newton	Starkville	Verona 1	Verona 2
PD	0.001	0.011	0.001	0.001
Hybrid	0.001	0.001	0.001	0.001
PD × hybrid	0.001	<u>0.049^{††}</u>	0.001	<u>0.001</u>
N rate	0.239	0.122	0.001	0.350
PD × N rate	0.906	0.483	0.118	0.940
Hybrid × N rate	0.097	<u>0.068</u>	0.001	<u>0.015</u>
PD × hybrid × N rate	<u>0.043</u>	<u>0.209</u>	<u>0.045</u>	<u>0.953</u>

[†] SV – Source of variation.
^{††} Significant effects that needed multiple means comparison are underlined.

Verona 2 locations. The three-way interaction (planting date × hybrid × N rate) was significant at the Newton and Verona 1 locations (Table 4). Generally, there was a trend for increased oleic acid concentration with earlier planting dates, the trend being more pronounced for hybrids with lower oleic acid concentration in the original seed (Figure 4). Another important observation is that earlier planting dates of ‘DKF3901’ and ‘DKF2990’ (the hybrid with the lowest oleic acid in original seed) actually had 200 to 300 g kg^{−1} higher concentration of oleic acid when grown in Mississippi compared to the original seed, produced in northern California.

Panel variable: N Rate

FIGURE 4 Interaction plot of adjusted oleic (g kg^{−1}) versus planting date for the four hybrids and the four N rates at the Newton location. Means sharing the same letter are not significantly different at the 1% level of significance.

The oleic acid of 'DKF3875' in earlier planting dates tended to be up to 100 g kg^{-1} higher, whereas in later planting dates it was up to 100 g kg^{-1} lower than in the original seed (Figure 4). The oleic acid of 'DKF3510' (the hybrid with mid-oleic acid) tended to be similar to the original seed.

At Starkville, the oleic acid of 'DKF2990' and 'DKF3901' was 200 to 300 g kg^{-1} higher than their respective concentrations in the original seed (Figure 5). At the Verona 2 location, this effect was less pronounced and similar to the results in Newton, where the oleic acid concentration of 'DKF3510' (the highest oleic acid hybrid) did not fluctuate relative to the original seed (Figure 5). The oleic acid concentration of 'DKF3901' at earlier planting date at Starkville was higher than the later planting date (Figure 5). Nitrogen at 67 kg ha^{-1} to 'DKF2990' at Starkville increased oleic acid concentration relative to the 0 or 134 kg ha^{-1} N rates, but was not different from the 202 kg ha^{-1} N rate. Earlier planting of 'DKF2990', 'DKF3901', and 'DKF3875' in the Verona 2 location resulted in a higher concentration of oleic acid than the third planting date (Figure 5). The 134 kg ha^{-1} N rate had higher oleic acid concentration in 'DKF2990' than the 67 kg ha^{-1} N rate, but not different from the 0 or 202 kg ha^{-1} N rates (Figure 5). Generally, in the Newton, Starkville, and Verona 2 locations, the lower oleic acid hybrids ('DKF2990', 'DKF3875', and 'DKF3901') had higher concentrations of oleic acid when grown in Mississippi relative to the respective original seed (Figures 4 and 5).

FIGURE 5 Interaction plot of adjusted oleic (kg ha^{-1}) versus planting date and N rate for the four hybrids at the Starkville and Verona 2 locations. Within each location, means sharing the same letter are not significantly different at the 1% level of significance.

An observation in this study and a previous report (Zheljazkov et al., 2008) was that low and mid-oleic acid sunflower hybrids grown in Mississippi will increase the concentration of oleic acid relative to their respective original seed (used for planting and produced in a more northern latitude, at 38°41' N lat). This increase in the accumulation of oleic acid of traditional sunflower transforms them into mid-oleic acid or NuSun hybrids (National Sunflower Association, 2009). The increase in oleic acid concentration could bring a higher price for producers and an increased marketability for producers and processors. An earlier report demonstrated the increase in oleic acid in sunflower from northern to southern locations (Robertson et al., 1978). Sobrino et al. (2003) found that an important factor for oleic acid accumulation was the temperature during development and maturation of sunflower achenes.

CONCLUSIONS

Overall, this and previous studies in Mississippi (Homenauth et al., 1986; Zheljazkov et al., 2008) demonstrated that sunflower could be a viable crop in the hot humid southeastern United States. However, with the exception of Zheljazkov et al. (2008), previous reports were on old sunflower varieties that are no longer available. Seed and oil yields in this study were similar to previous reports on the same hybrids (National Sunflower Association, 2009; Zheljazkov et al., 2008); however, in this study there was an additional factor, planting date. Seed yields in this study were generally comparable to sunflower seed yields in the northern United States, the traditional sunflower production area (National Sunflower Association, 2009).

Generally, our results suggest that sunflower hybrids in more southern locations should be planted earlier, in April, whereas sunflower hybrids in more northern locations would be better suited for a later planting date in May and would provide high yields. Further delay in planting dates in Mississippi may significantly reduce seed and oil yields. This finding is important with respect to fitting the sunflower in rotations with winter field crops such as winter canola (*Brassica napus* L.), which is harvested in the first half of May.

This study and the previous one in Mississippi (Zheljazkov et al., 2008) suggested that modern sunflower hybrids could provide ample yields even when the N fertilization is relatively low (67 kg ha⁻¹) or none. This finding would come as no surprise in some areas of the world (e.g., Argentina) where sunflower is grown without fertilizers (Mercau et al., 2001; Ruiz and Maddonni, 2006). Sunflower is known to have a well-developed and deeply penetrating root system (Jaafar et al., 1993; Nielsen, 1998; Angadi and Entz, 2002; Stone et al., 2002) with efficient nutrient utilization (Valchovski, 2002). Hence, if sunflower is grown after winter oilseed crops, it could utilize the available residual nutrients efficiently, a clear benefit for the environment

and the producers. The nonlinear pattern observed in the response of seed or oil yields to the N rates suggest that there may be other factors in the environment that were not controlled but may need to be included in future experiments.

The observed increase in oleic acid concentration relative to the original seed is an important finding; oleic acid has apparent nutritional benefits with regard to reduction of coronary heart disease (Jing et al., 1997; Krajcovicova-Kudlackova et al., 1997; Hu et al., 2001). Further, sunflower oils high in oleic acid do not need to be hydrogenated (a process resulting in unhealthy trans fats) and hence, have health advantages over other vegetable oils (canola, soybean). This makes high-oleic acid sunflower oil produced in Mississippi (and presumably in other southern locations) preferable to other vegetable oils.

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