

Cuphea Plant Nitrate Content and Seed Yield Response to Nitrogen Fertilizer

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Cuphea (*Cuphea viscosissima* Jacq. × *C. lanceolata* W.T. Aiton, PSR23, Lythraceae), is a new oilseed crop being developed in the Upper Midwest. Cuphea species are summer-annual plants native to North, Central, and South America. The only species native to the USA is *C. viscosissima* Jacq. (Knapp 1993). Cuphea seed oil is rich in medium-chain fatty acids (MCFA) such as caprylic (C8:0), capric (C10:0), lauric (C12:0), and myristic (C14:0) (Graham and Kleiman 1992). *Cuphea lanceolata* has high capric acid (70%) (Kleiman 1990), and *C. llavea* has about the highest level of capric acid (92 %) (Phippen et al. 2006). *Cuphea viscosissima* × *C. lanceolata* line PSR23 seed has 270 to 300 g kg⁻¹ of oil. The typical fatty acid distribution includes 69.9% capric, 2.9% lauric, 4.4% myristic, 5.9% palmitic, 9.4% oleic, and 4.8% linoleic acids. Cuphea oil has an iodine value of 19.7, and a high oxidative stability of 157 hr at 110°C comparable to that of coconut oil (*Cocos nucifera* L., Arecaceae). The content of free fatty acids (4%–4.25%) and chlorophyll (200–260 mg kg⁻¹) in the crude oil are high (Evangelista and Manthey 2006).

Medium-chain fatty acids can be used to replace saturated fatty acids and plasticizers in chewing gum. Cuphea oil also works well as a flow carrier and solvent in the candy industry, and as a defoaming agent and booster in soap and detergent manufacturing (Ag Innovation News 2003). Cuphea oil can be used in cosmetic products such as lipsticks, lotions and creams, and bath oils. The oil has a high oxidative stability, is low to medium spreading with a low slip value all of which provide the desirable non-slippery characteristic for use in sunscreens (Rheins et al. 2006).

The properties of cuphea oil also make it ideal for biofuel products (Cermak and Isbell 2002), including biodiesel and jet fuel. The addition of cuphea oil to jet fuel reduces the fuel's freezing point avoiding fuel-gelling problems at temperatures below –20°C. Oil from *C. viscosissima* VS-320 has a low viscosity, greater than number 2 diesel fuel, but less than rapeseed (*Brassica napus* L., Brassicaceae) oil (Geller et al. 1999).

The world market for lauric oil is 4.5 million tonnes (t) annually and the US consumption is 1.5 million t or one-third of annual production (Zenk 2006). Most oils rich in lauric acid come from Malaysia. Total coconut, palm, and palm kernel (*Elaeis guineensis* Jacq., Arecaceae) oil imports for the USA reached 939,000 t in 2004 (Table 1). Prices for these oils fluctuate yearly having decreased 15%, from 1999 to 2004 to an average price of \$600 t (Table 2), with an average yearly imported total value of 569 million dollars (FAOSTAT 2006) (Table 3).

An estimated 6.3 million ha of high-lauric cuphea production (based on an average oil yield of 150 kg ha⁻¹) would be required to substitute for current palm and palm kernel, and coconut oil imports. Currently, contracting companies are paying farmers \$1.19 kg⁻¹ of seed, with that, a gross income of \$1192 ha⁻¹ would be necessary to cover the production costs for a seed yield of 444 kg ha⁻¹ (Gesch et al. 2006). Net returns are expected to increase as seed yield and oil content increase with the development of new cuphea cultivars. Currently, the high value paid to the growers for cuphea seed makes oil much more costly to produce domestically than coconut or palm oil. Current cuphea lines are 10 or 20 times higher in capric acid than coconut (6%) and palm kernel oil

Table 1. United States import volume of coconut, palm, and palm kernel oil from 1999 to 2004 (FAOSTAT 2006).

Oil	Imported oil volume (1000 t)					
	1999	2000	2001	2002	2003	2004
Coconut	335	477	468	485	352	416
Palm kernel	208	168	149	173	220	250
Palm	143	165	171	219	211	273
Total	687	810	788	877	783	939

(3%) (Isbell et al. 2004), and they may have a higher market value. This is one of the challenges of bringing a new crop to the market.

Cuphea is being grown commercially in the Midwest mainly in Minnesota and eastern North Dakota, with a contracted area of about 300 ha in 2006. Cuphea still has several limitations as a commercial crop, particularly with regard to traits that affect stand establishment and harvest. Low stands are common in commercial fields due to deep seeding, low germination and vigor, and low soil temperatures in the spring. Cuphea has an indeterminate growth habit where the plant continues to flower and develop seed until frost. At harvest, the seeds shatter easily and have high moisture content.

Several agronomic studies have been conducted to determine the adapted area (latitudinal zone) (Forcella et al. 2005b), sowing date (Gesch et al. 2002), seeding rate, row spacing, plant density (Gesch et al. 2003a), weed control (Forcella et al. 2005a), water requirements (Sharratt and Gesch 2002, 2004), harvest date, harvest methods (Gesch et al. 2003a), and post harvest drying for cuphea in the North Central Corn Belt (Cermak et al. 2005).

There is little reported on the effect of nitrogen fertility on cuphea seed yield, oil content, or oil composition. The Cuphea Grower's Guide, created to give guidelines to farmers in Minnesota, recommends using band applications of fertilizer 5 cm to the side and 5 cm below the planting depth (Gesch et al. 2003b). For most soils the recommendation is to apply 45 kg ha⁻¹ of potassium sulfate (0–0–20–7) along with 224 kg ha⁻¹ of diammonium phosphate (39–92–0) and 112 kg ha⁻¹ of urea (46–0–0) (Gesch et al. 2003b). These estimates were based upon fertilizer needs of other crops, as no relevant research has been performed and/or reported specifically for cuphea. Consequently, the objective of this study was to determine the optimum nitrogen fertility for maximum seed yield and oil content in cuphea.

MATERIALS AND METHODS

Field Establishment and Experimental Design

This research was conducted at the North Dakota State University Agronomy Seed Farm at Casselton, on a Bearden silty-clay loam (fine-silty, mixed, superactive, Frigid Aeric, Calciaquolls) and in a farmer's field near Glyndon, Minnesota, on a Glyndon loam (coarse-silty, mixed, superactive, Frigid Aeric, Calciaquolls) in 2005 and on a Borup loam (coarse-silty, mixed, superactive, Frigid Typic, Calciaquolls) in 2006. The experiment also was conducted at Morris, Minnesota at the USDA-ARS Swan Lake Research Farm in 2005 on a Hamerly clay loam (fine-loamy, mixed, superactive, Frigid Aeric, Calciaquolls) mixed with Parnell (fine, smectitic, frigid Vertic, Argiaquolls), and Flom (Fine-loamy, mixed, superactive, Frigid Typic, Endoaquolls) in the lower areas of the field. Monthly precipitation amounts were recorded at all environments.

Table 2. Average price for imported oils in the USA (coconut, palm kernels, and palm) from 1999 to 2005 (FAOSTAT 2006).

Oil	Average imported oil price (\$/t)					
	1999	2000	2001	2002	2003	2004
Coconut	754	576	377	356	498	640
Palm kernel	764	668	476	459	523	645
Palm	518	361	343	306	423	519

Table 3. United States import value of coconut, palm, and palm kernel oil between 1999 and 2004 (FAOSTAT 2006).

Oil	Import value (million \$)					
	1999	2000	2001	2002	2003	2004
Coconut	253	275	176	173	175	266
Palm kernel	158	112	71	80	115	161
Palm	74	59	59	67	89	142
Total	485	446	306	320	379	569

Previous crops were sugarbeet (*Beta vulgaris* var. *saccharifera* L., Chenopodiaceae) at Glyndon in 2005 and 2006, wheat (*Triticum aestivum* L., Poaceae) at Casselton in 2005, soybean [*Glycine max* (L.) Merr., Fabaceae] at Morris in 2005, and sudangrass [*Sorghum bicolor* (L.) Moench. Poaceae] at Morris in 2006.

Cuphea (PSR23, *C. viscosissima* × *C. lanceolata* f. *silenoides* W.T. Aiton) was sown with a plot planter. Planting dates at Glyndon in 2005 and 2006, and Casselton in 2005 were 23 May, 22 May, and 6 June, respectively. At Morris, planting dates were 17 May in 2005 and 20 May in 2006. Seeding rates were 21 kg ha⁻¹ at all locations except for Morris where the seeding rate was 11 kg ha⁻¹ both years.

Nitrogen fertility treatments consisted of a check (non-fertilized treatment), as well as 60, 80, 100, 150, and 200 kg N ha⁻¹ (soil N + N fertilizer) treatments at Glyndon in 2005 and 2006 and Casselton in 2005. The source of nitrogen was urea [CO(NH₂)₂], which was hand-broadcast in each individual plot and then incorporated with a harrow. At Morris in 2005 and 2006 nitrogen fertility treatments were a check (non-fertilized treatments), as well as 171, 216, and 260 kg N ha⁻¹ (soil N + N fertilizer) treatments.

The experimental design at all sites was a randomized complete block with four replicates. Experimental units were 2.0 m wide by 7.6 m long with six rows separated by 30 cm spacings at Glyndon in 2005 and 2006 and at Casselton in 2005. At Morris, both years, experimental units were 3.6 m wide and 6.1 m long with six rows separated by 61 cm spacings.

Weed control, at Glyndon and Casselton included preplant incorporation of trifluralin (α,α,α -trifluoro-2,6-dinitro-N,N-dipropyl-p-toluidine) (0.5 kg a.i. ha⁻¹) followed by hand-weeding as needed. At Morris, both years, plots were treated with isoxaflutole (80 g a.i. ha⁻¹) immediately after planting. PSR23 cuphea tolerates both herbicides well (Forcella et al. 2005a).

Plant Sampling and Character Evaluations

Dependent variables evaluated were NO₃-N at vegetative stage, NO₃-N at bloom stage, NO₃-N at harvest maturity, plant and seed total nitrogen content, biomass and seed yields, harvest index, test weight, oil content, and soil NO₃-N at 0–30, 30–60, 60–90, and 90–120 cm depths.

Whole plants were collected at three developmental stages (vegetative, bloom, and harvest maturity) from each experimental unit to determine plant NO₃-N. The colorimetric determination of nitrate in plant tissue by the nitration of salicylic acid method was used (Cataldo et al. 1975).

Immediately before harvest, seed and biomass samples were collected from each plot and analyzed by the Kjeldahl procedure to determine total nitrogen content of biomass and seeds. Nitrogen uptake was determined by multiplying biomass and seed nitrogen contents by biomass and seed yields. Biomass samples were taken from 1-m² areas within each plot, where plants were cut at the bases of their stems. Heights were measured for five plants in each plot. Thereafter, four rows of each plot were harvested with a self-propelled plot combine (Hege) to estimate seed yield. Harvest index was calculated as the percent of dry seed weight divided by the total dry above ground biomass. Test weight was calculated by determining the weight of 40 mL of seed.

Seed oil content was determined with a Newport 4000 Nuclear Magnetic Resonance (NMR) Analyzer, Oxford Institute Limited. Samples were dried in an oven at 110°C for 3 hr and then cooled to room temperature to equilibrate seed moisture content before the analysis.

Soil samples were collected from the 0–30, 30–60, 60–90, and 90–120 cm depths from all plots at Glyndon and Casselton after harvest. The soil samples were analyzed for NO₃-N using the transnitration of salicylic acid method Vendrell and Zupancic (1990) by the Soil and Plant Analysis Laboratory, North Dakota State University. Soil samples were collected from 0–30 and 30–60 cm depths from all plots at Morris in the fall after harvesting. These samples were analyzed at the USDA North Central Soil Conservation Research Laboratory by the spectrophotometric method described by Bremner and Mulvaney (1956) and Mulvaney (1996).

Statistical Analysis

Statistical analysis was conducted by using standard procedures for a randomized complete block design. Each location-year combination was defined as an “environment” and was considered a random effect in the statistical analysis. Nitrogen rates were considered fixed effects. Environments were analyzed in two different experiments since nitrogen fertility treatments and plant spacing were different. Experiment 1 (Expt. 1) included the Glyndon 2005 and 2006, and the Casselton 2005 environments combined. Analysis for Expt. 1

was performed across these three environments. Experiment 2 (Expt. 2) included the Morris 2005 and 2006 environments combined. Analysis for Expt. 2 was performed across these two environments. Regression analysis was considered for character responses when there was a significant main effect for nitrogen fertility treatment or interaction. Linear, quadratic, and cubic regression models were tested. The regression models presented and all parameter estimates were significant at $P \leq 0.05$.

RESULTS AND DISCUSSION

Precipitation

Growing season precipitation varied considerably across environments (Table 4). Growing season precipitation was greater at Morris and Casselton than Glyndon in 2005. The 2006 growing season was below average for rainfall at Glyndon and Morris, which may have restrained plant growth and seed yield at these environments.

Plant $\text{NO}_3\text{-N}$

The environment by nitrogen interaction, for the combined analysis for Expt. 1 was not significant for $\text{NO}_3\text{-N}$ at the vegetative stage, $\text{NO}_3\text{-N}$ at the bloom stage, and $\text{NO}_3\text{-N}$ at harvest maturity. The nitrogen fertility main effect was significant for plant $\text{NO}_3\text{-N}$ at the vegetative stage, bloom stage, and harvest maturity, indicating that as nitrogen fertility was increased, nitrate content in plant tissue also increased (Fig. 1). The response of tissue nitrate to additional nitrogen was linear for the three developmental stages (Table 5).

Intercepts for the regression equations for plant $\text{NO}_3\text{-N}$ at the vegetative, bloom and harvest maturity were 3977, 2503, and 954 mg kg^{-1} , respectively (Fig. 1 and Table 5). The total nitrate content appeared to decrease as developmental stages advanced (Fig. 1). Nitrate-absorption curves lead dry-matter-production curves until reproductive development in most plant species (Black 1992); thereafter dry matter accumulation is much faster than nitrogen absorption and plant $\text{NO}_3\text{-N}$ content declines due to a dilution effect. Nevertheless, there was a tendency for the slopes of each relationship to increase with developmental stage. This may indicate that plant $\text{NO}_3\text{-N}$ was more responsive to N fertilizer rate at harvest than at earlier stages of development (Fig. 1).

In Expt. 2, the Morris environments, the environment by nitrogen interaction was significant for plant $\text{NO}_3\text{-N}$ at the vegetative stage, and bloom stages. Regression models were fitted for $\text{NO}_3\text{-N}$ at the vegetative stage and bloom stage for each environment separately (Table 6). In 2005, a linear response was observed at the vegetative stage while in 2006 the response was quadratic. Both years, tissue nitrate concentration almost doubled when nitrogen fertility increased from 126 kg N ha^{-1} to 260 kg N ha^{-1} (Fig. 2A). In the bloom stage, linear and quadratic responses were observed in 2005 and 2006, respectively (Fig. 2B).

Table 4. Growing season rainfall (mm) for Casselton, 2005, and Glyndon and Morris, 2005 and 2006 and the deviation (Dev.) from long-term averages.

Month	Casselton		Glyndon				Morris			
	2005		2005		2006		2005		2006	
	Total	Dev.	Total	Dev.	Total	Dev.	Total	Dev.	Total	Dev.
May	64	-4	52	-5	48	-9	55	8	47	-24
June	161	70	150	57	24	-69	173	145	28	-67
July	34	-48	50	-54	63	-41	77	50	27	-59
Aug.	113	45	147	82	35	-30	92	57	35	-48
Sept.	104	50	38	-24	92	30	97	-19	116	55
Total	476		437		262		494		253	

Plant and Seed Nitrogen

Plant total nitrogen content at the end of the season was not influenced by nitrogen fertility rates at any environment (data not shown). The nitrogen main effect was significant for seed nitrogen content for Expt. 1. Seed nitrogen content increased to as high as 2.9% wt/wt as nitrogen fertility levels increased (Fig. 3).

Total Plant Nitrogen Uptake

The nitrogen main effect and environment by nitrogen interactions were not significant for total plant nitrogen uptake for the combined analysis for Expt. 1 and Expt. 2. Mean nitrogen uptake was 139 kg N ha⁻¹ for Expt. 1, and 123 kg N ha⁻¹ for Expt. 2. Interestingly, despite greater initial soil N levels in Expt. 2 at Morris, total nitrogen uptake was less than that for Expt. 1. Perhaps this indicates a higher leaching of nitrate into the soil profile for the sandier soils at the Morris environments.

Plant Height

No main effects or interactions were significant for this character for either combined analysis for Expt. 1 and 2. Nitrogen did not influence plant height. Mean cuphea plant height was 82 cm. The ability of cuphea to branch and efficiently utilize the space surrounding the plant could have played a role in maintaining a similar height despite different nitrogen availability in the soil.

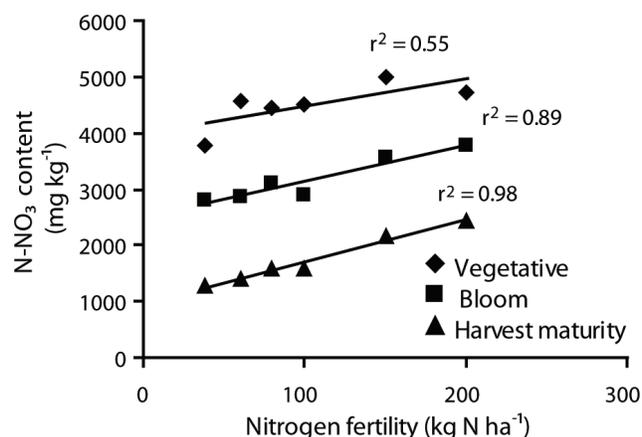


Fig. 1. Predicted regression lines for plant NO₃-N at vegetative bloom, and harvest maturity stages with different nitrogen fertility treatments for Expt. 1.

Table 5. Regression equations and r² value for the influence of nitrogen rates on plant NO₃-N at vegetative, bloom, and harvest maturity stages for Expt. 1.

Stage	Equation	r ²
Vegetative	NO ₃ -N = 3977 + 5.0N ^z	0.54* ^y
Bloom	NO ₃ -N = 2503 + 6.4N	0.89*
Harvest maturity	NO ₃ -N = 954 + 7.5N	0.97*

^zRegression equations for each stage are for the combined analysis for Expt. 1.

^ySignificant at the 0.05.

Table 6. Regression equations and r² value for the influence of nitrogen rates on plant NO₃-N at vegetative and bloom stages for Expt. 2.

Stage	Year	Equation	r ²
Vegetative	2005	NO ₃ -N = 485 + 15.5N ^z	1.00* ^y
	2006	NO ₃ -N = 2746 + 47.0N - 0.12N ²	0.96*
Bloom	2005	NO ₃ -N = -5197 + 71.0N - 0.014N ²	0.99*
	2006	NO ₃ -N = 994 + 5.6N	0.88*

^zThe Interaction environment by nitrogen was significant so regression models for each environment are presented separately.

^ySignificant at the 0.05.

Biomass and Seed Yield

The nitrogen main effect and the environment by nitrogen interaction were not significant for biomass and seed yield for the combined analysis for Expt. 1. Cuphea mean biomass yield was 7,392 kg ha⁻¹ averaged across nitrogen treatments and environments. However, a significant response was observed for the nitrogen effect for Expt. 2 when both Morris environments were combined. In Expt. 2, a significant linear response for seed yield was observed as nitrogen fertilizer was increased (Fig. 4). The response was observed only in Expt. 2, probably because the soil was a sandy-loam, which may have allowed nitrate leaching beyond cuphea's shallow root system (Sharrat and Gesch 2004) in the treatments with lower nitrogen fertility. Also, loam soils have lower organic matter content decreasing the release of nitrate by mineralization. Soil at the other environments may have had enough nitrates in the soil to meet plant requirements.

Harvest Index

The nitrogen treatment and the environment by nitrogen interaction were not significant for Expt. 1 and 2 combined. The mean value for harvest index was 2.8% averaged across treatments and environments (data not

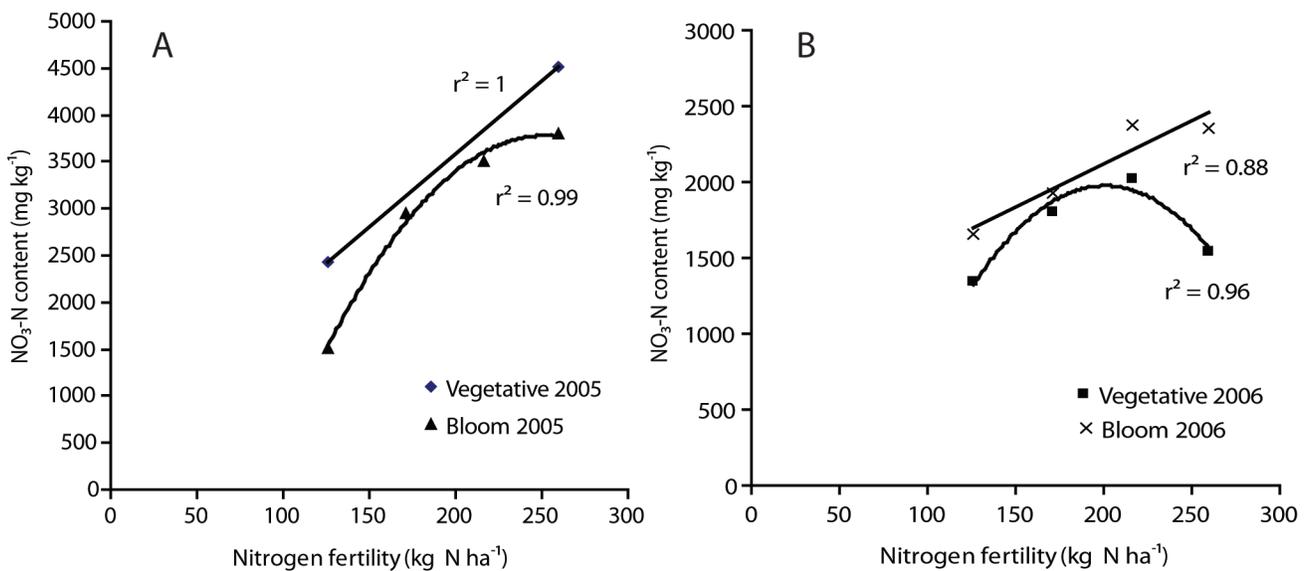


Fig. 2. Predicted regression lines for (A) Plant NO₃-N at vegetative stage (B) Plant NO₃-N at bloom, with different nitrogen fertility treatments for Expt. 2. Interaction between environment and nitrogen treatments was significant for both stages of development.

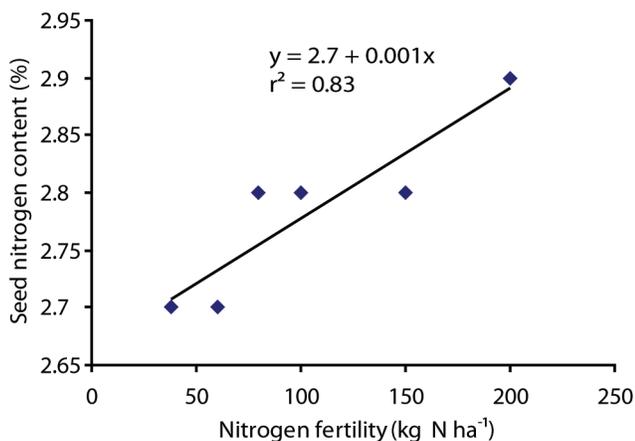


Fig. 3. Predicted regression line for seed nitrogen content as affected by nitrogen fertility for Expt. 1 environments combined.

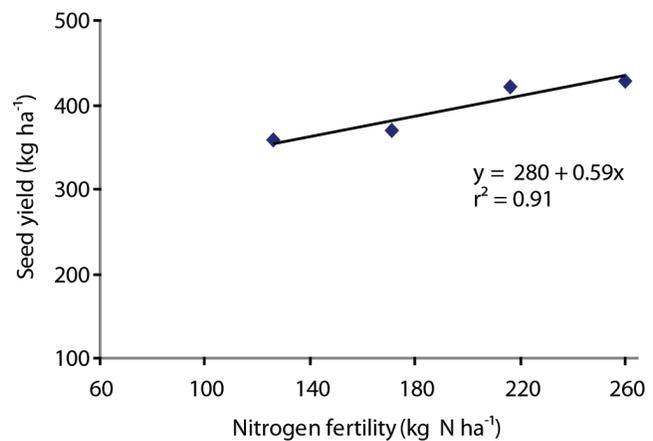


Fig. 4. Predicted regression line for seed yield as affected by nitrogen fertility for Expt. 2 environments combined.

shown). Harvest index in cuphea is much lower than other crops growing in the region. Cuphea needs genetic improvement to increase harvest index to be comparable with other oilseed crops, such as canola (*Brassica napus* L., Brassicaceae), sunflower (*Helianthus annuus* L., Asteraceae), and soybean.

Seed Oil Content and Test Weight

The nitrogen treatment and the environment by nitrogen interaction were not significant for seed oil content for Expt. 1 and 2. Mean seed oil content was 297 g kg⁻¹ and similar among all treatments.

The nitrogen fertility main effect was significant for test weight in Expt. 1. Test weight increased as nitrogen fertility increased (Fig. 5). The nitrogen and the environment by nitrogen interaction were not significant for test weight for the combined analysis across environments in Expt. 2.

Residual Soil Nitrate

Residual soil nitrate was influenced by the nitrogen fertility treatments. The environment by nitrogen, environment by soil depth, and nitrogen by soil depth interactions for the combined analysis for Expt. 1 were significant (Table 7). No significant effects were observed.

Residual soil nitrate increased linearly with added nitrogen fertilizer in Expt. 1 (Fig. 6). Soil nitrate increased with nitrogen fertility at 0–30 cm and 30–60 cm depths (Fig. 7). Nitrogen fertility did not affect soil NO₃-N below 60 cm. The lack of differences of residual NO₃-N at the two deepest increments indicates that the majority of the cuphea root mass is located in the upper 60 cm of the soil profile. Crops typically absorb nitrate only to sufficiency levels, thereafter nitrate accumulates and leaches to deeper layers in the soil (Black 1992).

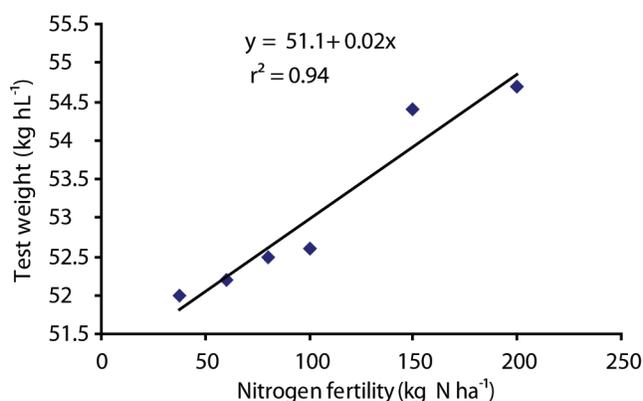


Fig. 5. Predicted regression line for the effect of nitrogen fertility in cuphea seed test weight content for Expt. 1 environments combined.

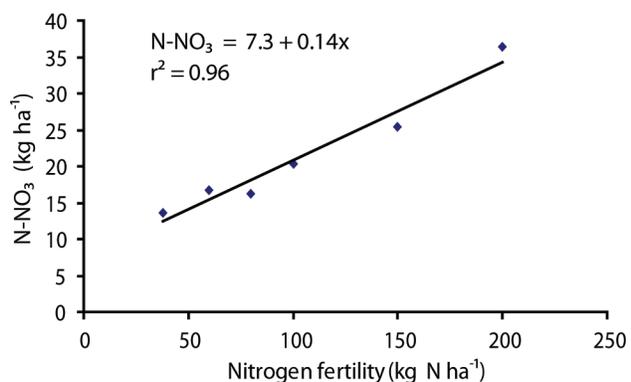


Fig. 6. Predicted regression line for residual soil nitrate content as affected by nitrogen fertility and four soil depths (0–30 cm, 30–60 cm, 60–90 cm, and 90–120 cm) for Expt. 1 environments combined.

Table 7. Regression equations, r^2 value, for the influence of nitrogen rates on soil nitrate content at four different soil depths for Expt. 1.

Soil depth	Equation	r^2
0–30	$\text{NO}_3\text{-N} = 2.9 + 0.37\text{N}^z$	0.91* ^y
30–60	$\text{NO}_3\text{-N} = 4.0 + 0.15\text{N}$	0.94*
60–90	$\text{NO}_3\text{-N} = 12.7 + 0.02\text{N}$	0.2*
90–120	$\text{NO}_3\text{-N} = 16.6 - 0.06\text{N}$	0.02*

^zThe Interaction nitrogen fertility by soil depth was significant so regression models for each soil depth are presented separately.

^ySignificant at the 0.05.

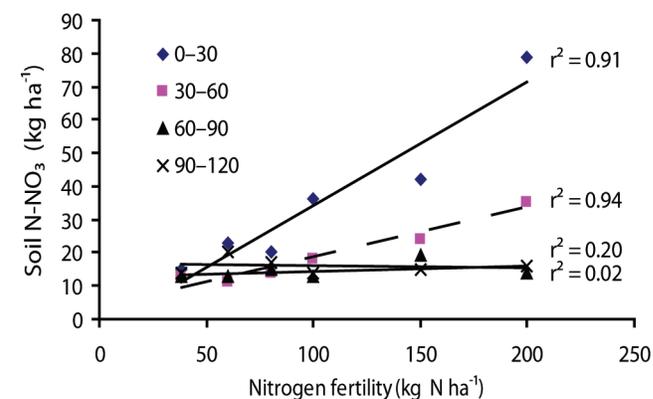


Fig. 7. Predicted regression line for residual soil nitrate content at different soil depths as affected by nitrogen fertility for Expt. 1 environments combined.

This did not seem to happen for this crop since nitrate accumulated only in the upper layers of the soil profile at the highest nitrogen fertility treatments. Other studies indicate that cuphea's rooting depth is restricted to the upper 40 cm of the soil profile where 65% to 85% of the roots are found within the upper 20 cm of the soil (Sharratt and Gesch 2002, 2004).

CONCLUSIONS

Plant nitrate levels showed the greatest response to nitrogen fertility treatments. As nitrogen fertility was increased, $\text{NO}_3\text{-N}$ increased in plant tissue at vegetative, bloom, and harvest maturity. At later stages $\text{NO}_3\text{-N}$ seemed to decrease due to a dilution effect from dry matter accumulation. Maximum total nitrogen uptake at harvest was 139 kg N ha^{-1} . Seed yield was enhanced with nitrogen fertility only at the Morris environments, where maximum seed yield was obtained with 216 kg N ha^{-1} . Soil residual $\text{NO}_3\text{-N}$ accumulated as nitrogen fertility increased and was evident only in the top 60 cm of the soil profile. According to the average total nitrogen uptake and residual soil nitrate accumulation, the nitrogen fertility recommendation would be 100 to 140 kg N ha^{-1} to optimize seed yield.

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