Effects of Manure and Fertilizer Applications on Canola Oil Content and Fatty Acid Composition

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ABSTRACT

Increasing fertilizer costs have resulted in more growers evaluating the use of alternative nutrient sources such as manure. Coincidentally, the questions about fertilizer effects on oil yield and oil fatty acid composition have been a concern. A 2-yr study was conducted to investigate nutrient source (fertilizer urea plus S and manure) and N level (0, 84, and 168 kg N ha^{-1}) effects on canola seed yield, total oil content, and oil composition at East Lansing and Chatham, MI. Results indicated nutrient applications were not necessary to increase canola yield (865–1991 kg ha^{-1}) in fertile fields. However, N fertilizer appeared to reduce total oil content (444–536 mL kg^{-1}), and at similar N levels, total oil content in canola with fertilizer was sometimes lower than that with manure application. Compared with the no nutrient control treatment, fertilizer application sometimes decreased linolenic acid (LN) content, and increased palmitic acid (P) and arachidic acid (A) at Chatham, while it appeared to decrease oleic acid (O) and increase P, linoleic (L) and A at East Lansing. Fertilizer applications often increased canola total saturated fatty acid content (6.80–8.32%) and decreased ratio of O/(L+LN) (2.04–2.52). Manure application had milder effects on oil composition than fertilizer application. Compared with less N (84 kg N ha^{-1}) applications, greater N level (168 kg N ha^{-1}) tended to lower oil quality by increasing total saturated fatty acid content and decreasing the O/(L+LN) ratio.

RAPESEED, TOGETHER WITH SOYBEAN [*Glycine max* (L.) Merr.], palm (*Elaeis guineensis*) oil, peanut (*Arachis hypogaea*), and sunflower (*Helianthus annuus* L.), is an important world vegetable oil source. Rapeseed contains high oil content (about 40%) and the seed meal remaining after extraction can be used as animal feed or as a crop nutrient source when returned to the field. Beginning in the 1960s, double low contents (low erucic acid, low glucosinolates) rapeseed, named "canola", was released in Canada which stimulated rapid growth of rapeseed throughout the world. Canola oil is widely used as cooking oil and salad oil, and can be processed into margarine. Rapeseed oil with high erucic acid content can be used as industrial lubricant oil. Due to the shortage and volatility of the world petroleum supply at the end of the 20th century, high oil content crops have gained substantial attention as important alternative bioenergy resources. In the European Union, 61.7% of canola oil was used to produce biodiesel in 2008 (ISTA, 2008; MVO, 2008).

Fertilizer was widely applied to increase canola seed yield. Canola yield responds to fertilizer application, especially available N (Ramsey and Callinan, 1994; Hocking et al., 1997; Rathke et al., 2005). To achieve maximum seed yield, *rapa* canola needs 106 kg N ha^{-1}, *alba* mustard and *napus* canola need 135 kg N ha^{-1}, and *juncea* canola need 162 kg N ha^{-1} in the northern Great Plains area of Canada (Gan et al., 2007). In Michigan, 140 kg N ha^{-1} is recommended to produce optimum yield (Copeland et al., 2001). However, N application has reportedly decreased canola seed oil content and increased seed protein content (Brennan et al., 2000; Karamanos et al., 2005; Rathke et al., 2005). Sulfur application can increase N use efficiency (Fismes et al., 2000) and has markedly increased canola yield in S-deficient soil (Janzen and Bettany, 1984; Nuttall et al., 1987; Jan et al., 2002). Canola seed oil and protein content have also been shown to increase with S fertilization (Jan et al., 2002; Malhi et al., 2007). Most nutrient management research to date has focused on canola grain yield and oil content response to fertilizer (N and S) application (Ramsey and Callinan, 1994; Brennan et al., 2000; Pennock et al., 2001; Jan et al., 2002; Thavarajah et al., 2003; Karamanos et al., 2005). However, limited studies can be found about oil fatty acid composition, which is directly related to oil quality, including oil stability and temperature tolerance. Except for one study which showed no significant effect on oil fatty acid composition of N fertilizer applications in Egypt (Ibrahim et al., 1989), there is no detailed research about fertilizer on canola oil yield and oil fatty acid composition and their relationships in the United States.

On the other side, overapplication of fertilizer not only increases input costs, but increases the potential for environmental concerns including water pollution and soil

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Abbreviations: A, arachidic acid; CT, plots in Chatham, MI; E, eicosenoic acid; EL, plots in East Lansing, MI; F-1, treatment with fertilizer nitrogen level 84 kg N ha^{-1}; F-2, treatment with fertilizer nitrogen level 168 kg N ha^{-1}; L, linoleic acid; LN, linolenic acid; M-1, treatment with manure nitrogen level 84 kg N ha^{-1}; M-2, treatment with manure nitrogen level 168 kg N ha^{-1}; O, oleic acid; P, palmitic acid; S, stearic acid.

Table 1. Site description including soil properties, plant varieties, planting, and harvest dates in 2007 and 2008.

Site†	Soil description	SOM‡	Sand	Clay	Silt	Canola variety	Planting date	Harvest date
		g kg^{-1}						
EL 2007 42°42′ N, 84°28′ W	Riddles-Hillsdale sandy loam; fine-loamy, mixed, mesic Typic Hapludalfs	13	680	90	217	DKL 38–25 RR	25 Oct. 2006	31 July 2007
EL 2008 42°41′ N, 84°29′ W	Capac loam; fine-loamy, mixed, mesic Aeric Ochraqualfs	40	450	140	370	Cropland Hyclass 910RR	22 Apr. 2008	31 July 2008
CT 2007, 2008 46°41′ N, 84°29′ W	Eben very cobbly sandy loam; sandy-skeletal, mixed, frigid Pachic Hapludolls	30	670	90	210	DKL 34–55	11 May 2007 8 May 2008	7 Aug. 2007 8 Aug. 2007

† CT, Chatham, MI; EL, East Lansing, MI.

‡ SOM = Soil organic matter.

degradation. Manure is a good source of organic nutrients, containing both macro- and micronutrients, and its application can improve soil organic carbon content (Fronning et al., 2008) and further improve soil physical and chemical properties (Eghball, 2002). At the same time, manure application could have some environmental issues, including antibiotics (Gao and Pedersen, 2005, 2009; Gu and Karthikeyan, 2005, 2008), N, P, and salt leaching into surface or groundwater (Jacob, 1995) and the risk of spreading weed seed (Blackshaw and Rode, 1991; Larney and Blackshaw, 2003). The disadvantages of manure application also include variable nutrient content, inconvenient pre-/postmanagement (Jacob, 1995). Limited research is available regarding anaerobically treated manure application on canola yield and oil quality. As the enlarging demand of biodiesel as an alternative biofuel source, balances between nutrient applications and sustainable environment, oil yield and oil quality are urgent issues for farmers and researchers.

Canola is well adopted in the Great Lakes Region. Spring canola can be grown throughout the region and winter canola can grow in southern areas (Copeland et al., 2001). The objectives of this study were to evaluate the effects of manure (anaerobic treated swine or dairy) and fertilizer applications on canola yield, total oil content and oil composition, and to determine the relationships among oil quality traits.

MATERIALS AND METHODS

Site Description and Experimental Design

Field experiments were conducted in 2007 and 2008 on two research farms at Michigan State University (MSU): East Lansing (EL, southern Michigan) and Chatham (CT, northern Michigan). Winter canola was planted at EL (42°42′ N, 84°28′ W) on 25 Oct. 2006; however, the second season crop did not survive the winter in 2007. Instead, an experiment with spring canola was replanted in a nearby field (42°41′ N, 84°29′ W) on 22 Apr. 2008 (Table 1). Two seasons of spring canola were planted at CT (46° 20′ N, 86° 54′ W) on 11 May 2007 and 12 May 2008 (Table 1). The preceding crop was soybean at EL fields and corn (*Zea mays* L.) at CT in both years. The experimental locations, soil types, canola varieties, planting and harvest dates are summarized in Table 1.

Plots were arranged in a randomized complete block design (RCBD). There were three blocks with three treatments in 2007 at EL, treatments including 0 kg N ha^{-1} application (control), 84 kg N ha^{-1} manure application (M-1); 168 kg N ha^{-1} manure application (M-2). The plot size was 4.6 by 15.3 m. There were six blocks with five treatments in 2008 at EL in a RCBD, including a control, M-1, M-2, 84 kg N ha^{-1} urea fertilizer with 22.4 kg S ha^{-1} (F-1) and 168 kg N ha^{-1} urea fertilizer with 22.4 kg S ha^{-1} (F-2). The plot size was 3.7 by 18.3 m. At CT, the experiment with five treatments, including control, M-1, M-2, F-1, and F-2, was arranged in a RCBD with four replications in both 2007 and 2008. The plot size was 15.2 by 12.2 m. In 2008 at CT, plots receiving the F-2 treatments were destroyed by fertilizer application injury, thus only four treatments were used for analysis. In all experiments, the amounts of manure application were based on nutrients analysis conducted in a commercial lab (A & L Great Lake Laboratories, Inc., Fort Wayne, IN) and manure management sheets (Jacobs et al., 1992). The N levels of manure application (first year N availability) matched the N levels of fertilizer applications. Manure was broadcast with a Houle 3150 manure spreader (J. Houle & Fils Inc., Drummondville QC, Canada) and then incorporated in topsoil with a field cultivator. Plots were then rolled with a cultipacker and seeded at 0.19 m row spacing (EL) or 0.18 spacing (CT) with a no-till drill calibrated for 7.3 kg seed ha^{-1}. Fertilizer was broadcast with a LESCO rotary/drop spreader (LESCO Inc., Troy, MI) and then incorporated in topsoil with a field cultivator. Treatment descriptions are provided in Table 2. Total monthly precipitation and average daily temperature at two locations in year 2007 and 2008 and 30-yr average values are reported in Table 3. Soil core samples were randomly collected right after harvesting in each plot at depths of 15 and 30 cm with a soil probe. The soil samples were air-dried and sieved through a 2-mm sieve, then extracted with 1 mol L^{-1} KCl at 1:5 soil/KCl ratio. After filtration, NO_3^-–N and NH_4^+–N were colorimetrically determined in a commercial lab. The results of soil samples are shown in Table 4.

Table 2. Fertilizer and manure treatments to canola at two Michigan locations in 2007 and 2008.

Treatment	Description	Available N applied
		kg N ha^{-1}
Control	no fertilizer	0
F-1	urea (46–0–0) with S (22.4 kg ha^{-1})	84
F-2	urea (46–0–0) with S (22.4 kg ha^{-1})	168
M-1	liquid anaerobic swine manure 46770 L ha^{-1} (EL) † liquid anaerobic dairy manure 56124 L ha^{-1} (CT)	84
M-2	liquid anaerobic swine manure 93540 L ha^{-1} (EL) liquid anaerobic dairy manure 84186 L ha^{-1} (CT)	168

† EL, East Lansing, MI; CT, Chatham, MI.

Table 3. Precipitation and temperature data for two locations in Michigan in 2007 and 2008.

	Chatham						East Lansing					
	Precipitation			Temperature			Precipitation			Temperature		
Month	2007	2008	30-yr†	2007	2008	30-yr†	2007	2008	30-yr†	2007	2008	30-yr†
	mm			°C			mm			°C		
Apr.	163	160	62.5	3.0	3.9	4.1	66.3	43.4	83.0	7.0	9.9	7.6
May	55.1	92.5	80.0	12.3	8.2	11.2	97.0	29.5	69.1	15.6	12.6	14.1
June	52.3	97.8	91.7	17.2	15.4	16.0	89.2	112	81.0	19.8	19.9	19.2
July	44.2	44.7	90.4	19.1	18.4	18.7	12.4	96.3	75.7	20.5	21.5	21.4
Aug.	19.6	27.2	90.2	18.3	17.9	18.1	140	16.5	87.5	21.4	20.6	20.3
Total	334	422	415				405	298	396			
Mean				14.0	12.8	13.6				16.9	16.9	16.5

† Average precipitation (mm) and temperature (°C) of recent 30 yr.

Total Oil Content

Total oil content was measured following the method of Matthäus and Brühl (Matthaus and Brühl, 2001). In this study, 3 g of powdered canola seeds from each plot in 2007 and 2008 was weighed and extracted with an accelerated solvent extractor ASE 200 (Dionex Corporation, Sunnyvale, CA) equipped with 22 mL stainless-steel extractor cells. Beyond canola seed powder, the extra volume of the extractor cell was filled with Diatomaceous Earth (Dionex Corporation, Sunnyvale, CA) to reduce solvent usage. The following conditions (Gao et al., 2009) were set on the ASE system: preheat for 6 min, heat for 6 min, oven temperature at 105°C, static time for 10 min, flush volume 70%, purge time for 60 s, two static cycles, and extraction pressure at 7000 KPa. After processing, the extraction solvent hexane was removed by purging O_2 free compressed N_2 (AGA Gas Inc., Cleveland, OH) above the surface at 50°C. The residue hexane was further removed in an oven at 105°C for about 70 min. The total oil content was calculated using AOCS Official Method AM 2-93 (AOCS, 2000) as follow:

$$C = \frac{\mathrm{O_w}}{\mathrm{W} \times (1 - \mathrm{m\%}) \times 0.91} \qquad [1]$$

where C (mL kg^{-1}) is total oil content based on seed dry mass, $\mathrm{O_w}$ (g) is the weight of total extracted oil, 0.91 (g mL^{-1}) is density of canola oil (Vadke et al., 1988; Lang et al., 1992), W (kg) is the weight of ground sample, and m% is the moisture percentage of the ground sample as measured by a Moisture Analyzer A&D MF-50 (A&D Company Ltd, San Jose, CA). All samples were run in triplicate.

Table 4. Soil NO_3^- and NH_4^+ analysis after harvesting at two locations in Michigan in 2007 and 2008.†

		Chatham		East Lansing	
Year	Treatment‡	NO_3^-–N	NH_4^+–N	NO_3^-–N	NH_4^+–N
		mg kg^{-1}			
2007	Control	3.70ab§	2.78	2.98	3.97
	F-1	3.39b	2.67	–¶	–
	F-2	3.41b	2.52	–	–
	M-1	5.10ab	3.06	2.81	3.78
	M-2	7.25a	2.93	3.43	3.89
2008	Control	13.69	2.24	1.08b	2.15
	F-1	11.50	1.96	1.31ab	2.05
	F-2	–	–	1.59a	2.33
	M-1	14.75	1.92	1.48a	2.23
	M-2	16.78	2.21	1.68a	2.30

† Concentrations of NO_3^-–N and NH_4^+–N were average values at depths of 0 to 15 and 15 to 30 cm.

‡ Control, treatment with zero N application; F-1, treatment with urea fertilizer 84 kg N ha^{-1}; F-2, treatment with urea fertilizer 168 kg N ha^{-1}; M-1, treatment with manure 84 kg ha^{-1}; M-2, treatment with 168 kg N ha^{-1}.

§ Statistics were conducted within year and location. Means followed by the same letter are not statistically different (α = 0.05).

¶ A dash means no data.

Fatty Acid Methyl Ester Reaction

To obtain oil composition information, in situ fatty acid methyl ester (FAME) reaction was conducted and analyzed by gas chromatography (GC). The FAME reactions were conducted in basic conditions following the method of Hammond (1991). Approximately 1 g of canola seed was added to a 50-well aluminum tray and crushed by a Hydraulic Press (P.H.I, Pasadena, CA) at the pressure of 27,560 KPa. Then 1 mL of hexane (GC grade, EMD Chemicals Inc., Gibbstown, NJ) was added to each well. After a minimum 2 h extraction, 100 µL supernatant was transferred into 0.5 mL 1 M sodium methoxide (Sigma-Aldrich Inc., Allentown, PA) solution. The reaction mixture was shaken at 150 rpm at room temperature for half an hour and the reaction was then stopped by adding 2 mL distilled water. When the reaction mixture separated into two clear layers (about half an hour), the upper layer containing the FAME reaction products was pipetted into 2 mL vial and stored at 4°C until analysis, typically in 2 days. The transesterification rate was > 98% and results were not significantly different from those obtained from powdered samples in an acid condition (Liu et al., 1995a; Liu et al., 1995b). All samples were analyzed in triplicate.

Gas Chromatography with Flame Ionization Detector

The FAME products were analyzed using a 6890 Agilent gas chromatography (GC)-Flame Ionization Detector (FID) equipped with a 30 m by 0.53 mm by 0.5 µm Supelcowax 10 capillary column (SUPELCO Inc., Bellefonte, PA) and HP 7673 autosampler (Agilent Technologies, Santa Clara, CA). Gas chromatograph running conditions were described in previous research (Gao et al., 2009). In brief, oven temperature was increased from 195 to 240°C at 4°C min^{-1} and held at 240°C for 2 min; injection volume was 1 µL with split ratio 10:1, inlet temperature was 220°C and detector temperature was 290°C. Ultra high purity (99.999%) helium (AGA Gas Inc., Cleveland, OH) was used as the carrier gas at a flow rate of 3.3 mL min^{-1}. Mixture methyl esters of fatty acids (palmitic acid (P, C16:0), stearic acid (S, C18:0), oleic acid

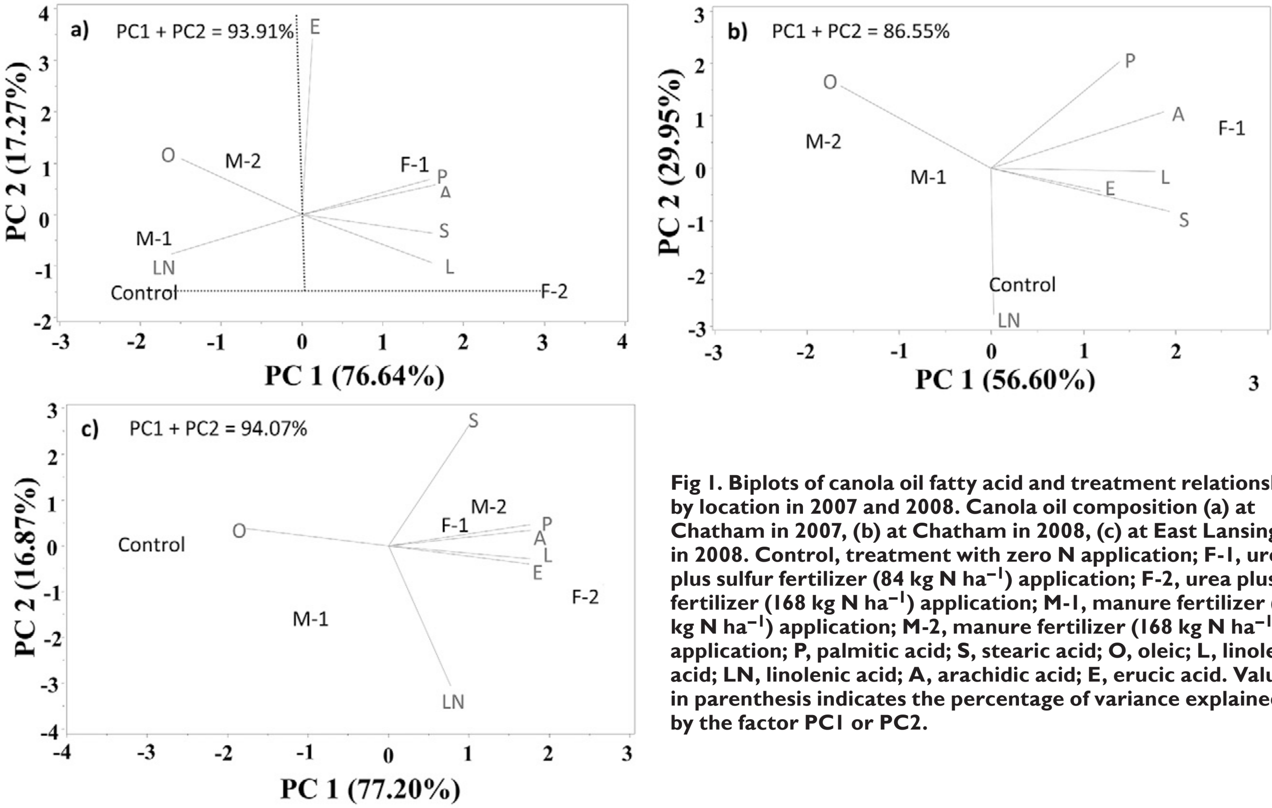

Fig 1. Biplots of canola oil fatty acid and treatment relationships by location in 2007 and 2008. Canola oil composition (a) at Chatham in 2007, (b) at Chatham in 2008, (c) at East Lansing in 2008. Control, treatment with zero N application; F-1, urea plus sulfur fertilizer (84 kg N ha^{-1}) application; F-2, urea plus S fertilizer (168 kg N ha^{-1}) application; M-1, manure fertilizer (84 kg N ha^{-1}) application; M-2, manure fertilizer (168 kg N ha^{-1}) application; P, palmitic acid; S, stearic acid; O, oleic; L, linoleic acid; LN, linolenic acid; A, arachidic acid; E, erucic acid. Value in parenthesis indicates the percentage of variance explained by the factor PC1 or PC2.

(O, C18:1), linoleic acid (L, C18:2), linolenic acid (LN, C18:3), arachidic acid (A, C20:0), and eicosenoic acid (E, C20:1) (Supeclo, Bellefonte, PA) were used to calibrate compound retention time. These seven fatty acids compose more than 98% of canola oil (Codex, 2003) and erucic acid concentration was <0.5% in this study. Oil composition (percent of fatty acid in total canola seed oil) was calculated from the compound peak areas (Liu et al., 1995a, 1995b; Codex, 2003; Latif and Anwar, 2009).

Statistical Analyses

Canola seed yield, total oil content, and fatty acid composition were analyzed separately in each year and each location using a mixed model. This analysis was conducted with PROC MIXED in SAS (SAS 9.1.3, SAS Inst., Cary, NC).

The relationships between fatty acid composition in oil and nutrient application treatments were graphed by a biplot macro using PROC IML (SAS 9.1.3, SAS Inst., Cary, NC) (Friendly, 2008). A biplot is a graphic representation of the information for a data matrix on a plane by means of two sets of points representing rows and columns (Gabriel, 1971, 1978; Yan et al., 2000; Le Roux and Gardner, 2005; Yan and Tinker, 2006). In general, the data matrix can be written as:

$$X_{m\times n} = U_{m\times r}\Delta_{r\times r}V'_{n\times r} = \sum_{i=1}^{r}\lambda_i u_i v'_i \qquad [2]$$

where r is the rank of X, Δ is the diagonal matrix containing singular variables of $\lambda_1 > \lambda_2 > \cdots > \lambda_r$, u_i and v_i is ith column of U and V respectively. A two-dimension approximation for X is

$$X_{m\times n} \approx (u_1, u_2)\begin{pmatrix}\lambda_1 & 0\\ 0 & \lambda_2\end{pmatrix}(v_1, v_2)' \qquad [3]$$

where X is column-center transformed, (v_1, v_2) are loadings of the first two principal components of the variance-covariance matrix while $(\lambda_1 u_1, \lambda_2 u_2)$ are the first two principal component scores accordingly. Depending on partition of the singular values into U and V, there are many possible ways to choose the coordinates of the biplot. In this study, column-metric preserving partition, in which the singular values were entirely partitioned into the V matrix, was selected and the biplot coordinates were each pairs of (u_1, u_2) and $(\lambda_1 v_1, \lambda_2 v_2)$. The goodness of approximated proportion was evaluated by the sum of the two squared singular values divided by the total sum of squared singular values, that is, $(\lambda_1^2 + \lambda_2^2)/\sum_{i=1}^{r}\lambda_i^2$. In this study, the column variables of the data matrix were fatty acid components and the row variables were nutrient application treatments. Analyses were conducted for each location and each year separately. Details about singular value decomposition, biplot creation, and interpretation of different kinds of biplots were described by Yan and Tinker (2006). In brief, the angle between variables represented their correlations (Gabriel, 1971; Yan et al., 2000). If the angle of two fatty acids was acute, they were positively related; if the angle was obtuse, they were negatively related; if the angle was a right angle, there was no relationship between them. To compare treatment effects, a line could be drawn between two treatments and a perpendicular line from the biplot origin could be drawn. If a fatty acid and one treatment were at the same side of the perpendicular line, this treatment could increase the fatty acid content when compared to the other one, or vice-versa (Yan et al., 2000). For example, (Fig. 1a), treatment F-2 increased fatty acids E, P, A, S, L contents in oil and decreased fatty acids O and LN contents in oil at CT in 2007 when compared to control treatment.

Table 5. Canola yield and total oil content response to fertilizer and manure treatments for two locations in Michigan in 2007 and 2008.

		Chatham			East Lansing		
Year	Treatment†	Yield	Total oil content‡	Oil yield	Yield§	Total oil content	Oil yield
		kg ha^{-1}	mL kg^{-1}	—— kg ha^{-1}	——	mL kg^{-1}	kg ha^{-1}
2007	Control	865c¶	514a	405c	271b	503a	124b
	F-1	1028bc	462bc	436bc	–#	–	–
	F-2	1119abc	444c	451bc	–	–	–
	M-1	1402a	511a	654a	712a	476b	309a
	M-2	1300ab	495ab	584ab	762a	481ab	335a
2008	Control	1718	536	838ab	1475	501ab	675
	F-1	1695	514	794b	1597	490ab	716
	F-2	–	–	–	1543	454b	633
	M-1	1937	536	948a	1426	511a	664
	M-2	1991	523	944a	1435	479ab	624

† Control, treatment with zero N application; F-1, treatment with urea fertilizer 84 kg N ha^{-1}; F-2, treatment with urea fertilizer 168 kg N ha^{-1}; M-1, treatment with manure 84 kg N ha^{-1}; M-2, treatment with 168 kg N ha^{-1}.

‡ Total oil content (mL kg^{-1}) is based on dry mass.

§ Bird damage reduced yield at East Lansing in 2007.

¶ Statistics were conducted within 1 yr and one location, means were followed by the same letter are not statistically different ($\alpha = 0.05$).

A dash means no data.

RESULTS AND DISCUSSION

Across the 2 yr, CT in 2007 had a higher average temperature (14.0°C) and drier field conditions (334 mm precipitation) than in 2008 (12.8°C, 422 mm), EL in 2007 had the same average temperature (16.9°C) and wetter field conditions (405 mm) than in 2008 (16.9°C and 298 mm). The previous research of Hocking et al. (1997) reported that canola seed oil content decreased 2.7 ± 0.6% (seed weight) per 1°C increasing during seedling fill period in Southern Australia. High temperature (>30°C) during flowering period reduced canola yield sufficiently (Sovero, 1993). The results of research conducted in a dry area showed reduction in canola seed yield due to water deficient during flowering and silique development decreased canola yield and high water deficiency (75% available water depleted) also caused reduction in seed oil content (Sinaki et al., 2007). In our study, the canola yield at CT in 2008 (1695–1991 kg ha^{-1}) was substantially greater than that at EL in 2008 (1426–1597 kg ha^{-1}) and at CT in 2007 (865–1402 kg ha^{-1}). Greater precipitation during canola growing season and lower temperature during the flowering period (Table 3) likely resulted in the higher canola grain yield at CT in 2008 relative to CT in 2007 and EL at 2008 (Sovero, 1993; Hocking et al., 1997; Sinaki et al., 2007).

Table 5 shows the comparisons of yield, oil content, and total oil yield among different treatments in the 2 yr at both locations. Large differences in yield were observed between years at EL. The winter canola planted at EL in 2007 suffered substantial loss due to bird damage because of small plots and proximity to tree. Our yields were 271 to 762 kg ha^{-1}, much less than typical yields of 2240 to 3350 kg ha^{-1} reported for the state (Copeland et al., 2001). There was no research about bird damage effects on canola seed oil content and oil composition. Because bird damage happened in the very late stage when canola seed was ready to harvest, we concluded that there was no influence of bird damage on canola seed quality in this study. The spring canola planted in 2008 at EL yielded 1426 to 1597 kg ha^{-1} which was near the typical range of 1680 to 2790 kg ha^{-1} reported for the state (Copeland et al., 2001). Thus, yield data and oil yield from EL in 2007 did not correctly reflect true canola yield in field, while seed total oil content 476 to 503 mL kg^{-1} (43.3–45.8%) and oil composition were comparable to the reported values.

Canola Yield and Oil Content

In this study, canola yield was not necessarily increased with nutrient applications. At CT in 2007, manure applications increased canola yield (1402 kg ha^{-1} of M-1 and 1300 kg ha^{-1} of M-2) relative to the control (865 kg ha^{-1}); however, there was no difference between synthetic fertilizer and control treatments, and the N levels within the respective manure or fertilizer nutrient source had no effect on canola yield. In 2008, there was no difference in canola yield among five nutrient applications at both CT and EL. Depending on soil analysis after harvest (Table 4), the plots with all applications had similar levels of nitrate and ammonium concentrations in the top 0 to 30 cm soil layer, excluding the plots at CT in 2007 where manure application (M-2) had higher nitrate concentration than fertilizer applications (F-1 and F-2) and EL in 2008 where control application had lower nitrate concentration than F-2, M-1, and M-2. Surface soil nitrate and ammonium analyses showed the available N level in soil after the season and no over nutrient application in this study (Marx et al., 1999). The results of yield, treatments, and soil mineral N levels indicated that nutrients application did not have a linear relationship with canola yield.

In this study, the range of canola total oil content was from 444 to 536 mL kg^{-1} (40.4–48.8%), which is comparable to the reported values 40 to 45% (O'Brien, 2004). Compared to the control treatment with no N input, fertilizer applications F-1 and F-2 at CT in 2007 and the manure application M-1 at EL in 2007 were found to decrease canola total oil content ($P < 0.05$) (Table 5). Canola total oil content with fertilizer application F-2 was less than that with the manure application M-1 at EL in 2008 ($P < 0.05$). Previous researchers have also reported that total oil content decreased with N fertilizer applications (Brennan et al., 2000; Karamanos et al., 2005, 2007; Rathke et al., 2005). On a land area basis, oil yield (yield × total oil content) was increased with manure application treatments at CT in 2007 (Table 5). The canola oil yield with fertilizer application

Table 6. Canola oil fatty acid composition in response to manure and fertilizer treatment for two locations in Michigan in 2007 and 2008.

Site†/year	Treatment‡	Fatty acid §						
		P	S	O	L	LN	A	E
		%¶						
CT/2007	control	4.42d#	2.40bc	65.1ab	19.6b	6.84a	0.64c	0.98
	F-1	4.72b	2.51b	64.8ab	20.1a	6.22bc	0.71ab	1.02
	F-2	4.93a	2.66a	64.6b	20.2a	5.89c	0.74a	0.98
	M-1	4.53bcd	2.32c	65.1ab	19.6b	6.75a	0.65c	1.00
	M-2	4.69bc	2.36c	65.3a	19.6b	6.35b	0.68bc	1.01
CT/2008	control	4.40b	2.10a	62.2	20.1	9.56a	0.63b	0.98
	F-1	4.60a	2.12a	62.3	20.2	9.17b	0.66a	0.99
	M-1	4.49ab	2.05b	62.5	20.1	9.26b	0.63b	0.97
	M-2	4.45b	2.03b	62.8	20.0	9.17b	0.62b	0.98
EL/2007	control	4.59	1.85	61.9	19.8	8.43	0.60b	2.83
	M-1	4.69	1.92	61.5	19.8	8.14	0.67a	3.31
	M-2	4.61	1.88	61.6	19.7	8.16	0.66a	3.44
EL/2008	control	4.26b	1.93	63.2a	21.2d	7.72b	0.61c	1.09c
	F-1	4.43a	1.94	62.1c	22.0b	7.73ab	0.65ab	1.18ab
	F-2	4.47a	1.94	61.5d	22.3a	7.87a	0.66a	1.23a
	M-1	4.31b	1.89	62.6b	21.7c	7.79ab	0.62 bc	1.15b
	M-2	4.45a	1.94	61.9cd	22.1ab	7.71b	0.65ab	1.20ab

† CT, plots in Chatham, MI; EL, plots in East Lansing, MI.

‡ Control, treatment with zero N application; F-1, treatment with fertilizer N level 84 kg N ha^{-1}; F-2, treatment with fertilizer N level 168 kg N ha^{-1}; M-1, treatment with manure N level 84 kg N ha^{-1}; M-2, treatment with manure N level 168 kg N ha^{-1}.

§ P, palmitic acid; S, stearic acid; O, oleic acid; L, linoleic acid; LN, linolenic acid; A, arachidic acid; E, eicosenoic acid. Comparison were conducted within location and year.

¶ %, percent of fatty acid in total oil is based on fatty acid GC peak area in total peak area.

Means followed by the same letter are not statistically different (α = 0.05).

(F-1) was lower than that with manure application (M-1, M-2) at CT in 2008. No difference was found in oil yield among treatments at EL in 2008. Manure application had higher canola oil yield than fertilizer application at CT in both years.

Canola Oil Composition

Oil composition is an important indicator of canola oil quality for both food and fuel markets. Concentrations of seven major fatty acids (P, S, O, L, LN, A, E) in canola oil were analyzed by GC-FID after transesterification and expressed as the percentage of total fatty acids. The ranges of these seven fatty acid concentrations were as follows: P, 4.26–4.93%; S, 1.85–2.66%; O, 61.5–65.3%; L, 19.6–22.3%; LN, 5.89–9.56%; A, 0.60–0.74%; E, 0.98–3.44% (Table 6). These ranges were consistent with previously reported data for canola oil (Gunstone, 2004). The results of oil composition showed that fatty acid E was relatively stable in this study compared with other fatty acid components. Manure and fertilizer applications increased E concentration only at EL in 2008 ($P < 0.05$). At EL in 2007, treatments had no effect on oil composition except for fatty acid A, which increased with manure applications (M-1 and M-2).

Oleic acid is a fatty acid with an 18-C chain and one unsaturated bond at δ-9. It is a desired fatty acid in biodiesel production, because it has lower melting point temperature than saturated fatty acids P and S and is more stable than polyunsaturated fatty acids L and LN. Statistical analysis showed that the O concentration was less in canola oil with the F-2 treatment relative to M-2 at CT in 2007 (Table 6). Data from EL in 2008 showed that all nutrient applications decreased the O concentration and treatment F-1 resulted in lower O content than treatment M-1; higher N level nutrient applications decreased O content more than lower N level applications. In this study, fertilizer had a trend to decrease canola oil O content more than manure applications. Further research is needed to confirm this result, and metabolic research is also needed to elucidate the fertilizer and manure effects on O levels in canola oil.

Linoleic acid and LN are the two major polyunsaturated fatty acids which have two and three unsaturated bonds respectively in their carbon structures. Nutrient applications had affects on L in canola oil. At CT in 2007, treatments F-1 and F-2 increased L concentration compared with the control and manure treatments, while there was no difference between the manure and control treatments. At EL in 2008, all nutrient applications increased L concentration in canola oil, with ranking of control < M-1 < F-1 ≤ M-2 ≤ F-2, with F-1 < F-2 ($P < 0.05$). There was no treatment effect on L concentration at CT in 2008 and EL in 2007 (Table 6). However, manure and fertilizer applications decreased LN concentration at CT in 2007 and 2008. No treatment effect on LN concentration was detected at EL in 2007 and 2008 with the single exception being that F-2 resulted in higher LN level than the control in 2008.

The O/(L+LN) ratio (Gao et al., 2009) indicates oil quality of major unsaturated fatty acids with higher values indicative of greater fuel stability. In Table 7, the average value of O/(L+LN) in canola oil was 2.22 ± 0.17. Soybean oil is widely used in food industry and biodiesel. Compared the O/(L+LN) value of soybean oil 0.39 ± 0.06 (Gao et al., 2009), canola oil is more stable than soybean oil. No treatment effect was found at CT in both years and EL in 2007. At EL in 2008, all treatments reduced this ratio compared to the control (Table 7);

Table 7. Oil quality in response to manure and fertilizer treatments for two locations in Michigan in 2007 and 2008.

Site†/year	Treatment‡	P+S+A§	O/(L+LN)
		%¶	
CT/2007	control	7.46c#	2.47
	F-1	7.93b	2.47
	F-2	8.32a	2.48
	M-1	7.50c	2.47
	M-2	7.73bc	2.52
CT/2008	control	7.12b	2.10
	F-1	7.38a	2.12
	M-1	7.16b	2.13
	M-2	7.10b	2.15
EL/2007	control	7.04b	2.19
	M-1	7.27a	2.20
	M-2	7.15ab	2.21
EL/2008	control	6.80b	2.19d
	F-1	7.02a	2.09b
	F-2	7.08a	2.04a
	M-1	6.82b	2.13c
	M-2	7.04a	2.08b

† CT, plots in Chatham, MI; EL, plots in East Lansing, MI.

‡ Control, treatment with zero N application; F-1, treatment with fertilizer N level 84 kg N ha^{-1}; F-2, treatment with fertilizer N level 168 kg N ha^{-1}; M-1, treatment with manure N level 84 kg N ha^{-1}; M-2, treatment with manure N level 168 kg N ha^{-1}.

§ P, palmitic acid; S, stearic acid; O, oleic acid; L, linoleic acid; LN, linolenic acid; A, arachidic acid; E, eicosenoic acid.

¶ %, percent of fatty acid in total oil is based on fatty acid GC peak area in total peak area.

Means followed by the same letter are not statistically different ($\alpha = 0.05$).

at the same N level, fertilizer applications could decrease the O/(L+LN) ratio more than manure applications.

Palmitic acid, S and A are three major saturated fatty acids in canola oil, and the sum of these three saturated fatty acids was from 6.7 to 8.6% (Tables 6 and 7). Treatments had significant effects on these fatty acid concentrations. Fertilizer application increased P and A more than manure application at CT in both years and at EL in 2008. The concentration of P in canola oil was greater with the F-2 treatment than with the F-1 treatment at CT in 2007, and was higher with M-2 than with M-1 treatment at EL in 2008. Fertilizer application increased S in canola oil at CT in 2007 while manure application decreased S in canola oil in 2008. No treatment effects were detected at EL in both years.

The sum of P, S, and A indicated total saturated fatty acids in canola oil, and a lower value of (P+S+A) is desired for biodiesel usage because of their high melting points (62, 69.3, and 75.5°C) associated with cold flow issues as fuel. The average value of (P+S+A) in this study was 7.25 ± 0.40% (Table 6), which was smaller than reported (P+S) value 15.88 ± 0.82% for soybean oil (Gao et al., 2009). At CT in 2007, only treatments F-1 and F-2 increased saturated fatty acid content (P+S+A) in oil when compared to the control; at CT in 2008, only treatment F-1 increased total saturated fatty acids content (P+S+A). At EL, treatment M-1 in 2007 and F-1, F-2, M-2 in 2008 increased total saturated fatty acid content. Nutrient N applications could increase total saturated fatty acid content in canola oil.

Correlations of Canola Oil Fatty Acids

Biplots of these canola oil fatty acid levels and nutrient application treatments are shown in Fig. 1. Because oil composition at EL in 2007 is relatively consistent and only three nutrient application treatments were conducted, the biplot of this site in 2007 was not shown. From plots in Fig. 1, fatty acids P, S, L, and A were consistently positively related and E was positively related with P, S, L, and A at CT and EL in 2008, O had negative relation with these four fatty acids and O and L were strongly negatively related with r values from −0.85 to −0.99 ($P < 0.0001$). The similar relationship between O and L has been reported in soybean oil (Oliva et al., 2006; Gao et al., 2009). Compared to the control treatment, all treatments decreased canola oil LN concentrations at CT in 2007 (except M-1 treatment) and 2008, while all treatments decreased O concentrations at EL in 2008 (Table 6 and Fig. 1).

CONCLUSIONS

In this study, fertilizer and manure applications did not consistently increase canola grain yield while they could decrease total canola oil content. Manure application tended to increase canola oil yield in this study. At the same time, fertilizer application sometimes caused reduction in fatty acids LN at CT or O at EL and increased fatty acids P, A, and L at both sites. With fertilizer applications, total saturated fatty acid content (P+S+A) could increase and the oil quality index ratio O/(L+LN) could decrease, which indicated that fertilizer application may decrease canola oil quality as biodiesel. Although manure application sometimes resulted in decreased levels of LN or O and increased levels of L and E, manure had less influence on canola oil fatty acid composition than fertilizer. Our results did not show any benefit of greater than 84 kg N ha^{-1} from nutrient applications on canola oil yield and oil quality. Furthermore, high N rate (168 kg N ha^{-1}) appeared to show a negative effect on total oil content and oil quality. The results confirm the need for judicious use of N fertilizer to minimize the potential reductions in canola oil quality and support the use of manure applications as a viable N alternative to synthetic fertilizer in canola. This is the first study about nutrient application effect on canola oil yield and oil fatty acid composition, and the results provided useful information for farmers and agronomists when they compare fertilizer and manure influence on oil quality associated with biodiesel.

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