Published online February 7, 2019

BIOFUELS

Brassica carinata Seeding Rate and Row Spacing Effects on Morphology, Yield, and Oil

Michael J. Mulvaney,* Ramon G. Leon,* Ramdeo Seepaul, David L. Wright, and Travis L. Hoffman

ABSTRACT

Carinata (*Brassica carinata* A. Braun) is an oilseed crop with potential as a winter crop in the southeastern United States to diversify crop rotations and provide biofuel production and livestock feed. The objective was to evaluate the effects of row spacing and seeding rate on carinata yield and oil composition. Field experiments were conducted in Jay and Quincy, FL, from 2013 to 2016 evaluating carinata growth, seed and oil yield, and oil composition grown in a factorial arrangement of four seeding rates (3, 6, 9, and 12 kg ha^{-1}) and four row spacings (18, 36, 53, and 89 cm). No interactions between seeding rate and row spacing were detected. Seeding rate did not influence any of the variables studied. In contrast, row spacing affected seed and oil yield, branch production, and pods per plant. Seed yield (ranked from highest to lowest) was 2761, 2286, 1851, and 1572 kg ha^{-1} for rows spaced at 36, 18, 53, and 89 cm, respectively. Branching and pods per plant increased with row spacing. Neither seeding rate nor row spacing affected oil concentration and quality. Oil concentration averaged 40%, of which more than a third was erucic acid (C22:1). Protein concentration was 31%, and glucosinolate concentration was 93 μmol g^{-1}. The results of the present study demonstrated that carinata can be successfully grown in the southeastern United States, reaching yields and oil quality similar to those reported at other latitudes, and can be a source of biofuel, protein for animal feed, and cropping system diversification for growers.

Core Ideas

- Carinata growth and yield was influenced more by row spacing than seeding rate.
- Rows spaced at 36 cm maximized carinata yield.
- Carinata branching was favored by wider row spacing.

Published in Agron. J. 111:1–8 (2019)
doi:10.2134/agronj2018.05.0316
Supplemental material available online
Available freely online through the author-supported open access option

Liquid biofuels are an important component for the transition to renewable energy–based systems (Perlack et al., 2005). Bioethanol derived from sugar fermentation processes from sugarcane (*Saccharum* L.), sugar beet (*Beta vulgaris* L.), and corn (*Zea mays* L.) represents the most common biofuel, which is usually mixed with gasoline (Perlack et al., 2005). When used as oilseed crops to produce biodiesel, soybean [*Glycine max* (L.) Merr.] and oilseed rape (*Brassica napus* L.) generate short hydrocarbon chains, so energy-consuming processes are required to generate the longer hydrocarbon chains needed to produce high-energy fuels (Bona et al., 1999; Perlack et al., 2005). Furthermore, there is concern about using traditional food crops for bioenergy production (Erb et al., 2012; Johansson and Azar, 2007).

Carinata (*Brassica carinata* A. Braun), also known as Ethiopian or Abyssinian mustard, is a species with high erucic acid concentration, which facilitates conversion to biofuel (Cardone et al., 2003). It is thought to have originated in northeastern Africa, where it has been cultivated since at least 3000 BC (Alemayehu and Becker, 2002; Simmonds, 1979). This species is the result of chromosome duplication after a hybrid cross between *Brassica nigra* (L.) W.D.J. Koch and *Brassica oleracea* L. (Alemayehu and Becker, 2002; Gómez-Campo and Prakash, 1999; Nagaharu, 1935). Although related species, such as canola and oilseed rape (*Brassica napus* L.), have been commonly grown for oil production in Eurasia and North America (Bona et al., 1999; Perlack et al., 2005), there is current interest in the use of carinata as a winter crop to produce biofuel in subtropical regions. Carinata seems to be more tolerant to warmer environments than canola and oilseed rape (Seepaul et al., 2016). It also has greater shattering tolerance than canola and has a high concentration of very-long-chain fatty acids, such as erucic acid, which allows the production of high-energy biofuels, such as jet fuel, with less energy required during refinement (Choudhary et al., 2000; Prakash and Chopra, 1988; Seepaul et al., 2016). Furthermore, because carinata exhibits glucosinolate levels that might negatively affect human health, it is not considered a food crop. However, through breeding and/or refinement, glucosinolate concentrations can be reduced to levels that allow the use of carinata as seed meal for animal feed after oil extraction (Rosenthal et al., 2017).

M.J. Mulvaney, West Florida Research and Education Center, Univ. of Florida, 4253 Experiment Drive, Highway 182, Jay, FL 32565; R.G. Leon, Dep. of Crop and Soil Sciences, North Carolina State Univ., Campus Box 7620, Raleigh, NC 27695; R. Seepaul, D.L. Wright, North Florida Research and Education Center, Univ. of Florida, 155 Research Road, Quincy, FL 32251; T.L. Hoffman, Agrisoma Biosciences Inc., 110 Gymnasium Place, Saskatoon, SK, Canada, S7N 0W9. Received 11 May 2018. Accepted Oct. 25, 2018.
*Corresponding authors (m.mulvaney@ufl.edu, rleon@ncsu.edu).

Unlike related species such as oilseed rape, there is limited information about production requirements for carinata, particularly in the southeastern United States, where carinata production has been recently introduced on a commercial scale (Agrisoma Biosciences Inc., 2017). The lack of basic agronomic information, including planting, fertilization, and pest management strategies, limits production of this new biofuel crop. Previous research has been conducted on carinata as a spring/summer crop in Canada (Pan et al., 2012; Warwick et al., 2006) and as a winter crop in Mediterranean climates and India (Cardone et al., 2003; Kaur and Sidhu, 2004; Setia et al., 1995; Zanetti et al., 2009). Most of the information generated in those studies indicated that oil concentration and composition tend to be more stable traits than yield. Therefore, agronomic cultural practices that maximize yield need to be identified, particularly for production in the southeastern United States.

Row spacing and seeding rate are cultural practices that may influence yield and oil concentration and are among the first management decisions carinata growers face. Compatibility with existing planting equipment and carinata row spacing requirements facilitates carinata in existing crop rotations. Also, because carinata has limited herbicide options, fast growth and canopy closure using adequate seeding rates and row spacings can favor weed suppression (Leon et al., 2017; O'Donovan, 1994). Optimization of seeding rate and row spacing are critical to maximize harvested yield in oilseed rape (Kuai et al., 2015). Responses to row spacing and seeding rate vary considerably across *Brassica* species and depend on environmental conditions. For example, brown sarson (*Brassica campestris* L. var. *dichotoma* Watt.) seed yield did not respond to changes in row spacing under limited soil moisture conditions, but oil concentration increased proportionally to row spacing. Conversely, under adequate water conditions, seed yield increased and oil concentration decreased at wider row spacing (Singh and Yusuf, 1979). Oilseed rape yield had a positive response to increasing planting density until populations reached 50 to 60 plants m^{-2}, with no further yield gains until populations reached 150 plants m^{-2}, at which point yield decreased (Leach et al., 1999). In Canada, maximum seed yield of carinata was achieved over a wider range, from 34 to 114 plants m^{-2} (Pan et al., 2012), which provides evidence of carinata yield resilience to a wide range of plant stand densities. At 20 cm row spacing, white mustard (*Sinapis alba* L.) exhibited a negative yield response to increases in plant density, although branching and pod number had a positive relationship with plant density (Hassan and Arif, 2012).

Row crop growers in the southeastern United States are exploring opportunities to grow carinata as a winter crop to diversify their cropping systems and increase profitability. The biofuel industry is interested in promoting the production of bioenergy crops during the winter to complement summer production in temperate regions. However, there is little published information about carinata growth and performance from field trials in the southeastern United States.

The objective of the present research was to evaluate seed yield, oil yield, and oil composition of carinata grown in the southeastern United States as a winter crop under several seeding rates and row spacings. These variables were selected because studies in other related *Brassica* species determined that they are agronomic practices that highly influence yield potential and oil characteristics (Morrison et al., 1990; Wang et al., 2015; Zhang et al., 2012).

MATERIALS AND METHODS

Field trials were conducted during 5 site-years from 2013 - 2014 to 2015 - 2016. *Brassica carinata* 'Avanza' was planted in mid-November each year, and harvest was conducted in late May to early June the following year.

Trials were located at Quincy, FL (2013–2014, 2014–2015, and 2015–2016) and Jay, FL (2014–2015 and 2015–2016). The Quincy site was located at the North Florida Research and Education Center on a Dothan sandy loam (fine, loamy siliceous thermic Plinthic Kandiudults; 30.540822, −84.584114; 74 masl). The Jay site was situated at the West Florida Research and Education Center on a Red Bay sandy loam, 0 to 2% slopes (fine-loamy, kaolinitic, thermic Rhodic Kandiudults; 30.775672, −87.138864; 63 masl). Weather stations were located within 1 km of the research sites.

The experiments were designed as a factorial randomized complete block with four replications. Seeding rates were 3, 6, 9, and 12 kg seed ha^{-1} (2.7, 5.4, 8.1, and 10.8 lbs seed ac^{-1}), representing 846,500, 1,693,000, 2,540,000, and 3,386,000 seed ha^{-1}, assuming a thousand seed weight of 3.544 g. Row spacings were 18, 36, 53, and 89 cm (7, 14, 21, and 35 inches) apart. Plots were 3.66 m wide and 6.1 m long.

Soil was prepared by two passes of a disk cultivator in both locations and with a rotavator pass after disking in Jay, FL. Carinata seeds were planted at 1.3-cm depth with a vegetable planter (Jang Seeder JPH-U, Jang Automation Co. Ltd.) in Jay and at 0.6-cm depth with a Hege 1000 series offset cone planter in Quincy. Soils were limed and fertilized according to soil test recommendations for canola, with the exception of nitrogen (N), which was applied at a rate of 14 kg N ha^{-1} (12 lbs N ac^{-1}) as ammonium sulfate at planting and an additional 76 kg N ha^{-1} (68 lbs N ac^{-1}) as ammonium sulfate topdressed at bolting. The relatively low N rate at planting was meant to reduce frost susceptibility during early growth (Ragan and Nylund, 1977), which could be a problem in the Southeast when temperatures are below −7°C (20°F), based on preliminary trials. The total N rate was applied based on preliminary research conducted in Florida.

The number of days to 50% flowering, bolting, and maturity were recorded. Maturity was based on 50% of seed color change from yellow to orange-brown. Plant height was recorded by measuring the general top of the canopy within the middle of the plot. The number of primary and secondary branches was determined by collecting three representative plants in the middle of the plots at maturity and then counting the number of branches. These same three plants were used to determine the number of pods per plant, number of seeds per pod, seed weight per pod, pod length (average of 10 random pods), and number of nodes on the main stem. Seed weight per pod and dry weight per plant data were based on 1 site-year. Yield was determined using a plot combine in the middle rows of the plots and adjusting for harvested area (which varied depending on row width) and seed moisture at 8%. Oil yield was calculated by multiplying yield (at 8% moisture) by oil concentration and then dividing by an oil density of 0.9218 kg L^{-1} (7.693 lbs gal^{-1}), which is the density of canola oil (Sieverding et al., 2016).

Total glucosinolates, protein and oil concentration, and fatty acid composition were predicted using near-infrared reflectance spectroscopy. Samples were analyzed using a FOSS XDS Rapid Content Analyzer (FOSS Inc.) using a scanning range of 400 to 2498 nm in 0.5-nm increments. Sample spectra were evaluated using the ISIscan program (FOSS Analytical) using a proprietary carinata calibration (Agrisoma Biosciences Inc.) and included periodic calibration and validation samples. The proprietary prediction model developed by Agrisoma Biosciences Inc. used ^{1}H nuclear magnetic resonance spectroscopy (Oxford MARAN Ultra Benchtop NMR System, Oxford Instruments) as well as gas chromatography (Agilent 6890N; Agilent Technologies) to develop the underlying equation (O'Neill et al., 2003; Taylor et al., 1992).

Statistical analyses were conducted using PROC MIXED as implemented in SAS 9.4 (SAS, 2016) at the 95% confidence level unless otherwise indicated. Full models considered seed rate and row spacing and their interactions as fixed effects. Replication and replication interactions with fixed effects as well as site-year and site-year interactions with fixed effects were considered random. Means and SEM were generated using PROC MEANS. Multiple pairwise means separation tests were performed with the %PDMIX800 macro (Saxton, 1998) within SAS 9.4 using the least significant difference method at the 95% confidence interval within PROC MIXED. Correlation analyses were conducted using PROC CORR and were considered significant at $P < 0.05$.

Climate

During winter, the Florida panhandle is often subject to sudden freezing temperatures after a period of relatively warm weather (Fig. 1), which can increase frost susceptibility of the crop because there are insufficient chilling hours to harden off the crop after a period of rapid growth. In the first 2 yr of the study, there were a few nights during which temperature fell below 0°C, causing frost injury of foliar tissue predominantly on leaf margins and tips. The growing points of the majority of plants were not affected, and plant recovery was observed by production of new leaves. Recent breeding efforts and selection in northern Florida have focused on improving carinata "cold shock" tolerance in conditions representative of the southeastern United States. This region is also prone to sudden heavy rainfall events, which can affect crop stands particularly early in the season if the crop is located on soils that are not well drained.

RESULTS AND DISCUSSION

Main Effects

F-tests of fixed effects indicated interactions between site-years and main treatment effects (Supplemental Table S1). The interactions by site-year were the result of the magnitude of differences between treatments, but the trends of the responses to seeding rate and row spacing were similar across site-years. Therefore, data were analyzed pooling site-years and considering them as a random effect in the model. Although both seeding rate and row spacing influence plant access to resources and intraspecific competition, in *Brassica* species the interaction between these factors is not as important as their independent effect. Morrison et al. (1990) and Ozer (2003) reported no interactions between seeding rate and row spacing on yield or yield components for summer/spring oilseed rape. Similarly, Wang et al. (2015) found no interactions between those factors for seed yield in canola.

Yield and Yield Components

In the present study, the optimum row spacing of 36 cm averaged 2761 ± 251 (mean ± SE) kg ha^{-1} (Fig. 2), similar to yields reported for carinata grown during the winter in Italy (Cardone et al., 2003) and during the summer in Canada (Pan et al., 2012). The ability to obtain similar yields under temperate and subtropical conditions gives carinata a great deal of potential to balance bioenergy supply throughout the year if production areas were to be established across a wide latitudinal gradient. Furthermore, unlike oilseed rape (Momoh and Zhou, 2001), seed oil concentration was not decreased by increasing seeding rates. Likewise, in Canada, summer-grown carinata lines had similar oil concentration regardless of seeding rate (Pan et al., 2012).

Row spacing had a greater effect on yield and yield components than did seeding rate (Fig. 2). Oil and seed yields were greatest with 36-cm row spacing but not significantly different from 18-cm spacing, with a numerical advantage at the 36-cm spacing. Row spacings of 53 and 89 cm had the lowest oil and seed yields. As row spacing increased, yield was reduced and corresponded with increased secondary branching and numbers of pods per plant. This implies that increased secondary branching did not contribute to yield as much as primary branching, nor did greater numbers of pods per plant. It seems that the increased photosynthate production needed for secondary branching and pod formation comes with a yield penalty. The numbers of secondary branching and pods per plant increased with increasing row spacing, indicating the ability of the crop to promote secondary branching and pod formation as the distance between rows increased.

Within the range of treatments in this study, increased seeding rate numerically decreased yield, primary branches, and the number of pods per plant. Previous research has shown that intraspecific competition can reduce carinata yield. For example, using 15-cm row spacing, Pan et al. (2012) determined that carinata yield was maximized at 100 to 200 seeds m^{-2}, but increasing planting density to 400 seeds m^{-2} decreased yield up to 31% during some site-years. Carinata yield response to seeding rate was inconsistent during 9 site-years in Canada, where low-yielding environments were less responsive to seeding rate than high-yielding environments (Hossain et al., 2018). In oilseed rape, seed yield tends to be stable across increasing planting densities, usually due to compensations in branch and pod number per plant and seed number per pod (Degenhardt and Kondra, 1981; Pahkala et al., 1994; Sierts et al., 1987). However, at high densities the stability of the yield trait, measured as ecovalence, has been shown to decrease, and reduction in yield may occur (Sierts et al., 1987). It is possible that, in the present study, seeding rate did not affect yield as reported by Pan et al. (2012) because most row spacing treatments were considerably wider and intraspecific competition was more likely in the present study. Industry currently recommends a seeding rate of 4.5 to 6.7 kg ha^{-1} (4–6 lbs ac^{-1}) and a row spacing of 38 to 76 cm (15–30 inches) or twin rowed on 76-cm centers for a plant stand density of 65 to 108 plants m^{-2} (6–10 plants ft^{-2}) (Agrisoma Biosciences Inc., 2017). Maximum yield, obtained at 36-cm row spacing,

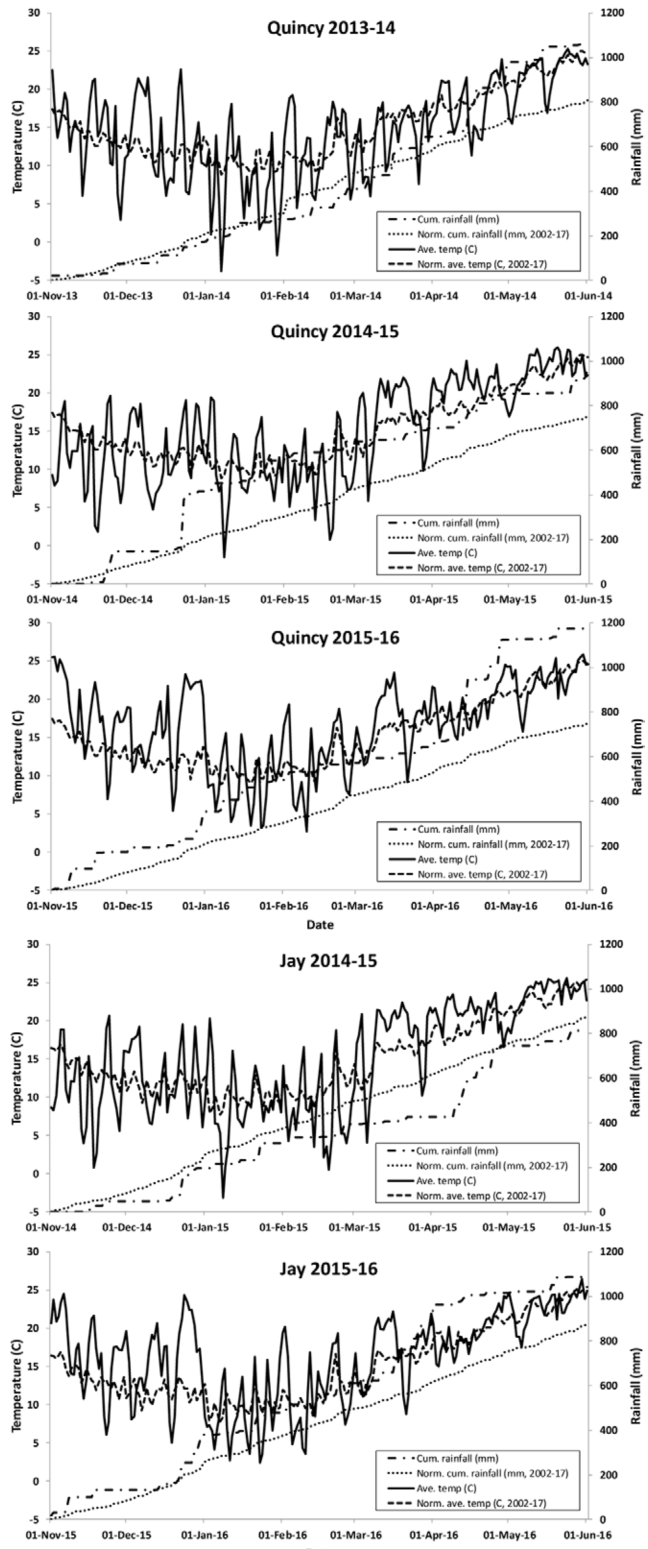

Fig. 1. Daily average temperatures and cumulative rainfall compared with 15-yr normals during 5 site-years in north Florida.

corresponded to a final plant stand density at harvest of 74 ± 6 plants m^{-2} (mean ± SE) (Fig. 2), corroborating those recommendations. It is important to note, however, that all emerged seedlings do not necessarily survive to the end of the season, so initial stand densities are not typically representative of final stands at harvest. Decreased plant populations with increased row spacing at harvest indicate that intraspecific competition "self-thinned" the crop. Indeed, the final stand density of 740,000 plants ha^{-1} at 36-cm row spacing represents a seeding rate of 3.0 kg ha^{-1} if all plants successfully established, assuming a seed weight of 250,000 treated seeds kg^{-1}. Because the lowest seeding rate was 3 kg ha^{-1}, this may be why seeding rate responses were not generally significant in the present study. However, given the small seed size and difficulty of precise depth placement, lower seeding rates are challenging with the equipment used in this trial as well as the equipment used in commercial production.

Most of the studies conducted in related *Brassica* crop species showed a positive relation between row spacing and branching. For example, in oilseed rape, it has been extensively documented that increasing row spacing favors production of primary and secondary branches (Kondra, 1977; McGregor, 1987; Morrison et al., 1990). In the present study, carinata exhibited a similar response, and, as row spacing increased, plant architecture responded with a linear increase in secondary branching over the treatment range [no. secondary branches plant^{-1} = 34.4 + 0.24 × row spacing (cm); R^2 = 0.91] (Fig. 2). Primary branching was maximized at 36-cm row spacing, which corresponded to the greatest yield. Similar to results reported by Angadi et al. (2003) and Clarke and Simpson (1978), increased plant spacing favored secondary branching and the production of more pods per plant, but this did not increase yield. In fact, yield was lowest at the two widest row spacings in the present study. Although correlation analyses showed a negative correlation between row spacing and yield (−0.20; P = 0.0003), row spacing was positively correlated with primary (0.22; P = 0.0023) and secondary (0.19; P = 0.0090) branching. Primary and secondary branching were also weakly positively correlated with yield (0.27 and 0.16, respectively; P < 0.03). Although increased row spacing increased branching, the resulting yield advantage from increased branching has limits. It seems that, under our experimental conditions, the increased photoassimilate demand created by secondary branching and pod formation came at the expense of photoassimilate translocation to seeds. Indeed, plant biomass was not altered by treatments (Table 1), even though secondary branching increased with increasing row spacing (Fig. 2). Setia et al. (1995) proposed that maintaining a functional leaf area during seed production is critical to supply the necessary photoassimilates to maintain greater numbers of branches and pods per plant.

Within the range of treatments in this study, higher seeding rates did not translate into higher yields, nor was plant architecture affected by this factor (Fig. 2). Zhang et al. (2012) reported that oilseed rape exhibited higher seed yield and oil concentration when increasing seeding rate, but carinata maintained constant seed yield and oil concentration regardless of seeding rate. The fact that planting arrangement played a more important role than seeding rate for the aforementioned growth parameters indicates that carinata exhibits a strong response to light availability and has enough phenotypic plasticity to modulate plant architecture to optimize light interception. This plasticity could also affect intraspecific competition, imposing a limit to the potential to increase yield by using higher population densities, as has been done in other crops (Egli, 1988; Wang et al., 2015). Our results indicate that lower planting densities would provide adequate populations to maximize yield. Thus, the recommendation for optimal yield in the southeastern United States is a seed rate of 3 kg ha^{-1} and 36-cm row spacing, although 18-cm

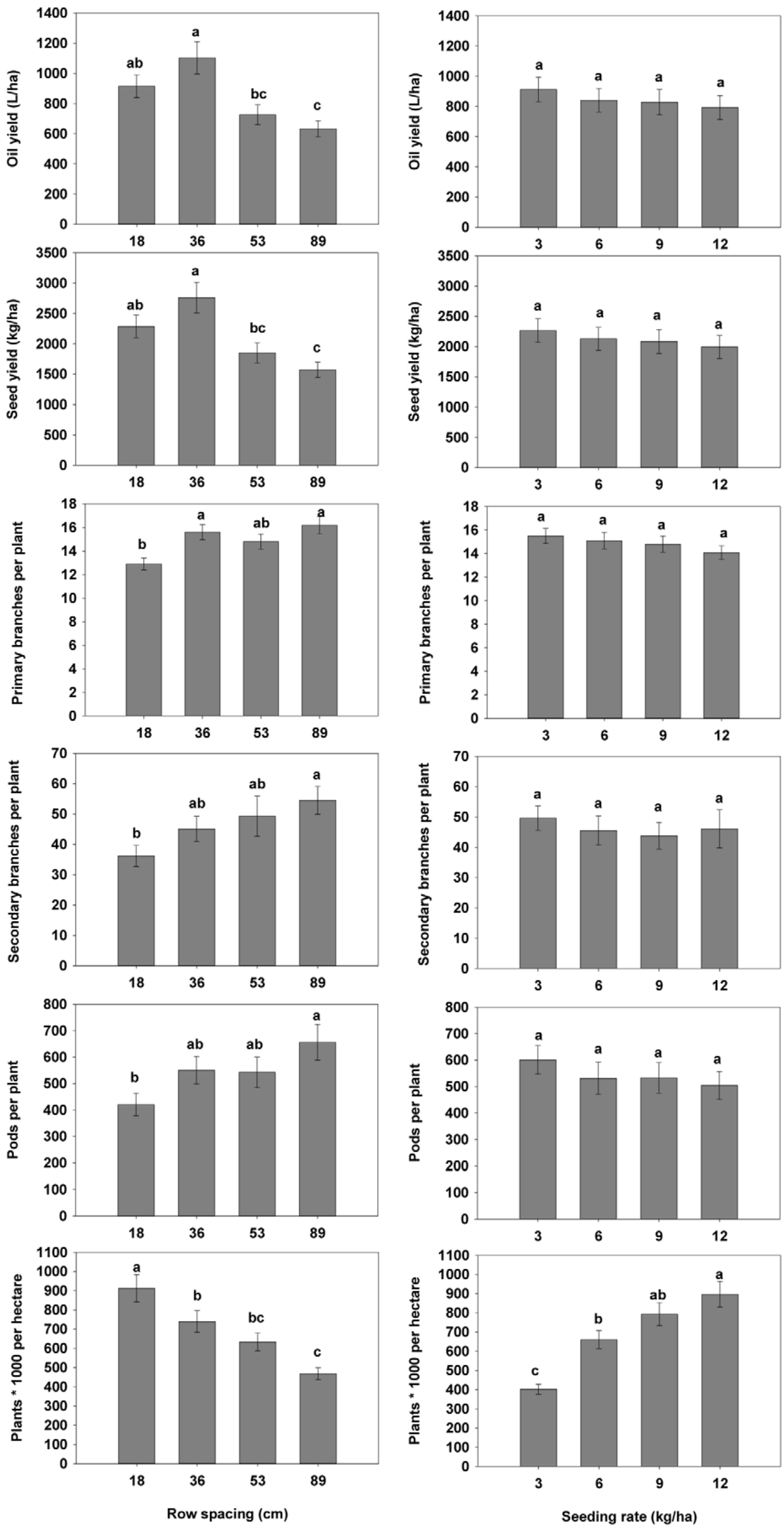

Fig. 2. Carinata yield at 8% moisture and yield components at four seed rates and four row spacings during 5 site-years in northern Florida. Plant population data are shown at harvest, not after emergence. Error bars represent SEM. Different letters represent significantly different means (LSD, $P < 0.05$).

Table 1. Morphological and yield components of carinata grown at four row spacings and four seeding rates. Means are the average of data collected across 5 site-years in northern Florida. No statistical differences were observed across seeding rates and row spacing treatments, so only means and SEM are presented.

Parameter	*n*	Mean	SEM
Seed weight per pod, g	48	0.060	0.002
Nodes per plant, n	141	24.33	0.613
Seed per pod, n	192	13.73	0.147
Dry matter per plant, g	48	78.04	5.416
Plant height, cm	253	122.6	1.76
Pod length, cm	192	5.622	0.47
Thousand seed weight, g	192	3.544	0.046

row spacing may also perform well. These row spacings are commonly available on currently used grain drills in the southeastern United States by leaving all seed tubes open or blocking off every other one. However, given the small seed size and difficulty of precise depth placement, lower seeding rates are challenging, so growers may choose to use higher seeding rates to simplify planting because higher seeding rates did not reduce yield.

Plant height, dry weight per plant, number of seeds per pod, pod length, number of nodes, and thousand seed weight were not affected by treatments (Table 1). Note that not all nodes produced branches (Table 1; Fig. 2) such that the number of nodes was greater than the number of primary branches. Seepaul et al. (2016) also found that the number of nodes exceeded the number of branches and further found that the number of nodes and branches depended on N rate, confirming that environmental factors influence node and branching characteristics in carinata. Because wider rows had not only the same plant dry weight and the same number of seeds per pod but also the same number of pods per plant as narrower row spacings, the yield reductions observed in wider spacings were likely associated with lower plant populations (Fig. 2). However, it cannot be ruled out that seed size was reduced at wider row spacings and that our sample size was not large enough to detect those differences. Clarke and Simpson (1978) proposed that, because bottom branches tend to produce lower seed weight than upper branches, increased branching could have limited seed weight by creating unequal photoassimilate supply to pods distributed throughout the canopy.

Oil Concentration and Composition

None of the experimental variables affected oil concentration and composition ($P > 0.05$). Therefore, overall means are presented in Table 2. In general, seeds had 40% oil concentration, of which half were long-chain fatty acids (C14–C18) and half were very long-chain fatty acids (>C19). Erucic acid (C22:1) represented 36% of the total oil; C18 fatty acids represented 45% of the total oil.

The value of carinata seed meal for animal nutrition is an important component for the incorporation of this crop into agroecosystems. Although protein is an important N source in meal, glucosinolates can affect thyroid function in animals. Glucosinolate concentration was 93 μmol g^{-1}, and protein concentration was 31%, only 10 to 12% lower than soybean (Bellaloui et al., 2015). By comparison, the European Union allows up to 1.5 μmol glucosinolates g^{-1} of feed for monogastric animals, and the US Food and Drug Administration allows up to 100 g glucosinolates kg^{-1} of dietary dry matter for feedlot beef cattle (Colombini et al., 2014). To use carinata meal as animal feed, glucosinolate concentrations must be reduced either by breeding or by solvent or mechanical extraction. Of the extraction processes, solvent extraction appears the most promising to reduce glucosinolate concentrations in feed (Rosenthal et al., 2017), although great progress has been made using various breeding technologies to transform rapeseed into canola (Carlsson et al., 2007), which must contain <30 μmol glucosinolates g^{-1} of air-dried, oil-free meal (Canola Council of Canada, 2015).

Table 2. Seed chemical characterization of carinata grown at four row spacings and four seeding rates. Means are the average of data collected across 5 site-years in north Florida. No statistical differences were observed across seeding rates and row spacing treatments, so only means and SEM are presented.

Parameter	*n*	Mean	SEM
Oil concentration, %	304	39.7	0.2
Protein in seed, %	304	31.6	0.2
Saturated fatty acids, %	304	6.2	0.0
Monounsaturated fatty acids, %	304	57.2	0.1
Polyunsaturated fatty acids, %	304	35.9	0.1
Long-chain fatty acids, % with chain length 14–18C	304	49.8	0.2
Very long-chain fatty acids, % with chain length >19C	304	52.7	0.2
Other fatty acids in seed, %	128	0.6	0.0
C16:0 (palmitic acid), %	304	3.4	0.0
C16:1 (palmitoleic acid), %	128	0.2	0.0
C18:1 (oleic acid), %	304	12.7	0.1
C18:0 (stearic acid), %	304	1.1	0.0
C18:2 (linoleic acid), %	304	18.3	0.1
C18:3 (linolenic acid), %	304	12.9	0.1
C20:0 (arachidic acid), %	128	0.8	0.0
C20:1 (eicosenoic acid), %	304	8.6	0.1
C16:0 (eicosadienoic acid), %	128	1.4	0.1
C22:0 (behenic acid), %	128	0.5	0.0
C22:1 (erucic acid), %	304	36.4	0.1
C22:2 (docosadienoic acid), %	128	0.5	0.0
C24:0 (lignoceric acid), %	128	0.3	0.0
C24:1 (nervonic acid), %	128	1.4	0.0
Glucosinolates, μmol g^{-1}	304	92.9	1.0
Iodine value	304	113.7	0.1

Oil concentration and composition were within ranges reported in previous studies conducted with multiple carinata lines (Alemayehu and Becker, 2002; Pan et al., 2012; Warwick et al., 2006; Zanetti et al., 2009). Studies characterizing variability in carinata germplasm for multiple plant and seed traits reported ranges from 25 to 50% for oil concentration, 25 to 41% for protein concentration, and 88 to 139 μmol g^{-1} glucosinolate content (Alemayehu and Becker, 2002; Warwick et al., 2006). In the present study, although oil concentration was at the low end of the ranges reported for carinata germplasm, protein concentration and glucosinolates were at the high and low ends of the ranges, respectively, which favors seed meal use for animal nutrition. The level of N fertilization used in the present study (90 kg ha^{-1}) has been shown to increase yields but also to decrease the oil/protein ratio in the seed (Pan et al., 2012). This might explain why oil levels were at the lower range of reports for carinata germplasm.

'Avanza', the variety used in the present study, had 23, 72, and 85% more primary and secondary branches and pods per plant than the highest values reported for germplasm evaluated by Alemayehu and Becker (2002). Therefore, breeding efforts appear to have targeted yield potential by increasing branching and pod production (Setia et al., 1995) with minimal impact on oil profile but with considerable improvements for the use of carinata seed meal for animal nutrition. The fact that carinata oil concentration, composition, protein, and glucosinolate concentrations were stable across different planting strategies and 5 site-years simplifies the management of carinata as a bioenergy crop because growers can focus on yield goals while expecting a minimum risk of affecting seed quality with agronomic practices.

CONCLUSIONS

Five site-years of data showed that carinata grown in the southeastern United States had greater yield and yield component responses to row spacing than to seeding rate. Neither row spacing nor seeding rate affected carinata oil concentration, seed weight per pod, nodes per plant, number of seeds per pod, biomass per plant, plant height, or pod length. Yield and primary branching were greatest at 36-cm row spacing, whereas secondary branching increased linearly across all row spacings tested. Based on these data, it is recommended that carinata production in the southeastern United States use 36-cm spacing and 3 kg seed ha^{-1}. Higher seeding rates may be required under conditions of soil crusting or in high-residue systems. Seeding rates up to 12 kg seed ha^{-1} had no significant reduction in yield.

ACKNOWLEDGMENTS

This material is based on work that is supported by the National Institute of Food and Agriculture, USDA, under award number 2016-11231 and by the Florida Department of Agriculture and Consumer Services Office of Energy Grant SRD0007. This work was also supported in part by the USDA National Institute of Food and Agriculture Hatch project numbers 1007448, 005248, and 233849. The authors thank L. Ledbetter-Kish for her perseverance in the laboratory as well as G.K. Obrien, M. Douglas, C. Dickson, and the staff of Extension Agronomy at the NFREC for their technical support throughout this project. Agrisoma Biosciences Inc. was instrumental for the provision of seed and oil profile analyses.

REFERENCES

Agrisoma Biosciences Inc. 2017. Carinata management handbook, southeastern US 2017-18. Agrisoma Biosciences Inc. USA, Tifton, GA.

Alemayehu, N., and H. Becker. 2002. Genotypic diversity and patterns of variation in a germplasm material of Ethiopian mustard (*Brassica carinata* A. Braun). Genet. Resour. Crop Evol. 49:573–582. doi:10.1023/A:1021204412404

Angadi, S., H. Cutforth, B. McConkey, and Y. Gan. 2003. Yield adjustment by canola grown at different plant populations under semiarid conditions. Crop Sci. 43:1358–1366. doi:10.2135/cropsci2003.1358

Bellaloui, N., H.A. Bruns, H.K. Abbas, A. Mengistu, D.K. Fisher, and K.N. Reddy. 2015. Agricultural practices altered soybean seed protein, oil, fatty acids, sugars, and minerals in the Midsouth USA. Front. Plant Sci. 6:31. doi:10.3389/fpls.2015.00031

Bona, S., G. Mosca, and T. Vamerali. 1999. Oil crops for biodiesel production in Italy. Renew. Energy 16:1053–1056. doi:10.1016/S0960-1481(98)00370-X

Canola Council of Canada. 2015. Canola meal feeding guide. Feed Industry Guide. Canola Council of Canada, Winnipeg, Manitoba, Canada.

Cardone, M., M. Mazzoncini, S. Menini, V. Rocco, A. Senatore, M. Seggiani, and S. Vitolo. 2003. *Brassica carinata* as an alternative oil crop for the production of biodiesel in Italy: Agronomic evaluation, fuel production by transesterification and characterization. Biomass Bioenergy 25:623–636. doi:10.1016/S0961-9534(03)00058-8

Carlsson, A.S., D. Clayton, M. Toonen, and E. Salentijn. 2007. Oil crop platforms for industrial uses. CPL Press, Thatcham, UK.

Choudhary, B., P. Joshi, and S. Ramarao. 2000. Interspecific hybridization between *Brassica carinata* and *Brassica rapa*. Plant Breed. 119:417–420. doi:10.1046/j.1439-0523.2000.00503.x

Clarke, J., and G. Simpson. 1978. Influence of irrigation and seeding rates on yield and yield components of *Brassica napus* cv. Tower. Can. J. Plant Sci. 58:731–737. doi:10.4141/cjps78-108

Colombini, S., G.A. Broderick, I. Galasso, T. Martinelli, L. Rapetti, R. Russo, and R. Reggiani. 2014. Evaluation of *Camelina sativa* (L.) Crantz meal as an alternative protein source in ruminant rations. J. Sci. Food Agric. 94:736–743. doi:10.1002/jsfa.6408

Degenhardt, D., and Z. Kondra. 1981. The influence of seeding date and seeding rate on seed yield and yield components of five genotypes of *Brassica napus*. Can. J. Plant Sci. 61:175–183. doi:10.4141/cjps81-027

Egli, D. 1988. Plant density and soybean yield. Crop Sci. 28:977–981. doi:10.2135/cropsci1988.0011183X002800060023x

Erb, K.-H., H. Haberl, and C. Plutzar. 2012. Dependency of global primary bioenergy crop potentials in 2050 on food systems, yields, biodiversity conservation and political stability. Energy Policy 47:260–269. doi:10.1016/j.enpol.2012.04.066

Gómez-Campo, C., and S. Prakash. 1999. Origin and domestication. In: C. Gómez-Campo, editor, Developments in plant genetics and breeding. Elsevier, Amsterdam. p. 33–58.

Hassan, F., and M. Arif. 2012. Response of white mustard (*Sinapis alba* L.) to spacing under rainfed conditions. J. Anim. Plant Sci. 22:137–141.

Hossain, Z., E.N. Johnson, R.E. Blackshaw, K. Liu, A. Kapiniak, C. Gampe, L. Molnar, L. Luan, L. Poppy, and Y. Gan. 2018. Agronomic responses of *Brassica carinata* to herbicide, seeding rate, and nitrogen on the Northern Great Plains. Crop Sci. 58:2633–2643. doi:10.2135/cropsci2018.05.0312

Johansson, D.J.A., and C. Azar. 2007. A scenario based analysis of land competition between food and bioenergy production in the US. Clim. Change 82:267–291. doi:10.1007/s10584-006-9208-1

Kaur, P., and M. Sidhu. 2004. Effect of sowing date, nitrogen level and row spacing on the growth and yield of African sarson (*Brassica carinata* A. Br.). J. Res. Punjab Agric. Univ. 41:27–34.

Kondra, Z. 1977. Effects of planted seed size and seeding rate on rapeseed. Can. J. Plant Sci. 57:277–280. doi:10.4141/cjps77-038

Kuai, J., Y. Sun, Q. Zuo, H. Huang, Q. Liao, C. Wu, J. Lu, J. Wu, and G. Zhou. 2015. The yield of mechanically harvested rapeseed (*Brassica napus* L.) can be increased by optimum plant density and row spacing. Sci. Rep. 5:18835. doi:10.1038/srep18835

Leach, J., H. Stevenson, A. Rainbow, and L. Mullen. 1999. Effects of high plant populations on the growth and yield of winter oilseed rape (*Brassica napus*). J. Agric. Sci. 132:173–180. doi:10.1017/S0021859698006091

Leon, R.G., J.A. Ferrell, and M.J. Mulvaney. 2017. Carinata tolerance to preemergence and postemergence herbicides. Weed Technol. 31:877–882. doi:10.1017/wet.2017.62

McGregor, D. 1987. Effect of plant density on development and yield of rapeseed and its significance to recovery from hail injury. Can. J. Plant Sci. 67:43–51. doi:10.4141/cjps87-005

Momoh, E., and W. Zhou. 2001. Growth and yield responses to plant density and stage of transplanting in winter oilseed rape (*Brassica napus* L.). J. Agron. Crop Sci. 186:253–259. doi:10.1046/j.1439-037x.2001.00476.x

Morrison, M., P. McVetty, and R. Scarth. 1990. Effect of row spacing and seeding rates on summer rape in southern Manitoba. Can. J. Plant Sci. 70:127–137. doi:10.4141/cjps90-015

Nagaharu, U. 1935. Genome analysis in *Brassica* with special reference to the experimental formation of *B. napus* and peculiar mode of fertilization. J. Jap. Bot. 7:389–452.

O'Donovan, J.T. 1994. Canola (*Brassica rapa*) plant density influences tartary buckwheat (*Fagopyrum tataricum*) interference, biomass, and seed yield. Weed Sci. 42:385–389.

O'Neill, C.M., S. Gill, D. Hobbs, C. Morgan, and I. Bancroft. 2003. Natural variation for seed oil composition in *Arabidopsis thaliana*. Phytochemistry 64:1077–1090. doi:10.1016/S0031-9422(03)00351-0

Ozer, H. 2003. The effect of plant population densities on growth, yield and yield components of two spring rapeseed cultivars. Plant Soil Environ. 49:422–426. doi:10.17221/4151-PSE

Pahkala, K., H. Sankari, and E. Ketoja. 1994. The relation between stand density and the structure of spring rape (*Brassica napus* L.). J. Agron. Crop Sci. 172:269–278. doi:10.1111/j.1439-037X.1994.tb00178.x

Pan, X., C. Caldwell, K. Falk, and R. Lada. 2012. The effect of cultivar, seeding rate and applied nitrogen on *Brassica carinata* seed yield and quality in contrasting environments. Can. J. Plant Sci. 92:961–971. doi:10.4141/cjps2011-169

Perlack, R.D., L.L. Wright, A.F. Turhollow, R.L. Graham, B.J. Stokes, and D.C. Erbach. 2005. Biomass as feedstock for a bioenergy and bioproducts industry: The technical feasibility of a billion-ton annual supply. Oak Ridge National Lab., Oak Ridge, TN. doi:10.2172/1216415

Prakash, S., and V. Chopra. 1988. Introgression of resistance to shattering in *Brassica napus* from *Brassica juncea* through non-homologous recombination. Plant Breed. 101:167–168. doi:10.1111/j.1439-0523.1988.tb00283.x

Ragan, P., and R. Nylund. 1977. Influence of NPK fertilizers on low temperature tolerance of cabbage seedlings. HortScience 12:320–321.

Rosenthal, E., J. Clapper, G. Perry, and D. Brake. 2017. Effects of solvent-or mechanically extracted *Brassica carinata* meal on performance of cows. J. Anim. Sci. 95:311. doi:10.2527/asasann.2017.635

SAS. 2016. SAS System for Windows 9.4. SAS, Cary, NC.

Saxton, A. 1998. A macro for converting mean separation output to letter groupings in Proc Mixed. Proceedings of the 23rd SAS Users Group International, Nashville, TN. 22–25 Mar. 1998. SAS, Cary, NC. p. 1243–1246.

Seepaul, R., S. George, and D.L. Wright. 2016. Comparative response of *Brassica carinata* and *B. napus* vegetative growth, development and photosynthesis to nitrogen nutrition. Ind. Crops Prod. 94:872–883. doi:10.1016/j.indcrop.2016.09.054

Setia, R., G. Bhathal, and N. Setia. 1995. Influence of paclobutrazol on growth and yield of *Brassica carinata* A. Plant Growth Regulation 16:121–127. doi:10.1007/BF00029532

Sierts, H.P., G. Geisler, J. Leon, and W. Diepenbrock. 1987. Stability of yield components from winter oil-seed rape (*Brassica napus* L.). J. Agron. Crop Sci. 158:107–113. doi:10.1111/j.1439-037X.1987.tb01153.x

Sieverding, H.L., X. Zhao, L. Wei, and J.J. Stone. 2016. Life-cycle assessment of oilseeds for biojet production using localized cold-press extraction. J. Environ. Qual. 45:967–976. doi:10.2134/jeq2015.06.0313

Simmonds, N.W. 1979. Principles of crop improvement. Longman, London, UK.

Singh, S., and M. Yusuf. 1979. Effect of water, nitrogen and row spacing on yield and oil content of brown sarson. Can. J. Plant Sci. 59:437–444. doi:10.4141/cjps79-068

Taylor, D.C., D.L. Barton, K.P. Rioux, S.L. MacKenzie, D.W. Reed, E.W. Underhill, M.K. Pomeroy, and N. Weber. 1992. Biosynthesis of acyl lipids containing very-long chain fatty acids in microspore-derived and zygotic embryos of *Brassica napus* L. cv Reston. Plant Physiol. 99:1609–1618. doi:10.1104/pp.99.4.1609

Wang, R., T. Cheng, and L. Hu. 2015. Effect of wide–narrow row arrangement and plant density on yield and radiation use efficiency of mechanized direct-seeded canola in Central China. Field Crops Res. 172:42–52. doi:10.1016/j.fcr.2014.12.005

Warwick, S., R. Gugel, T. McDonald, and K. Falk. 2006. Genetic variation of Ethiopian mustard (*Brassica carinata* A. Braun) germplasm in western Canada. Genet. Resour. Crop Evol. 53:297–312. doi:10.1007/s10722-004-6108-y

Zanetti, F., T. Vamerali, and G. Mosca. 2009. Yield and oil variability in modern varieties of high-erucic winter oilseed rape (*Brassica napus* L. var. oleifera) and Ethiopian mustard (*Brassica carinata* A. Braun) under reduced agricultural inputs. Ind. Crops Prod. 30:265–270. doi:10.1016/j.indcrop.2009.05.002

Zhang, S., X. Liao, C. Zhang, and H. Xu. 2012. Influences of plant density on the seed yield and oil content of winter oilseed rape (*Brassica napus* L.). Ind. Crop. Prod. 40:27–32. doi:https://doi.org/10.1016/j.indcrop.2012.02.016