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Carinata Dry Matter Accumulation and Nutrient Uptake Responses to Nitrogen Fertilization

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ABSTRACT

Brassica carinata is grown as a winter crop in the Southeast United States and it is a non-edible oilseed feedstock for 'drop-in' aviation and transportation fuels. The objective of this 2-yr study was to determine the effects of N application on dry matter (DM) production and the accumulation of nutrients in above- and belowground biomass. Carinata var. 110994EM was treated with four N rates (0, 45, 90, and 135 kg N ha^{-1}) in 2014 and 2015 at Quincy, Florida. Above- and belowground biomass were collected and analyzed for macro- and micronutrients. The allocation of DM among root, leaves, stems, flowers/pods, and seed did not differ with N rate. Carinata was highly responsive to N with maximum yield (2798 kg ha^{-1}) produced at 102.3 kg N ha^{-1}, while the economic optimum N rate occurred at 93 kg N ha^{-1}. Maximum N uptake occurred between 50% bolting and 50% flowering while all other elements had maximum uptake between 50% flowering and pod formation. Nitrogen concentration in seed and straw increased with N rate. These results were attributed to the strong relationship between uptake and dry matter production. Total N uptake exceeded applied N by 11 to 160%, suggesting that carinata is highly efficient at scavenging and utilizing residual soil N. The identification of growth stages associated with maximum nutrient uptake may aid in aligning time of N application to critical growth stages corresponding to maximum N uptake.

Core Ideas

- This is the first report of carinata dry matter accumulation and allocation responses to nitrogen.
- Carinata growth, resource allocation, seed, and straw nitrogen concentration and uptake are highly responsive to nitrogen application in North Florida.
- Dry matter accumulation increases with nitrogen rate; however, the allocation of dry matter to roots, leaves, stems, flower/pods and seeds are similar regardless of nitrogen rate.
- Maximum nitrogen uptake occurred between 50% bolting and 50% flowering while all other elements had maximum uptake later in the season between 50% flowering and pod formation.
- Total nitrogen uptake exceeded applied N by 11 to 160%, suggesting that carinata is highly efficient at scavenging and utilizing residual soil nitrogen.
- Adequate nitrogen is required (93 kg N ha^{-1}) for optimizing carinata productivity in sandy loam soils in North Florida.

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The Renewable Fuel Standard under the Energy Independence and Security Act of 2007 targets the production of 136 billion liters of renewable biofuels by 2022, mandating 58% derived from advanced biofuels that achieve a 50% reduction in greenhouse gas emissions (Schnepf and Yacobucci 2012). Advanced or second-generation biofuel feedstocks must utilize competitively natural resources for fuel production without taking lands out of food/feed/fiber production. One approach to meet these targets is the use of advanced biofuels derived from non-food feedstocks that include lignocellulosic biomass and industrial oilseeds.

Carinata (*Brassica carinata* A. Braun) is a promising and competitive second-generation dedicated feedstock for bio-based fuel industries. Carinata oil can be transesterified/methanolysed to produce biodiesel (Cardone et al., 2003) or hydrotreated to produce jet fuel (Gesch et al., 2015) with physical and chemical properties and performance similar to petroleum-based products (Cardone et al., 2003, 2002). In the Southeast United States, carinata has been commercially grown to produce 'drop in' aviation fuels (ARA, 2018) on fallowed lands. Carinata has superior agronomic traits (drought and heat tolerance (Malik, 1990), pod shatter resistance (Banga et al., 2011), non-dormancy, and fits into current agricultural infrastructure for harvesting, handling, and storage, thus positioning carinata for commercialization within the United States (D. Wright personal communication, 2018). However, there is a dearth of information on the production and management of carinata, particularly in the Southeast United States.

Nitrogen accounts for the largest energy input and production cost in oilseed production (Gan et al., 2008), therefore optimizing N application rate and timing for optimum productivity, economic feasibility, and environmental stewardship is critical for commercial success of this relatively new bioenergy crop. A carinata production guide recommends the application of 90 N, 45 P_2O_5, 90 K_2O, and 28 S (kg ha^{-1}) for a yield goal of 3300 to 3500 kg ha^{-1} on loamy sands in North Florida (Seepaul et al., 2016a). Brassica plant development is divided into vegetative (seedling, rosette), transition (bolting), and reproductive growth stages (flowering, pod development, and seed ripening). Modifications of fertilizer application practices through synchronous N

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Abbreviations: DAP, days after planting; DM, dry matter; LRL, lateral root length; RDW, root dry weight; RSR, root shoot ratio; TDM, total dry matter; TRL, taproot length.

application with these growth stages will optimize N uptake and utilization and may also reduce run-off and leaching.

Brassicas including oilseed rape (*Brassica napus* L.), juncea [*Brassica juncea* (L.) Czern.], and carinata are highly responsive to N fertilizer and require relatively high rates of mineral fertilizers for optimum seed yields (Gan et al., 2007; Johnson et al., 2013; Montemurro et al., 2016; Prakash et al., 1999). Maximum seed yield (2204 kg ha^{-1}) of spring planted carinata was produced at 150 kg N ha^{-1} in the Canadian prairies (Pan et al., 2012). In Italy, application of 100 kg N ha^{-1} produced 1770 kg seed ha^{-1}, 29% greater than the 0 N control (Montemurro et al., 2016). In India, winter carinata dry matter accumulation increased linearly with N rate as high as 150 kg N ha^{-1}, but there was no benefit to seed yield above 100 kg N ha^{-1} (Kaur and Sidhu, 2004). In Canada, mean straw yield also showed a positive response to N application, reaching peak at 200 kg N ha^{-1} (Johnson et al., 2013) or 100 kg N ha^{-1} (Gan et al., 2007) in various *Brassica* species.

Nitrogen concentration and uptake in brassica seeds and straw increase with increasing N availability (Prakash et al., 2000; Johnson et al., 2013). Increased nutrient uptake is related to greater biomass accumulation from increased growth and photosynthetic capacity under non-limiting N conditions (Seepaul et al., 2016b). Nitrogen uptake in carinata seeds (90.3 kg N ha^{-1}) is 115% greater than straw (42.0 kg N ha^{-1}) N uptake (Prakash et al., 2000). Similarly, seed P uptake (13.7 kg N ha^{-1}) is greater (158%) than straw P uptake (5.3 kg N ha^{-1}) (Prakash et al., 2000). Nitrogen is translocated from leaves and stems to the developing seed, hence the greater nutrient uptake in seeds. Seasonal nutrient assimilation and distribution of carinata in response to N availability are lacking in the literature.

An understanding of the biomass accumulation and allocation, nutrient concentration, and uptake can aid in synchronizing in-field N application with crop growth for optimum uptake and utilization. The effect of applied N on carinata plant, seed, and straw nutrient concentration and uptake in the Southeast United States has not been previously reported. The objectives of this study were to (i) determine the seasonal accumulation and relative allocation of dry matter among the plant parts, and (ii) quantify the concentration and uptake of macro and micronutrients in above- and belowground tissue, seed, and straw of carinata in response to N rates. This study will improve our understanding of the N utilization potential of carinata in the soils of the Southeast United States where leaching potential is high, quantity and quality of residue left behind for the following crop, and overall sustainability of carinata incorporated into traditional southeastern cropping systems as a winter crop. In addition, these findings may aid in refining N management to increase the efficiency of utilization, ensure sufficiency of N at critical times in the growing season.

MATERIALS AND METHODS

Site Characterization, Management and Experimental Design

Non-irrigated field trials were conducted during the 2014–2015 and 2015–2016 winter/spring growing seasons at the University of Florida North Florida Research and Education Center (30°32’44”N, 84°35’40.7”W), Quincy, FL, on a Norfolk loamy fine sand (fine-loamy, kaolinitic, thermic Typic Kandiudults) with 0 to 2% slopes in 2014–2015 and on a Orangeburg loamy sand, 2 to 5% slopes (Fine-loamy, kaolinitic, thermic Typic Kandiudults) in 2015–2016. The experimental design was a randomized complete block with four replications. Treatments were three rates of N (45, 90, and 135 kg N ha^{-1}) and a nonfertilized control. Plots were 1.5 × 20 m with 1.8 m between plots. In both years, alleys were planted with carinata and mowed at physiological maturity to reduce border and alley effects.

The soil was prepared by a single disk cultivator pass to a 15 cm depth followed by a cultipacker to create a firm and smooth seedbed. Carinata variety 110994EM sourced from Agrisoma Biosciences Inc. was planted on 21 Nov. 2014 and 4 Nov. 2015 using a Hege 1000 series cone planter (Wintersteiger Inc., Salt Lake City, UT) in 17.8 cm row spacing at a rate of 6.1 kg ha^{-1} (129 seeds m^{-2}). Block level soil samples were analyzed using Mehlich-III soil test extraction procedure followed by elemental quantification via inductively coupled plasma optical emission spectrometry (ICP–OES) by Waters Agricultural Laboratories, Camilla, GA. Carinata was not planted in the same field in the 2 yr of the study since rotation is recommended (Seepaul et al., 2016a). In year 1 of the study, the field was fallowed from October 2013 to November 2014. In year 2, the field was planted with oats (*Avena sativa* L.) from November 2014 to April 2015 and fallowed from April 2015 to November 2015. Field plots used in both years had comparable chemical characteristics (Table 1).

Pre-emergence herbicide pendimethalin (N-(1-ethylpropyl)-3,4-dimethyl-2,6-dinitrobenzenamine) and burndown herbicide paraquat dichloride (1,1’-dimethyl-4,4’-bipyridinium dichloride) were tank-mixed and applied at planting at an active ingredient rate of 0.73 L ha^{-1} and 0.63 L ha^{-1}, respectively, in both years. Diflubenzuron (N-[[(4-chlorophenyl)amino] carbonyl]-2,6-difluorobenzamide) applied at active ingredient rates of 0.05 L ha^{-1} was tank mixed with spinosad at 0.10 L ha^{-1} active ingredient and applied on 24 Apr. 2015 to control diamond back moths (*Plutella xylostella* L.). Bifenthrin (2 methyl[1,1–biphenyl]-3-yl) methyl 3-(2-chloro-3,3,3-trifluoro-1-propenyl)-2,2-dimethyl-cyclopropanecarboxylate) was applied at active ingredient rates of 0.05 L ha^{-1} to control aphids and diamond back moths on 7 Jan. 2016.

Soils were fertilized according to soil test recommendations for canola in both years (Buntin et al., 2010). Potassium (potassium chloride, 0–0–60) and P (triple superphosphate, 0–44–0) were preplant incorporated at 42 and 84 kg ha^{-1}, respectively. Ammonium nitrate $(NH_4)(NO_3)$ (34–0–0) was applied with a First Products Dry Fertilizer Applicator (First Products, Tifton, GA) at three rates of 45, 90, and 135 kg ha^{-1}, with 25% preplant incorporated on 21 Nov. 2014 and 4 Nov. 2015, 50% topdressed at bolting on 17 Feb. 2015 and 1 Feb. 2016 and 25% topdressed at flowering on 30 Mar. 2015 and 22 Feb. 2016.

Data Collection

Plants in one 0.25 m^2 quadrat were sampled for aboveground and belowground biomass at the following stages of phenological development: 10 leaf stage (vegetative), 50% bolting, 50% flowering, pod development, and seed maturation at, respectively,84, 96, 120, 139, and 167 d after planting (DAP) in 2015 and 77, 87, 115, 132, and 160 DAP in 2016. Roots were excavated with a 15.3 cm width× 116.3 cm length drain spade approximately 31–38 cm from the base of the plant. The length of cleaned tap roots and lateral roots were measured on four plants. All plants

Table 1. Soil chemical characteristics of fields planted with carinata in 2014 and 2015 at Quincy, FL. Soils were sampled at a 15 cm depth. CEC, cation exchange capacity.

Year	Soil	pH	CEC	P	K	Mg	Ca	S	B	Zn	Mn	Fe	Cu
			meq 100 g^{-1}	kg ha^{-1}									
2014	Typic Kandiudults	6.7	5.7	32	185	147	603	45	0.45	4	11	31	0.45
2015	Typic Kandiudults	6.6	4.7	96	155	111	753	46	0.45	5	13	38	1.01

in the 0.25 m^2 area were hand separated into roots, leaves (blade only), stems (stems and leaf petioles), and reproductive structures (flowers and pods), then oven dried at 55–60°C for 72 h for dry matter determination to estimate DM allocation and root/shoot ratio. Dry matter accumulation across growth stage was expressed per plant. Roots and aboveground tissue (leaves, stems and reproductive structures) were ground separately to pass a 2 mm stainless steel screen and analyzed for mineral content at Waters Agricultural Laboratories. At harvest, plots were trimmed on either ends to 7.62 m. Naturally desiccated carinata was harvested on 29 May 2015 and 18 May 2016 with a Wintersteiger Delta plot combine (Wintersteiger Inc.). Seeds were oven-dried at 50°C for 48 h and yields were corrected to 8% moisture. Seed moisture content and test weight were measured with a Steinlite Sl95 moisture meter (Steinlite, Atchison, KS) using ~0.75 kg seeds. Two samples of 1000 seeds were randomly subsampled and weighed. Seeds were analyzed for mineral concentration at Waters Agricultural Laboratories. Dried and ground seed and tissue samples were chemically digested in nitric acid in a digestion block (DigiBlock Digester ED36S, Labtech, Wilmington, MA) followed by elemental quantification via ICP–OES (ICP–OES, PerkinElmer optima 4300 DV). All residue exiting the combine harvester was collected, weighed fresh and a 1-kg subsample was taken and dried in a forced air oven at 55–60°C for 72 h for DM determination. This sample was ground to pass a 2 mm stainless steel screen and analyzed for mineral concentration at Waters Agricultural Laboratories. Nutrient uptake and removal were calculated by multiplying the aboveground DM yield by elemental tissue concentration. Harvest index was calculated as the ratio of seed to the total aboveground matter on the final destructive harvest at seed maturation. The economic optimum N rate, defined as the point where the last increment of N returns a yield increase large enough to pay for the additional N, was calculated by equating the first derivatives of the quadratic response equation to N fertilizer price ratio and solving for N (data not shown). The cost of production used in the calculation was (USD) \$675 ha^{-1} taking into consideration \$0.68 kg^{-1} as the cost of N. Revenue was calculated using a price of \$8.50 bu^{-1} (22.7 kg) of seed.

Statistical Analysis

The data were analyzed by fitting mixed models using PROC MIXED in SAS at the 95% confidence level (SAS Institute Inc., Cary, NC). Nitrogen application rate and plant growth stage and their interactions were considered fixed effects while replication and year were considered random effects. Interactions and main effects for all measured parameters were similar across years although there were differences in the magnitude of the response. As a result, the data was reanalyzed by pooling the 2 yr and considering year as a random effect in the model. Differences among N rates and growth stages were separated using the PDIFF option in PROC MIXED procedure, and responses were tested with orthogonal polynomial contrasts. When linear and quadratic or linear, quadratic, and cubic functions were detected, the functional form of the regression relation was determined by starting with the linear function, then adding successively higher order polynomials in conjunction with plotting the data and making visual observations. If it was determined that the higher order polynomial did not substantially improve the explanation of the response curve (based on r^2 values), then those higher order polynomials were ignored. Correlations among growth parameters, tissue nutrient concentration and content were determined by correlation analysis (PROC CORR). When correlations were detected, regressions were performed using PROC REG to quantify the relationship.

RESULTS AND DISCUSSION

Growing Season Conditions

Rainfall during the 2014–2015 and 2015–2016 growing seasons were 27 and 53 cm greater than the 30-yr long term growing season (November–May) rainfall (66 cm) (Fig. 1). The 3 mo preceding planting were slightly wetter in 2014 (24 cm) than in 2015 (20 cm). Rainfall during the vegetative growth phase (3 mo following planting) was 60 cm in both years, although December 2014 received 27cm of rainfall compared with 18 cm in

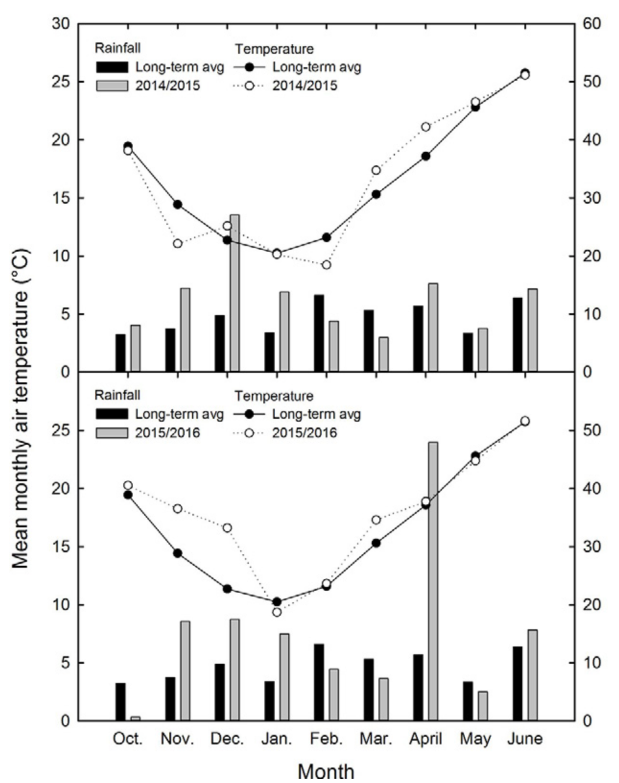

Fig. 1. Mean monthly air temperature, total monthly rainfall, and the 30-yr average by month for 2014-2015 and 2015-2016 winter/spring growing season in Quincy, FL.

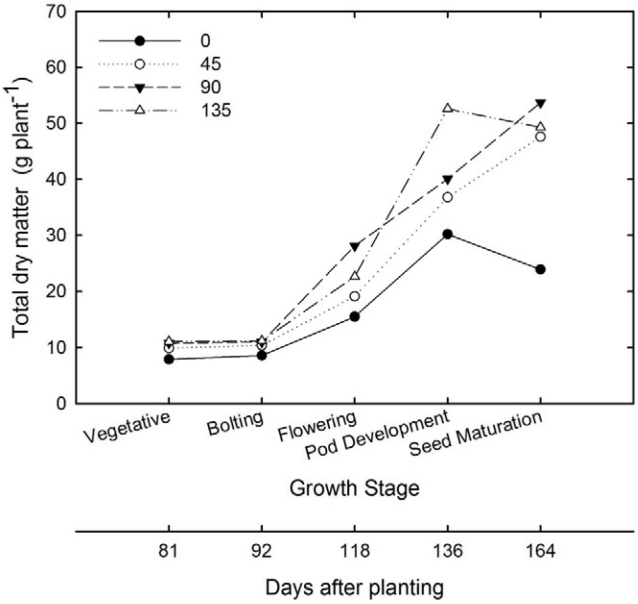

Fig. 2. Dynamics of carinata dry matter accumulation from vegetative to seed maturity as a function of nitrogen application rate. Data represents means of 2 yr and four N application rates.

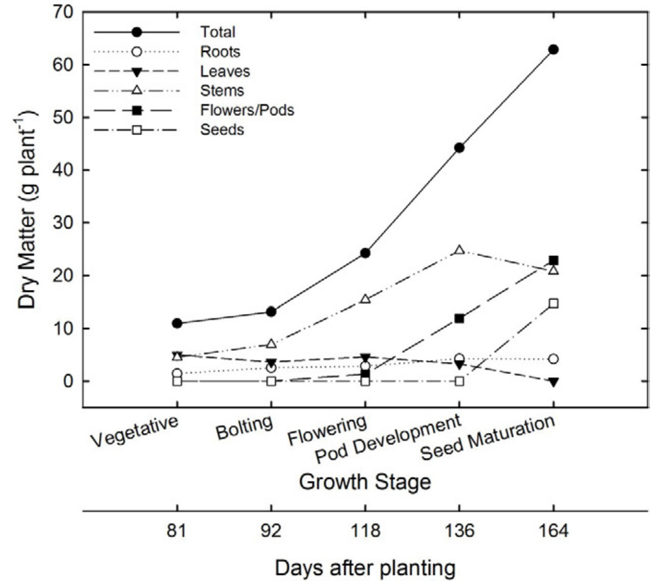

Fig. 3. Dry matter allocation to above- and belowground tissue as a function of nitrogen application. Data represent means of 2 yr and four N application rates.

December 2015. Pod development and seed maturation occurred during a wetter April in 2016 (48 cm) than 2015 (15 cm).

Mean temperatures during the growing season in 2014–2015 (14.9°C) were similar to the long-term mean (15.0°C) while in 2015–2016, temperatures were 1.5°C greater than the long-term mean (Fig. 1). The 3 mo following planting in 2015 were 3.5°C warmer than the same period in 2014. These variations in growing season conditions between the 2 yr did not have an effect on the response trends in carinata dry matter accumulation, allocation, seed and straw dry matter yield and nutrient uptake responses to N rates.

Carinata Dry Matter Accumulation

Plant density did not vary with N application rate and ranged from 67 to 72 plants m^{-2}, well within the 34 to 114 plants m^{-2} range associated with maximum seed yield (Pan et al., 2012). Carinata total dry matter (TDM) varied significantly with N rate × growth stage ($P = 0.043$). At the vegetative and bolting stages, TDM was similar across all N rates (Fig. 2). Postbolting DM accumulation generally increased with N application rate except at 0 and 135 kg N ha^{-1} where TDM was greatest at the pod development stage and declined thereafter. Across N rates, TDM accumulation increased by 107% from bolting to flowering, 87% from flowering to pod development and 9% from pod development to seed maturation.

Relative inter-growth stage accumulation of DM was modified by N availability (Fig. 2). When N was limited (0N), 33% of DM was accumulated from seedling to vegetative stage. An average of 21% of DM was accumulated during this growth period for all N treated plants. At 0, 45, and 135 kg N ha^{-1}, maximum (62, 37, and 61%, respectively) DM accumulation occurred from flowering through pod development stages. At 90 kg N ha^{-1}, 32% of TDM accumulated between bolting and flowering. At 0N and 135 kg N ha^{-1}, carinata DM decreased by 26 and 7% respectively from pod development to seed maturation. Final dry matter in the current study was more than 50% lower than previously reported with maximum (120 g plant^{-1}) accumulation at 150 kg N ha^{-1} (Kaur and Sidhu, 2004). Increased N availability stimulated traits related to dry matter including plant height, leaf area index, primary and secondary branching, and pod numbers similar to previous reports (Kaur and Sidhu, 2004; Punja et al., 2001).

Carinata Biomass Resource Allocation

The relative allocation of DM among carinata root, leaves, stems, flowers/pods, and seed did not differ with N application rate ($P > 0.05$), hence the data was pooled across N rate (Fig. 3). Leaf DM was 52% of TDM at the vegetative stage and sharply declined to 0 at the end of the growing season (Fig. 3). Stem DM increased from 48% at vegetative stage to 73% at flowering but declined to 37% at maturity. At the flowering stage, flower structures contributed 7% to TDM while at seed maturation, pods accounted for 39% of TDM. At harvest, seeds were 25% of TDM. The morphological plasticity and reallocation of resources confers carinata with the ability to withstand suboptimal conditions of nutrient availability is supported by previous findings (Seepaul et al., 2016b).

Carinata Root Characteristics

There was no interaction between growth stage and N rate, however, growth stage and N rate main effects on carinata root shoot ratio (RSR), root dry weight (RDW), lateral (LRL) and taproot length (TRL) existed ($P < 0.05$). Root shoot ratio was greatest at bolting (0.23) and declined gradually to 0.10 at seed maturation. The RDW, LRL, TRL increased by 185, 33, and 56% from vegetative to seed maturation growth stages (Table 2). The RSR decreased linearly with N rate while RDW, LRL, and TRL did not vary with N application (Table 2). A similar declining linear trend in root/shoot ratio response to N rate was previously reported, however the ratio in the current study is considerably lower (0.14–0.17) than that study (Seepaul et al., 2016b). Maximum lateral and tap root lengths also did not vary with N rate in a previous report (Seepaul et al., 2016b).

Table 2. Root to shoot ratio, root dry weight, and lateral and tap root length of carinata grown during 2014–2015 and 2015–2016 winter/spring growing seasons in Quincy, FL. Weights do not include root exudates.

Growth stage	Root:shoot ratio	Root dry weight	Lateral root length	Tap root length
		— g plant^{-1} —	— cm —	
Vegetative	0.15	1.48	17.4	17.2
Bolting	0.23	2.78	17.3	21.6
Flowering	0.14	3.16	17.7	21.6
Pod development	0.11	4.23	20.9	23.1
Seed maturation	0.10	4.22	23.2	26.8
OPC†	L***, Q***‡	L***	L***	L***
N rate (kg ha^{-1})				
0	0.17	3.34	20.2	23.3
45	0.16	3.16	19.7	21.1
90	0.13	3.10	18.6	21.8
135	0.14	3.09	19.8	22.0
Mean		3.17	19.6	22.1
OPC	L***	ns	ns	ns

† OPC, orthogonal polynomial contrasts (L, linear; Q, quadratic).
‡ *** represents linear (L), quadratic (Q) significant at the 0.001 level. ns, not significant.

Seed and Straw Dry Matter Yield and Harvest Index

Oilseed brassica are generally highly responsive to N fertilizer application (Gan et al., 2007; Johnson et al., 2013; Montemurro et al., 2016). The main effect of N rate on carinata seed ($P = 0.0261$) and straw ($P = 0.0012$) yields were significant. A quadratic equation expressed the relationship between N application rate and carinata seed and straw yield. Maximum carinata yields (2798 kg ha^{-1}) occurred at 102.3 kg N ha^{-1} (Fig. 4), which was considerably lower than 150 kg N ha^{-1} needed to produce 1780 kg ha^{-1} in spring planted carinata in Canada (Pan et al., 2012). The economic optimum N rate, which is the rate of N that maximizes profitability occurred at 93 kg N ha^{-1}, which produced 2791 kg seed ha^{-1} representing a \$306 ha^{-1} profit margin. Maximum carinata yields also occurred below the recommended N rates for winter canola in the SE which ranged from 140 to 160 kg ha^{-1} (Porter, 1993; Wright, 2010). In addition, the agronomic optimum N rate is on the lower end of the range (100 to 160 kg N ha^{-1}) reported for seed yields to plateau in various *Brassica* species in the Great Plains (Gan et al., 2007). In another study, maximum yield (1815 kg ha^{-1}) occurred at the highest N rate evaluated (90 kg N ha^{-1}), which was 47% greater than the control (Punja et al., 2001). In Italy, 100 kg N ha^{-1} produced 1770 kg seed ha^{-1}, 29% greater than the 0N control (Montemurro et al., 2016).

In the current study, maximum straw yield (7134 kg ha^{-1}) was estimated to be obtained at 112–115 kg N ha^{-1} (Fig. 4). In Canada, mean straw yield also showed a positive response to N application, reaching maximum 6127 kg ha^{-1} at 200 kg N ha^{-1} (Johnson et al., 2013) or 100 kg N ha^{-1} in various *Brassica* species (Gan et al., 2007). Seed yield positively correlated with straw yield ($r = 0.80365$, $P = 0.0002$) ($y = 0.3463x + 1356.6$; $r^2 = 0.875$), suggesting that high DM accumulation at all growth stages throughout the crop growth cycle in stress-free conditions is key to optimizing oilseed yield components and yield (Zhang and Flottmann, 2016). Harvest index increased curvilinearly ($y = -5\text{E-}06x^2 + 0.001x + 0.299$; $r^2 = 0.99$) with N application

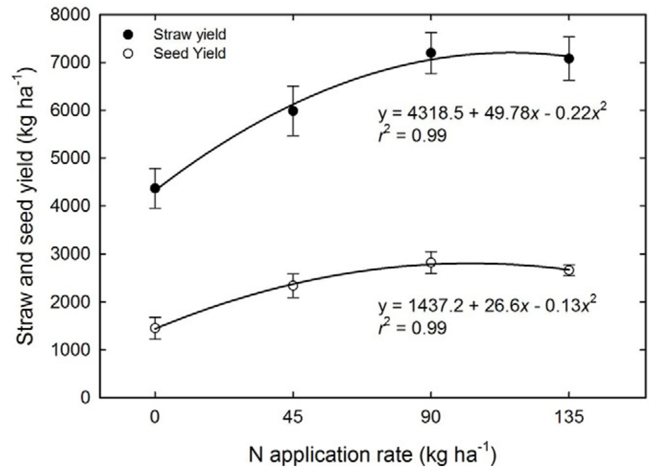

Fig. 4. Carinata seed and straw yield grown during 2014-2015 and 2015-2016 winter/spring growing seasons in Quincy, FL.

rate from 0.30 to 0.34 at 0 and 135 kg N ha^{-1}, respectively (data not shown).

Seasonal Tissue Nutrient Concentration

There was a growth stage and N rate interaction on aboveground ($P = 0.0157$) and belowground N concentrations ($P = 0.0347$) (Table 3). Across N rates, N concentrations in above and belowground tissue decreased across growth stages, however, the magnitude of the response differed among N rates, contributing to the interaction effect. For 0 N treatment, N concentration of aboveground tissue decreased by 66% from vegetative to seed maturation stages while at 135 kg N ha^{-1}, this decrease was 56% (Table 3). The seasonal decrease in N concentration of belowground tissue was similar (70.5%) at 0 and 135 kg N ha^{-1} (Table 3).

The growth stage × N rate interaction did not have an effect for all other measured above- or belowground tissue elemental (P, K, Ca, Mg, S, B, Mn, Fe, Zn, and Cu) concentrations ($P > 0.05$), however, growth stage and N rate main effects existed for several of the elements tested. Across the growing season, the concentrations of all other measured elements in the aboveground tissue decreased from the vegetative to seed maturation stages for all nutrients except for Zn and Cu (Table 4). Aboveground tissue K, S, and Mn concentrations varied with N rate (Table 4). Similar to the aboveground tissue concentration, root P, K, Ca, Mg, and S decreased with N rate over the growing season (Table 5). Root nutrient concentration was consistently lower than shoot concentration for all measured elements except for Fe and Cu (Table 5). With the exception of P, N rate did not have an effect on the root nutrient concentration (Table 5). This study has demonstrated the temporal changes in nutrient concentration of above- and belowground carinata tissue in response to N nutrition. The consistent decline in macro and micronutrient concentrations with plant age is typical of higher plants and is associated with nutrient dilution on a concentration basis of assimilated and stored nutrients by carbon rich DM as plant development advances (Marschner, 1995).

Seasonal Nutrient Uptake

The growth stage × N rate interaction did not show significant effects ($P > 0.05$) on nutrient uptake for all elements,

Table 3. Growth stage × N application rate interaction effect on aboveground tissue N concentration of carinata grown during 2014–2015 and 2015–2016 winter/spring growing seasons in Quincy, FL.

	N application rate (kg N ha^{-1})			
	0	45	90	135
Growth stage	Aboveground tissue concentration			
	g kg^{-1}			
Vegetative	23.5	23.5	24.6	24.8
Bolting	23.2	27.1	29.7	20.9
Flowering	12.9	20.9	23.3	20.1
Pod development	9.8	7.2	15.5	12.1
Seed maturation	7.9	9.5	10.5	10.8
OPC†	L***, Q*‡	L**	L**, Q**	L***, Q*
	Belowground tissue concentration			
Vegetative	13.3	13.0	11.1	15.6
Bolting	8.1	11.5	14.6	10.8
Flowering	6.0	8.2	9.0	7.9
Pod development	5.6	5.2	9.0	5.4
Seed maturation	4.0	4.4	5.9	4.5
OPC	L**, Q**‡	L***, Q**	L*, Q**	L***, Q**

† OPC, orthogonal polynomial contrasts (L, linear; Q, quadratic).
‡ *, **, and *** represent linear (L), quadratic (Q) significant at the 0.05, 0.01, and 0.001 levels, respectively.

however, significant growth stage and N rate main effects existed (Table 6). Maximum N uptake (73 kg N ha^{-1}) occurred between 50% bolting and 50% flowering while maximum P, K, Ca, Mg, S, B, Mn, Fe, Zn, and Cu uptake occurred between 50% flowering and pod formation (Table 6). Maximum N uptake occurred at 90 kg N ha^{-1} while all other elements had maximum uptake at 135 kg N ha^{-1} (Table 6). The trend in N uptake across the growing season was quadratic while the remainder of the measured elements linearly increased over the growing seasons.

The magnitude of nutrient uptake varied by element and was regulated by N availability. The rate of nutrient uptake in descending order was K > N > Ca > S > P > Mg > Cu > Fe > Zn > B > Mn. Nutrient uptake was closely related to biomass production (*r* ranged from 0.87 to 0.94). Increasing N availability increased nutrient uptake of measured elements primarily due to increased DM production.

Seed and Straw Nutrient Concentration

Seed N concentration varied with N application rate ($P < 0.0001$), increasing linearly with N rate while the remainder of the tested nutrients remain unchanged with N rate (Table 8). Averaged over N rates, seed nutrient concentrations on a DM basis ranked in descending order were N > S > K > P > Ca > Mg > Fe > Zn > Mn > B > Cu. Nitrogen and S are essential for the synthesis of lipid, protein, and glucosinolates in oilseed brassicas (Anjum et al., 2012; Malhi et al., 2007), hence the greater concentration of these elements in the seeds.

Nitrogen rate main effects were also significant for straw N ($P = 0.0341$), S ($P = 0.0235$), and Zn ($P = 0.0112$) nutrient concentrations while the remainder of the measured elements (P, K, Ca, Mg, B, Mn, Fe, and Cu) did not change with N rate (Table 7). Straw N, S, and Zn concentration increased linearly with N application rate. Prakash et al. (2000) also found that N rate had no effect on carinata seed or straw P concentration. Straw tissue concentration was consistently lesser than seed tissue concentration by a factor of 1–6, which can be attributed to post-bolting translocation of nutrients from leaves and stems to seeds (Papantoniou et al., 2013). Averaged over N rate, the straw tissue concentration on a DM basis ranked in descending order was K > Ca > N > S > Mg > P > Fe > Zn > Mn > B > Cu.

Seed, Straw, and Total Nutrient Uptake

Nitrogen rate main effects were significant for all calculated seed, straw and total nutrient uptake ($P > 0.05$) except for straw Cu and total uptake. The amount of nutrients in the seed followed a similar quadratic trend as seed yield response to N application rate (Table 8). Seed N uptake varied from 47 to 100 kg N ha^{-1}, was within the range previously reported for carinata and other *Brassica* species (Johnson et al., 2013; Prakash et al., 2000). Estimated maximum seed N uptake (100 kg N ha^{-1}) occurred between 100 and 105 kg N ha^{-1}. A previous study reported that seed nutrient uptake in response to N application did not plateau but rather increased linearly with 104 kg N ha^{-1} removed

Table 4. Growth stage and N application rate effect on aboveground tissue P, K, Ca, Mg, S, B, Mn, Fe, Zn, and Cu concentration of carinata grown during 2014–2015 and 2015–2016 winter/spring growing seasons in Quincy, FL.

Growth stage	P	K	Ca	Mg	S	B	Mn	Fe	Zn	Cu
	g kg^{-1}					mg kg^{-1}				
Vegetative	3.3	47.2	14.7	2.5	7.3	26.2	22.6	67.6	25.3	1.9
Bolting	3.3	32.3	11.0	2.2	6.1	18.8	17.1	51.9	25.7	3.4
Flowering	2.4	27.6	8.9	2.0	5.6	19.1	18.7	38.4	28.0	2.3
Pod Development	2.0	23.9	7.3	1.7	5.4	17.7	18.1	32.1	26.7	2.2
Seed Maturation	1.8	18.2	6.3	1.6	4.5	17.4	14.7	30.6	25.2	2.0
Mean									26.4	2.5
OPC†	L*, Q*‡	L**, Q*	L**, Q*	L***, Q*	L***, Q**	Q**	L**	L***, Q**		
N rate (kg N ha^{-1})										
0	2.6	25.4	9.4	1.9	4.8	19.0	15.7	43.9	21.3	2.4
45	2.6	30.1	9.6	2.0	5.9	20.1	18.8	48.0	28.2	2.1
90	2.4	32.8	9.9	2.0	6.5	20.2	19.5	45.7	28.8	2.6
135	2.6	31.0	9.8	2.0	5.9	20.1	18.9	44.8	26.4	2.4
Mean	2.5		9.6	2.0		19.8		45.6		2.3
OPC	ns	L**, Q*	ns	ns	L**, Q**	ns	L*	ns	L*, Q**	ns

† OPC, orthogonal polynomial contrasts (L, linear; Q, quadratic).
‡ *, **, ***, or ns represent linear (L), quadratic (Q) significant at the 0.05, 0.01 and 0.001 levels, respectively. ns, not significant.

Table 5. Growth stage and N application rate effect on belowground tissue P, K, Ca, Mg, S, B, Mn, Fe, Zn, and Cu concentration of carinata grown during 2014–2015 and 2015–2016 winter/spring growing seasons in Quincy, FL.

Growth stage	P	K	Ca	Mg	S	B	Mn	Fe	Zn	Cu
	g kg^{-1}					mg kg^{-1}				
Vegetative	2.8	29.7	3.5	1.3	3.5	13.4	17.1	595.3	21.9	2.3
Bolting	3.0	19.5	3.1	1.3	2.8	10.0	9.5	226.0	20.9	3.6
Flowering	1.6	11.3	2.8	1.1	1.9	6.5	11.6	172.2	22.7	3.2
Pod development	1.2	14.8	3.2	1.1	2.1	14.7	16.7	297.3	19.2	5.2
Seed maturation	0.6	12.9	3.7	1.0	2.3	11.3	11.1	323.3	15.6	3.0
Mean							12.2	254.7	19.6	3.8
OPC†	L***, Q**‡	L*	Q*	L*	Q*	ns	ns	ns	ns	ns
N rate (kg N ha^{-1})										
0	2.2	16.8	3.2	1.2	2.4	10.7	14.1	380.5	20.0	3.7
45	1.9	17.3	3.3	1.2	2.4	10.2	10.9	326.7	18.9	3.2
90	1.6	18.9	3.5	1.2	2.7	13.6	16.8	278.8	21.9	4.2
135	1.8	17.6	3.1	1.2	2.4	10.3	10.9	315.7	19.4	2.8
Mean		17.7	3.3	1.2	2.5	11.2	13.2	325.4	20.1	3.5
OPC	L**, Q**	ns	ns	ns	ns	ns	ns	ns	ns	ns

† OPC, orthogonal polynomial contrasts (L, linear; Q, quadratic).

‡ *, **, and *** represent linear (L), quadratic (Q) significant at the 0.05, 0.01 and 0.001 levelsrespectively. ns, not significant.

at an application rate of 120 kg N ha^{-1} (Prakash et al., 2000). Averaged over N rate, the seed nutrient uptake in descending order was N > K = S > P > Ca = Mg > Fe > Mn > B > Cu.

Straw nutrient uptake increased linearly (N, K, Ca, Mg, S, B, Mn, Fe, Zn) and curvilinearly (P) with N rate for all nutrients measured except for Cu (Table 8). Straw N uptake were comparable to a previous report (Prakash et al., 2000). Straw nutrient concentration in descending order was K > Ca > N > S > Mg > P > Fe > Zn > Mn > B > Cu.

Total (seed + straw uptake) nutrient uptake followed similar linear or quadratic trends in response to N rate as seed uptake (Table 8). Averaged over N rates, total nutrient uptake in descending order was N > K > Ca > S > P > Mg > Fe > Zn > Mn > B > Cu. Similar to oilseed rape (Rossato et al., 2001), carinata is efficient at extracting mineral N from the soil profile. At 0 and 45 kg N ha^{-1}, carinata extracted 73 and 72 kg ha^{-1} or 60% more N than applied, respectively. As N increased to 90 and 135 kg N ha^{-1}, N extracted was 57 (63%) and 16 (12%) kg N ha^{-1}, respectively, in excess of the N application rate.

In soils of the Southeastern United States where leaching potential is high, optimum and judicious nutrient applications informed by the 4 R Nutrient Stewardship Framework will have direct implications on nutrient use efficiency. Our results demonstrated that carinata requires N fertilizers to produce high seed yields, however, to increase the uptake and utilization efficiency, fertilizer application will have to be informed by soil test recommendations and timed with crop growth stages with maximum uptake. Carinata can be considered as a scavenger of soil N, utilizing up to 73 kg ha^{-1} from the soil pool that may be leached out of the root zone into groundwater. Post-harvest straw decomposition and mineralization of N and other elements may improve soil physical and chemical properties while providing nutrient credits for succeeding summer crops. This pre- and post-season flux of soil nutrients in carinata production systems will contribute to the overall sustainability of winter carinata in the Southeastern United States.

CONCLUSIONS

Carinata growth, resource allocation, seed, and straw N concentration and uptake are highly responsive to N application

Table 6. Growth stage and N application rate effects on seasonal N, P, K, Ca, Mg, S, B, Mn, Fe, Zn, and Cu nutrient uptake of carinata grown during 2014–2015 and 2015–2016 winter/spring growing seasons in Quincy, FL.

Growth stage	N	P	K	Ca	Mg	S	B	Mn	Fe	Zn	Cu
	kg ha^{-1}						g kg^{-1}				
Vegetative	37.4	5.0	73.3	22.8	3.8	11.3	39.9	35.2	103.3	38.7	103.3
Bolting	54.1	6.9	67.9	23.3	4.7	12.8	39.4	35.5	105.1	53.7	105.1
Flowering	73.0	8.6	100.2	32.3	7.2	20.2	69.4	67.6	138.5	100.1	138.5
Pod development	71.5	12.0	151.3	46.1	10.2	33.8	112.0	117.3	200.6	170.8	200.6
Seed maturation	65.4	11.4	121.9	41.5	10.2	30.2	113.4	97.8	112.2	163.8	274.6
Mean									131.9		
OPC†	L***, Q**‡	L***	L***	L***	L***	L***	L***	L***		L***	L***, Q*
N rate (kg N ha^{-1})											
0	40.6	7.6	69.3	25.5	5.6	13.6	56.1	44.9	98.1	64.0	122.8
45	51.1	7.9	95.8	30.8	6.7	20.4	69.5	67.0	125.7	102.5	146.0
90	75.3	8.6	116.2	35.0	7.5	25.0	77.9	78.7	125.1	120.1	189.0
135	74.2	11.0	130.5	41.6	9.1	27.6	95.7	92.0	178.8	135.2	200.0
OPC	L***	L***	L***	L***	L***	L***	L***	L***	L**	L***	L***

† OPC, orthogonal polynomial contrasts (L, linear; Q, quadratic).

‡ *, **, and *** represent linear (L), quadratic (Q) significant at the 0.05, 0.01 and 0.001 levels, respectively.

Table 7. Nitrogen application rate effects on seed and straw N, P, K, Ca, Mg, S, B, Mn, Fe, Zn, and Cu nutrient concentration of carinata grown during 2014–2015 and 2015–2016 winter/spring growing season in Quincy, FL.

N rate (kg N ha^{-1})	N	P	K	Ca	Mg	S	B	Mn	Fe	Zn	Cu
	Seed nutrient concentration										
	g kg^{-1}						mg kg^{-1}				
0	36.7	9.5	10.8	4.4	4.4	11.1	12.2	29.5	77.9	51.0	5.4
45	38.0	9.1	10.6	4.2	4.3	11.2	13.2	30.5	78.1	51.5	5.4
90	39.7	8.9	10.4	4.3	4.2	11.8	13.4	30.6	84.6	57.3	5.2
135	40.5	8.9	10.4	4.4	4.1	12.0	13.2	31.3	83.4	55.8	5.0
Mean		9.1	10.6	4.3	4.2	11.5	13.0	30.5	81.0	53.9	5.2
OPC†	L***‡	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
	Straw nutrient concentration										
0	6.4	0.79	7.7	6.9	1.4	2.1	12.4	16.9	31.7	20.4	3.9
45	6.3	0.59	7.8	6.7	1.3	2.2	12.7	13.1	23.0	20.3	2.1
90	6.9	0.61	9.6	7.1	1.3	2.8	13.8	14.1	29.8	27.3	2.8
135	7.8	0.81	8.2	7.1	1.4	3.0	13.3	16.1	37.8	27.9	2.8
Mean		0.7	8.3	6.9	1.3		13.0	15.1	30.6		2.9
OPC	L**	ns	ns	ns	ns	L*	ns	ns	ns	L*	ns

† OPC, orthogonal polynomial contrasts (L, linear; Q, quadratic).

‡ *, **, and *** represent linear (L), quadratic (Q) significant at the 0.05, 0.01 and 0.001 levels, respectively. ns, not significant.

in the seasonally high rainfall environment of North Florida. Carinata DM accumulation increased with increasing N application rate; however, the relative allocation of DM to roots, leaves, stems, flowers/pods and seed were similar regardless of the N application rate. Maximum carinata yields (2798 kg ha^{-1}) were produced at 102.3 kg N ha^{-1} while the economic optimum N rate occurred at 93 kg N ha^{-1}. Maximum N uptake occurred between 50% bolting and 50% flowering while maximum P, K, Ca, Mg, S, B, Mn, Fe, Zn, and Cu uptake occurred between 50% flowering and pod formation. Nitrogen concentration in seed and straw increased with N rate while there was little or no effect on P, K, Ca, Mg, S, B, Mn, Fe, Zn, and Cu concentrations. Seed and straw nutrient uptake increased with increased N rate primarily due to the strong relationship between uptake and dry matter production. At 90 kg N ha^{-1}, carinata total N uptake was 147 kg N ha^{-1}, which was 63% more N than what was applied, demonstrating its efficiency in extracting N from the soil profile.

Although carinata is a highly plastic species that compensates by reallocating DM to maintain root shoot balance when N is limited, adequate N is required (93 kg N ha^{-1}) for optimizing productivity in sandy loam soils in North Florida. This study identified that the maximum N uptake occurs between 50% bolting and 50% flowering. These findings will aid in refining N management strategies to time N application with critical growth stages and thereby optimize growth and productivity.

Table 8. Nitrogen application rate effects on seed, straw and total N, P, K, Ca, Mg, S, B, Mn, Fe, Zn, and Cu nutrient uptake of carinata grown during 2014–2015 and 2015–2016 winter/spring growing season in Quincy, FL.

N rate (kg N ha^{-1})	N	P	K	Ca	Mg	S	B	Mn	Fe	Zn	Cu
	Seed uptake										
	kg ha^{-1}						g ha^{-1}				
0	46.6	12.9	15.1	6.0	6.0	13.4	18.2	42.1	111.9	69.8	8.5
45	80.1	19.5	24.8	9.2	9.6	23.1	33.2	72.7	179.3	114.3	13.0
90	100.1	22.3	29.1	11.3	11.1	28.9	40.8	87.2	233.5	151.9	14.6
135	97.5	22.0	27.4	11.2	10.6	27.8	36.7	84.4	211.9	137.4	13.8
OPC†	L***, Q***‡	L***, Q***	L***, Q***	L***, Q**	L***, Q***	L***, Q***	L***, Q***	L***, Q***	L***, Q***	L***, Q***	L***, Q*
	Straw uptake										
0	26.1	3.0	28.3	29.6	5.2	7.1	52.2	59.4	124.8	78.1	18.7
45	36.9	2.7	42.0	39.9	6.5	11.5	76.8	69.1	126.1	108.4	10.7
90	46.8	3.6	60.7	51.7	8.2	17.1	99.5	92.9	199.7	174.6	19.9
135	53.1	5.4	56.2	49.7	9.2	20.3	93.3	111.4	245.2	190.6	19.6
Mean											17.2
OPC	L***	Q*	L***	L**	L***	L**	L***	L***	L*	L***	ns
	Total (seed + straw) uptake										
0	72.7	15.9	43.4	35.6	11.3	20.5	70.4	101.6	236.8	147.9	27.2
45	116.9	22.2	66.7	49.2	16.1	34.5	110.0	141.8	305.4	222.7	23.7
90	146.9	25.9	89.8	63.0	19.4	46.0	140.4	180.1	433.2	326.5	34.5
135	150.6	27.5	83.6	60.8	19.8	48.1	130.0	195.8	457.0	327.9	33.4
Mean											29.7
OPC	L***, Q***	L***, Q***	L**, Q***	L***, Q***	L***, Q***	L***, Q***	L**, Q***	L***, Q***	L***, Q***	L***, Q***	ns

† OPC, orthogonal polynomial contrasts (L, linear; Q, quadratic).

‡ *, **, and *** represent linear (L), quadratic (Q) significant at the 0.05, 0.01 and 0.001 levels, respectively. ns, not significant.

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