Tillage system and seeding rate effects on the performance of *Brassica carinata*

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**Abstract**

*Brassica carinata*, a nonfood oilseed crop, is used to produce renewable fuels because of its high oil content and favorable fatty acid profile. Production in the southeastern United States is relatively new, and information on agronomic management practices to optimize growth and yield is limited. Since optimal seeding rate may depend on the land preparation method for this small-seeded crop, a study was conducted to evaluate the effect of tillage system (conventional, no-till, broadcast-disc, and ripper-roller) and seeding rate (1.12, 5.60, 10.09, and 14.57 kg seed ha\(^{-1}\)) on the performance of *B. carinata*. A randomized complete block design with a strip-plot restriction on randomization and four replications was implemented in Headland, AL, Jay, FL, and Quincy, FL, over five site-years during the 2017–2018 and 2018–2019 growing seasons. Data were collected on soil residue cover; plant population; soil penetrometer resistance and moisture; biomass (including carbon and nitrogen); stalk diameter; yield and yield components; seed oil, protein, and glucosinolates concentration; and oil composition. Soil penetrometer resistance was significantly affected by tillage system, with the ripper-roller consistently having the lowest penetration resistance values across all site-years. Ripper-roller tillage had the highest oil content and lowest protein and glucosinolate contents. Yield response to tillage system was variable. Among seeding rate treatments, yield was lowest at 1.12 kg seed ha\(^{-1}\) and similar among 5.60, 10.09, and 14.57 kg seed ha\(^{-1}\) at all site-years. There was no tillage by seeding rate interaction for yield. Results indicate that among seeding rate treatments used, 5.6 kg seed ha\(^{-1}\) rate was optimal at all site-years regardless of land preparation method and is thus the recommended seeding rate for commercial carinata production in the Southeastern United States.

**KEYWORDS**

agronomic management, agronomic performance, land preparation, oilseed crop, penetrometer resistance
1 | INTRODUCTION

The global shift to alternative renewable energy sources is compelled by the need for energy independence, environmental stewardship, profitable economics, augmenting oil reserves, long-term unsustainability of nonrenewable energy sources, as well as global warming concerns (Fiorentino et al., 2014; Perlack & Stokes, 2011; Seepaul, Small, Marois, et al., 2019; Woods et al., 2010). This shift has received considerable policy support, for example, the Renewable Energy Directive of the European Union, which set targets to increase total energy consumption from alternative renewable energy sources to 20% by 2020 (Böhringer et al., 2009). Another example is the expanded Renewable Fuel Standard of the United States under the Energy Independence and Security Act of 2007, which set targets to produce 136 billion liters of biofuel by 2022, 58% of which must be second-generation biofuel to achieve a 50% reduction in greenhouse gas emissions (Schnepf & Yacobucci, 2013). These targets may not be met solely from soybean (Glycine max (L.) Merr.) and corn (Zea mays L.), which are considered first-generation biofuel feedstocks because they utilize food crops that are explicitly grown for ethanol and biodiesel production in the United States (U.S. Energy Information Administration, 2016). The use of food crops for biofuel creates a competition for land used for energy crop production. There is a need to identify new sources of biofuel, as well as agronomic practices that make production profitable. Second-generation feedstock, viz. lignocellulosic biomass and industrial oilseeds from nonfood crops, diversify renewable energy sources from agricultural crops. These second-generation feedstocks obviate the food versus fuel conflict, since there is concern about using food crops for bioenergy production (Bona et al., 1999; Mckendry, 2002; Perlack et al., 2005). Among the second-generation feedstock produced in the United States, carinata (Brassica carinata A. Braun) was selected for commercial winter production in the southeastern United States (SE US) because of its carbon sequestering value as a dedicated low carbon feedstock, higher biomass, and greater yield than other oilseed crops such as camelina (Camelina sativa L. Crantz), high erucic acid rapeseed (Brassica napus L.), and pennycress (Thlaspi arvense L.; Gesch et al., 2015; Seepaul, Small, Marois, et al., 2019), coupled with the fact that it does not displace primary crops grown in the SE US since it is grown during winter.

Originating in the Ethiopian plateau of northeastern Africa, where it is believed to have been cultivated since 4000 BC for use as a cooked leafy vegetable (Mekonnen et al., 2014), carinata has seen increasing interest as a winter crop in the humid subtropical region of the United States to produce biofuel. This is due in part to its desirable oil profile (Mulvaney et al., 2019) and greater adaptability than other industrial oilseed crops (Blackshaw et al., 2011; Gesch et al., 2015; Seepaul, Small, Marois, et al., 2019). In comparison to other oilseed crops in the region, carinata possesses valuable agronomic traits such as greater disease resistance, heat tolerance, and shattering tolerance; and a higher concentration of erucic acid (40%–45%), a fatty acid that produces two hydrocarbon molecules upon facile cleavage, allowing the production of two biofuel molecules from each erucic acid molecule (Seepaul, Small, Mulvaney, et al., 2019). These traits allow the production of carinata as a high-energy biofuel crop, with less energy required during processing and having similar physical and chemical properties and performance compared to petroleum-based jet fuel, with the potential to produce 50% less greenhouse gas emissions (Cardone et al., 2003; Choudhary et al., 2000; Seepaul et al., 2016; Seepaul, Small, Marois, et al., 2019). Due to its high protein content and low fiber content, carinata seed can also be used to produce meal for animal feed after oil extraction, provided that it has undergone processing to reduce glucosinolate content to levels safe for animal health (Rosenthal et al., 2017; Schulmeister et al., 2019; Xin & Yu, 2014). Carinata seeds are also a source of sinapic acid, a starting material for organic and polymer synthesis (Shen, 2019). Carinata has been certified as a sustainable biofuel crop in Canada, Argentina, and Uruguay (RSB, 2020) by Scientific Certification Systems global services, a certification body accredited by the Roundtable on Sustainable Biomaterials.

In the SE US, winter carinata production represents a potential opportunity for row crop growers to increase profitability by double cropping between summer crops, typically cotton (Gossypium hirsutum L.) and peanut (Arachis hypogaea L.). Approximately 19 million hectares of agricultural land is left fallow each winter in the SE US, equating to 5–6 months of fallow each year, leaving land susceptible to weed infestation, erosion, and nutrient leaching (SHI, 2019). Double cropping carinata between summer crops has the potential to increase grower profitability and land use efficiency in the region.

Although the market for carinata seed is well developed in Canada, where it is grown during the summer, the expanding second-generation biofuel market demands a source of feedstock during the winter months, making the SE US an attractive location. Carinata commercialization was first introduced to SE US in 2014 as a pilot project to assess its feasibility as a winter crop to produce jet fuel, seed meal, and other high-value co-products, but several challenges related to production and scaling emerged (Seepaul, Small, Mulvaney, et al., 2019). The successful development of a carinata supply chain in the SE US relies on the rotational fit of carinata into existing cropping systems. Since carinata is a relatively new crop to the region, information on agronomic management practices that maximize yield and improve rotation fit is crucial. Tillage and seeding rate are among the first management decisions growers face and are key management issues requiring resolution.
Most soils of the SE US are not naturally as productive as soils found in other regions of the United States because they are highly weathered and acidic, with less than 1\% organic matter contents and poor soil structure (Radcliffe et al., 1988). Conventional tillage practices that include various combinations of multiple surface tillage operations (e.g., moldboard plowing, chisel plowing, or discing) aggravate the poor physical (e.g., hard pans, coarse texture) and chemical nature (e.g., low soil organic matter) of these soils (Balkcom et al., 2013). Since conservation tillage is known to mitigate many of these problems (Balkcom et al., 2013; Langdale et al., 1990; Reeves, 1997), conservation tillage practices are often promoted in the region. While there is a great deal of interest in conservation tillage practices among crop growers, there is no information in the peer-reviewed literature about tillage effects on carinata production in the SE US.

Since carinata has limited weed management options (Leon et al., 2017), rapid growth leading to early canopy closure using optimal seeding rates to favor weed suppression is an important strategy for the successful implementation of commercial carinata. This is particularly important as carinata competes poorly with weeds at early growth stages but can outcompete most weeds once the crop is established. From the standpoint of weed control, optimal seeding rate of carinata is critical, and may affect yield and quality. The current seeding rate recommendation is 3 kg seed ha\(^{-1}\). However, this recommendation was based on the use of fine seedbeds and may be insufficient under conditions of soil crusting or in high-residue systems (Mulvaney et al., 2019). Seeding rates below 3 kg ha\(^{-1}\) are challenging with equipment used for commercial production in the SE US, given the small seed size and the difficulty of precise depth placement. Commercial production of carinata in the SE US requires a recommendation for row crop growers that commonly do not plant on fine seedbeds. Common land preparation methods practiced in the SE US include various forms of conservation tillage (i.e., no-till, mulch, or reduced tillage) and conventional tillage for traditional row crops, while planting is commonly accomplished using either a grain drill or broadcast followed by a shallow discing operation. This research tested the hypothesis that optimal seeding rate was not dependent on the land preparation regime used. The objective of this study was to evaluate the performance of carinata under different seeding rates and land preparation methods for conditions in the SE US.

2 | MATERIALS AND METHODS

2.1 | Study site

Field trials were conducted during the winter growing season at three locations for a total of five site-years: the E.V. Smith Research Center in Shorter, Alabama (2017/2018 and 2018/2019, referred to by harvest year as Shorter-2017 and Shorter-2019, respectively; 32.441665, −85.897558, 63 m a.s.l.); the North Florida Research and Education Center in Quincy, Florida (2018/2019, referred to by harvest year as Quincy-2018; 30.5456493, −84.588348, 75 m a.s.l.); and the West Florida Research and Education Center in Jay, Florida (2017/2018 and 2018/2019, referred to by harvest year as Jay-2017 and Jay-2019, respectively; 30.77542, −87.147662, 62 m a.s.l.). These sites were selected to represent differences in climatic conditions and soil types (USDA-NRCS, 2021) common to the region. Based on the carinata season (November–May), the experimental site in Shorter has historically cooler winter temperatures than Jay and Quincy, with a mean temperature of 13°C and rainfall of 841 mm. The Quincy site has a mean temperature and rainfall of 16°C and 798 mm, respectively, while the Jay site has an average of 14°C and 925 mm. The soil type in Shorter was a Luverne sandy loam (fine, mixed, semi-active, thermic Typic Hapludults), the Quincy site was a Dothan sandy loam (fine-loamy, kaolinitic, thermic Plinthic Kandiults), and the Jay site was a Red Bay fine sandy loam (fine-loamy, kaolinitic, thermic Rhodic Kandiults).

Daily weather data were obtained from weather stations located within 1 km of each study site and are summarized in Table 1. Growing degree days (GDD) were calculated as:

\[
GDD = \sum \left( \frac{T_{\text{max}} + T_{\text{min}}}{2} \right) - T_{\text{base}}
\]

where \(T_{\text{max}}\) and \(T_{\text{min}}\) are daily maximum and minimum air temperatures, respectively, and \(T_{\text{base}}\) is 5°C, the base temperature for canola (Brassica napus L.; NDAWN, 2020).

At each site-year, the previous crop was cotton cultivated under a strip-till tillage system. Soil chemical properties, based on samples taken from the upper 15 cm approximately 1 week prior to applying tillage treatments, are shown in Table 2. Plant available soil nutrients were determined using Mehlich-3 (Jay and Quincy sites) and Mehlich-1 (Shorter site) extractants, while soil pH was determined in 1:1 soil:water (v/v). Soil pH was within the recommended range (5.5–6.5) for growing carinata in the SE US (Seepaul, Small, Mulvaney, et al., 2019).

2.2 | Experimental design and procedure

The experiment was a 4 × 4 randomized complete block design with a strip-plot restriction on randomization with four replications. A strip-plot design was chosen because more precision was desired for measuring the interaction between seeding rate and tillage regime than for either fixed effect
TABLE 1  Monthly average air temperature, cumulative precipitation, and growing degree days (GDD) at each site-year, including their long-term averages (Shorter, 19 years; Jay, 12 years; Quincy, 15 years)

|               | Monthly Shorter-2018 | Shorter-2019 | Shorter-19-year average | Jay-2018 | Jay-2019 | Jay-12-year average | Quincy-2019 | Quincy-15-year average |
|---------------|----------------------|--------------|-------------------------|----------|----------|---------------------|-------------|------------------------|
| **Average temperature (°C)** | | | | | | | | |
| November      | 12.9                 | 10.7         | 13.0                    | 14.7     | 13.0     | 14.1                | 14.0        | 14.6                   |
| December      | 8.2                  | 9.9          | 9.1                     | 11.3     | 12.0     | 11.8                | 12.7        | 12.0                   |
| January       | 2.4                  | 8.7          | 7.6                     | 7.0      | 10.1     | 9.5                 | 11.1        | 10.4                   |
| February      | 9.1*                 | 14.1         | 9.2                     | 17.5     | 16.3     | 11.4                | 16.5        | 11.7                   |
| March         | 13.5*                | 13.1         | 13.5                    | 15.1     | 14.5     | 15.5                | 15.2        | 15.7                   |
| April         | 15.5                 | 17.9         | 17.8                    | 17.9     | 18.7     | 19.2                | 19.0        | 18.9                   |
| May           | 23.6                 | 24.0         | 22.0                    | 24.0     | 24.4     | 23.2                | 24.5        | 22.8                   |
| **Mean**      | **12.2**             | **14.1**     | **13.2**                | **15.3** | **15.6** | **14.9**            | **16.1**    | **15.2**               |
| **Precipitation (mm)** | | | | | | | | |
| November      | 28                   | 144          | 89                      | 5        | 201      | 79                  | 196         | 80                     |
| December      | 73                   | 210          | 119                     | 68       | 336      | 152                 | 264         | 120                    |
| January       | 10                   | 127          | 93                      | 70       | 84       | 115                 | 118         | 92                     |
| February      | 113*                 | 57           | 113                     | 141      | 46       | 126                 | 29          | 182                    |
| March         | 130*                 | 79           | 130                     | 34       | 51       | 124                 | 71          | 101                    |
| April         | 41                   | 181          | 104                     | 102      | 140      | 145                 | 155         | 144                    |
| May           | 92                   | 114          | 99                      | 95       | 82       | 112                 | 61          | 75                     |
| **Total**     | **487**              | **912**      | **747**                 | **515**  | **940**  | **853**             | **894**     | **794**                |
| **Growing degree days (°C d)** | | | | | | | | |
| November      | 261                  | 209          | 272                     | 328      | 276      | 305                 | 299         | 320                    |
| December      | 163                  | 191          | 192                     | 237      | 252      | 253                 | 268         | 266                    |
| January       | 28                   | 176          | 166                     | 172      | 213      | 202                 | 235         | 227                    |
| February      | 172*                 | 274          | 172                     | 365      | 326      | 212                 | 337         | 227                    |
| March         | 288*                 | 278          | 288                     | 333      | 320      | 341                 | 340         | 348                    |
| April         | 159                  | 389          | 387                     | 382      | 415      | 435                 | 420         | 422                    |
| May           | 578                  | 589          | 531                     | 608      | 606      | 575                 | 618         | 561                    |
| **Total**     | **1649**             | **2106**     | **2008**                | **2425** | **2408** | **2323**            | **2517**    | **2371**               |

†Site-2018 and site-2019 refer to site-harvest years.
‡Weather parameters were obtained from weather stations within 1 km of each study site.
*19-year average was used due to unavailable data.

TABLE 2  Initial chemical properties of the soil in each study site and year using Mehlich-3 (Jay and Quincy sites) and Mehlich-1 (Shorter site) extractants

|               | Site-year† | pH‡ | P₂O₅ kg ha⁻¹ | K₂O | Mg | Ca | S | CEC (meq/100 g) |
|---------------|------------|-----|---------------|-----|----|----|---|-----------------|
| Shorter-2018  | 5.8        | 15  | 81            | 182 | 770 | * | 4.6–9.0†       |
| Shorter-2019  | 5.5        | 31  | 82            | 103 | 581 | * | <4.6†          |
| Jay-2018      | 5.9        | 13  | 136           | 211 | 1318| 26 | 7.9           |
| Jay-2019      | 5.9        | 571 | 185           | 191 | 1372| 31 | 7.2           |
| Quincy-2019   | 6.2        | 81  | 113           | 138 | 550 | 29 | 3.1           |

†Site-2018 and site-2019 refer to site-harvest years.
‡Soil pH was determined in a 1:1 soil:water (v/v) extract.
*No data available.
†CEC was provided as a range at the experimental site in Shorter.
(seeding rate or tillage regime) alone (Gomez & Gomez, 1984). Seeding rates were randomly assigned to the vertical strip-plot while tillage regimes were randomly assigned to the horizontal strip-plot, for a total of 64 plots. Seeding rates were 1.12, 5.60, 10.09, and 14.57 kg seed ha\(^{-1}\) (referred to as 1, 6, 10, and 15 kg seed ha\(^{-1}\), respectively, for convenience). Tillage regimes were as follows: (1) Conventional (mow previous cotton stalks with a multi-spindle rotary cutter, disc to a depth of 7.62–15.24 cm, chisel to a depth of 15.24–30.48 cm, field cultivate to a depth of 7.62–10.16 cm, plant with no-till drill), (2) Ripper-roller (mow previous cotton stalks with a multi-spindle rotary cutter, plant with no-till drill, then rip-roll 90\(^\circ\) to planting with a rip roller to a depth of 33.02–35.56 cm), (3) No-till (mow previous cotton stalks with a multi-spindle rotary cutter, plant with no-till drill into shredded cotton stalks; the drill coulters ran to a depth of 3.81–7.62 cm), and (4) Broadcast-disc (mow previous cotton stalks with a multi-spindle rotary cutter, broadcast seed, disc to a depth of 7.62–15.24 cm, and culipack to break soil clods and to ensure good seed to soil contact). The ripper-roller tillage implement is a form of non-inversion deep tillage system that can fracture the soil up to 45 cm deep with shanks 30 cm apart and is used to break up hardpans that limit water infiltration during crop establishment. The ripper-roller was meant to facilitate drainage and eliminate standing water, which can be an issue for carinata during stand establishment based on experience. The reason for planting before ripping was to avoid compacting the plots with the no-till drill after ripping. The broadcast-disc treatment was added to the trial during the 2018/2019 season after receiving grower and industry feedback. The tillage regimes chosen for the present study are commonly used land preparation methods for small grain production in the SE US. Plots were 10.67 m long and 3.66 m wide. Conventional and the no-till drill tillage treatments consisted of nine rows spaced 38.1 cm apart (row spacing was achieved by planting by counting all emerged carinata plants from two representative areas within a 0.5-m\(^2\) quadrat and adjusted for row spacing for plots with rows (conventional and no-till).

## 2.3 Measurements

### 2.3.1 Surface residue cover and initial population counts

After planting but before carinata emergence, surface residue cover was determined using the line transect method (Morrison et al., 1993) by placing two 3-m long transects with 50 equally spaced markers at 45\(^\circ\) to the row and counting the number of marker points with crop residue on the soil surface. Surface residue cover data were based on four site-years (Shorter-2018, Jay-2018, Shorter-2019, and Jay-2019). Initial plant population counts were determined 3 weeks after planting by counting all emerged carinata plants from two representative areas within a 0.5-m\(^2\) quadrat and adjusted for row spacing for plots with rows (conventional and no-till).

### 2.3.2 Soil penetration and moisture

Soil cone index measurements served as a measure of soil penetration resistance. These measurements were collected after planting, with a tractor-mounted hydraulically driven multiprobe soil cone penetrometer (Raper et al., 1999) following procedures previously described in Balkcom et al. (2018). Briefly, cone index values were collected at the same time from five positions per plot using the multiprobe soil cone penetrometer with a cone base area of 130 mm\(^2\) (ASABE, 2018, 2019). The five positions, which were 22.5 cm apart, typically correspond to: +45 cm adjacent to the crop row in the non-trafficked middle; +22.5 cm adjacent to the crop row in the non-trafficked middle; in the crop row (0 cm); −22.5 cm adjacent to the crop row in the trafficked middle; and −45 cm adjacent to the crop row in the trafficked middle. However, the narrow row spacing of carinata prevented this specific pattern across carinata rows. The previous cotton rows were used as a guide for the center of the row whenever possible, while carinata rows were located inside the measurement area based on the drill spacing. Three single
insertions of the multiprobe soil cone penetrometer were collected from each plot at a constant sampling rate of 25 Hz into the soil profile in 5 cm increments to a sampling depth of 50 cm. Cone index measurement dates for each site and year are summarized in Table S1. Prior to statistical analysis, raw cone index measurements were summarized following a procedure previously described by Balkcom et al. (2016). In summary, the method involved calculating average cone index values across the sampled depths in each row position. Once averages were obtained by row position, an area under the curve for cone index (AUC$_{CL}$) was determined for each plot. Consolidating all cone index measurements into a single value for each plot prior to statistical analysis reduced the complexity of cone index analyses and allowed for screening of differences among treatments without the need to initially examine each depth and row position, thus simplifying penetration resistance interpretations across treatments (Balkcom et al., 2016). In addition, average cone index values by depth and row spacing were used to create contour graphs to visually represent how soil strength varied across the measurement zone for each treatment. Soil samples were collected from each plot with a 2.2-cm-diameter steel soil probe in order to provide information on soil moisture differences at the time of penetration resistance measurements. Five soil samples, randomly collected from each plot to a depth of 30 cm, were partitioned into 0–15 cm and 15–30 cm depths. Samples from each depth were composited, weighed, and oven-dried to constant weight at 105°C to determine gravimetric soil moisture content.

2.3.3 | Yield and yield components, aboveground biomass

To prepare for seed and aboveground biomass harvest, plots were trimmed to 9 m long to remove border effects and sprayed with diquat dibromide at a rate of 0.87 L ha$^{-1}$ as a desiccant and harvest aide. Excessive rainfall during the harvest period in Jay, FL 2018 and Shorter, AL 2019 prevented field access resulting in preharvest sprouting. For this reason, seed yield data from Jay-2018 and Shorter-2019 site-years were not available. Seed was harvested directly from the center 1.42 m of each plot using a plot combine. Carinata harvest dates and equipment used at each site are summarized in Table S1. Seed weight and seed moisture content were measured immediately after harvest. Seed yield was adjusted to a seed moisture content of 8.0% (Mulvaney & Devkota, 2020) and corrected for harvested area (which varied depending on the length and width of harvested area, accounting for the number of rows of carinata actually harvested in conventional and no-till treatments). Thousand seed weight (TSW) was estimated by weighing 500 seeds per sample and multiplying the weight by 2. Test weight (TW) of the seeds was measured using a handheld digital test weight scale (SmartScoop, 26HST, Seedburo, Illinois). Test weight data were based on three site-years (Shorter-2018, Jay-2019, and Quincy-2019), and TSW data were based on two site-years (Jay-2019 and Quincy-2019).

Immediately after seed harvest, aboveground biomass was determined by clipping all aboveground tissue from two representative areas per plot within a 1-m$^2$ quadrat outside of the seed harvest area and adjusted for row spacing for plots with rows (conventional and no-till). Samples were further oven-dried with forced air at 65°C to a constant weight. After weighing, subsamples were ground to pass a 2-mm sieve using a Wiley mill (Thomas-Wiley, Model 4, Thomas, Pennsylvania) and analyzed for total carbon (C) and total N concentration by dry combustion (AOAC International, 2006). The percentage of total C and N was multiplied by aboveground biomass yield to determine total biomass C and N uptake. No attempt was made to collect abscised leaves, which naturally abscise prior to maturity. Total biomass C and N uptake data were based on four site-years (Shorter-2018, Shorter-2019, Jay-2019, and Quincy-2019).

2.3.4 | Stalk diameter and final plant population

Stalk diameter was determined by measuring the diameter of 10 stalks in a row, 10 cm above the ground, from a representative area of the plot within the harvest area using a digital vernier caliper (Husky, 1467H, The Home Depot, Florida). Stalk diameter data were based on three site-years (Jay-2018, Jay-2019, and Quincy-2019). Final plant population was determined at the same time by counting all stalks from two representative areas within a 0.5-m$^2$ quadrat and adjusted for row spacing for plots with rows (conventional and no-till).

2.3.5 | Seed oil profile analysis and oil yield

Subsamples of seed (3–5 g) after harvest were analyzed for total glucosinolates, protein and oil concentration, and oil composition using a method previously described by Mulvaney et al. (2019). Briefly, samples were analyzed using a FOSS XDS Rapid Content Analyzer with XDS near infrared reflectance (NIR) spectroscopy (FOSS NIRSystems, Inc., MD, USA) and scanned over a range of 400 to 2499.5 nm in 0.5 nm increments. Resulting sample spectra were processed using the ISIscan routing analysis software (Program version 4.10.0.15326, Database version 4.6.0.14416, FOSS Analytical, Hoganas, Sweden) using a proprietary prediction model developed for carinata by Agrisoma Biosciences Inc. (now Nuseed-Carinata). The underlying NIR equations were calibrated using nuclear magnetic resonance spectroscopy
(Oxford MARAN Ultra Benchtop NMR System, Oxford Instruments, Oxfordshire, UK) and gas chromatography (Agilent 6890N; Agilent Technologies, Santa Clara, California, USA; O’Neill et al., 2003; Taylor et al., 1992). Every 50 samples, a calibration sample of a known value was analyzed for oil, protein, glucosinolates, and erucic acid concentration and results were entered into the calibration control chart to verify that they fall within 3 standard deviations of the expected value. Oil yield was determined by multiplying seed yield (adjusted to 8.0% moisture) by oil concentration and then dividing by an oil density of 0.878 kg L$^{-1}$, the density of carinata oil (Sieverding et al., 2016).

2.4 Data analyses

Data were subjected to analysis of variance (ANOVA) using PROC GLIMMIX in SAS version 9.4 (SAS Institute Inc., 2012). Initial analyses were performed on all dependent variables to determine the effect of site-year as a fixed effect. In these analyses, independent variables were site-year, tillage regime, and seeding rate. These preliminary analyses indicated significant interactions ($p < 0.05$) between site-year and treatments (tillage regime and seeding rate) for most response variables. The interactions were the result of magnitude of differences among treatments but with similar trends of response across site-years, except for soil cone index, soil moisture, seed, oil, and erucic acid yield (Table S2). Therefore, all subsequent analyses, excluding these dependent variables, were performed for all site-years combined as random effect. Soil cone index and moisture, seed, oil, and erucic acid yield data analyses were performed by site-year. Box plots and residual plots were evaluated to determine if data violated ANOVA assumptions. When assumptions were not satisfied, an appropriate transformation method was applied consisting of logarithmic, arc-sine, or square-root transformations (Gomez & Gomez, 1984). For combined site-year analyses, tillage regime, seeding rate, and their interactions were considered fixed effects. Site-year, replication nested within site-year, and their interactions with fixed effects were considered random effects. For analyses by site-year, tillage regimes, seeding rates, and their interactions were considered fixed effects, while replication and their interactions with fixed effects were considered random effects. In all cases, degrees of freedom were estimated using the Kenward-Roger method. The NOBOUND option was used to allow negative within subject variances to be estimated. Least square means of fixed effects were computed and differences among least square means were compared using the PDIFF option within PROC GLIMMIX. The SLICE option for the LSMEANS command was used to partition significant interactions among fixed effects. To compare and contrast trends, seeding rate and the seeding rate by tillage interaction for seed yield were further partitioned into linear, quadratic, and lack of fit partitions using PROC GLIMMIX. Treatment differences for all parameters were considered significant at $p < 0.05$. Pearson correlations were conducted among selected dependent variables for all site-years combined using PROC CORR in SAS version 9.4 (SAS Institute Inc., 2012) and were considered significant at $p < 0.01$. Figures were generated using R software version 4.0.0 (R Core Team, 2020).

3 RESULTS AND DISCUSSION

3.1 Weather

During the 2017/2018 growing season, conditions were unusually dry, with total precipitation 260 and 338 mm below long-term average at Shorter and Jay, respectively (Table 1). The 2018/2019 growing season at all sites was characterized by excessive rainfall, much of which can be attributed to an unusually wet December compared to the long-term average. The average temperature for the entire growing season for each site and year tended to be warmer than the long-term average, except at Shorter-2018. For each site-year, the highest mean monthly temperature was recorded in May, while the lowest temperature was recorded in January. It should be noted that temperatures above 29°C during flowering and pod-filling stages can lead to a reduction in the number of flowers and fertile pods in Brassica species (Angadi et al., 2000; Morrison & Stewart, 2002). Although monthly average temperature at Jay and Quincy site-years never exceeded this threshold temperature, temperatures above 29°C were observed for 136, 169, and 193 hours during the study period at Jay-2018, Jay-2019, and Quincy-2019, respectively, much of which occurred in May (data not shown). The number of accumulated GDD for each site-year was greater than the long-term average except at Shorter-2018 (Table 1).

3.2 Soil residue cover and carinata stands

As expected, tillage treatments had an effect on surface residue cover measured after carinata planting ($p < 0.0001$). All tillage treatments except conventional tillage met the definition of conservation tillage, a tillage operation that leaves at least 30% of residue cover on the soil surface after planting (SSSA, 2020; Table 3). Conservation tillage treatments would be expected to reduce erosion and improve soil structure, soil water holding capacity, and infiltration compared to conventional tillage (Balkcom et al., 2010; Dabney et al., 2001). Non-inversion deep tillage systems (e.g., the ripper-roller) are known to minimize surface soil disturbance and maximize belowground soil disruption of compacted subsoil layers, enabling root exploration of a larger
soil volume to obtain nutrients and moisture (Balkcom et al., 2007; Busscher et al., 1988; Schwab et al., 2002). Lopez-Fando et al. (2007) reported that the amount of soil residue cover after no-till and paraplow zone tillage was greater than that obtained after conventional tillage system. As expected, seeding rate \( (p = 0.2867) \) and its interaction with tillage treatments \( (p = 0.1134) \) had no influence on the amount of surface residue cover (Table 3) since measurement was done before carinata emergence.

Carinata initial and final plant population were affected by tillage treatments \( (p < 0.0001) \), being greater under conventional tillage compared to other tillage treatments (Table 3). Soil surface residue cover can affect seed–soil contact, causing a reduction in plant population, particularly in conservation tillage systems (Balkcom et al., 2018; Brandt, 1992). The finer seedbed after conventional tillage compared to other tillage treatments may have improved seed–soil contact, and therefore increased plant population. Results from previous studies have also shown higher plant populations after conventional tillage compared to conservation tillage systems (Weisz & Bowman, 1999; Lithourgidis et al., 2006; Lafond et al., 2006; and Rieger et al., 2008). Seeding rate also affected carinata initial and final population \( (p < 0.0001) \).

As seeding rate increased, initial and final population increased, but not proportionally to seeding rate (Table 3). This finding is consistent with observations reported in other studies on carinata (Hossain et al., 2018; Pan et al., 2012), canola (Harker et al., 2015; Kutcher et al., 2013), camelina (Agegnehu & Honermeier, 1997; Urbaniak et al., 2008), and soybean (Hamid et al., 2002). An interaction between tillage and seeding rate affected carinata initial and final populations \( (p < 0.0001) \); Table 3). Examination of this interaction showed that conventional tillage and no-till markedly increased plant populations at higher seeding rates \( (10 \text{ and } 15 \text{ kg seed ha}^{-1}) \) compared to broadcast-disc and ripper-roller tillage treatments, causing an interaction effect (Figure S1). This was likely caused by the more uniform depth of seed using conventional tillage and no-till, which becomes more apparent at high seeding rates.

### 3.3 Soil penetration and moisture

Soil cone index is an empirical measure of soil shear strength (Chen et al., 2005; Osubitan et al., 2005) and is widely used as a proxy for the ease of root penetration and for assessment of soil compaction (Bedard et al., 1997; Tessier et al., 1997). Cone index measurements were affected by tillage treatments during all five site-years \( (p \leq 0.0254) \) based on the AUC approach (AUCC.I.). No-till and broadcast-disc AUCC.I. values were consistently among the highest measured at all site-years (Figure 1). The disruption of deep soil compaction by the ripper-roller resulted in lower AUCC.I. values at all site-years and was not different from conventional tillage during three of the five site-years (Shorter-2018, Shorter-2019, and Jay-2018, Figure 1) when measurements were taken immediately following planting (Table S1). Lower soil penetration resistance measured soon after planting following the use of ripper-roller and conventional tillage compared to other tillage treatments is consistent with observations reported in
earlier studies (Chen et al., 2005; Gomez et al., 1999; Lopez-Fando et al., 2007; Mosaddeghi et al., 2009; Osunbitan et al., 2005; Salem et al., 2015; Wilkins et al., 2002). Conventional and no-till AUCC.I. values were similar at Jay-2019. Similarly, conventional, no-till, and broadcast-disc AUCC.I. values were similar at Quincy-2019 (Figure 1). These measurements were taken much later in the growing season at these site-years (Jay-2019 and Quincy-2019) compared to other site-years (Table S1). Raindrop impact, soil surface sealing, and human-induced or natural soil compaction may have led to an increase in soil strength in the conventional tillage as the season progressed. Osunbitan et al. (2005) also provided evidence of penetration resistance increase over time after conventional tillage operations, becoming comparable to no-till at 0–5 cm depth after 8 weeks. Thus, conventional tillage may not be an effective in-season tool for alleviating soil

FIGURE 1 Area under the curve for cone index (AUCC.I.) values calculated from cone index measurements collected from tillage treatments averaged over seeding rates for each site-year. Different letters represent significantly different means (LSD, $p < 0.05$) within a site-year.
compaction; and ripper-roller de-compaction effects remain longer into the season than conventional tillage, at least under sandy loam conditions.

A soil cone index value of 2 MPa is an accepted critical threshold level at which soil strength begins to hinder root growth and development in most agricultural crop species (Atwell, 1993; Taylor & Burnett, 1964). Soil cone index values averaged across all site-years, row positions, and depths were 1.46, 1.59, 2.00, and 2.04 MPa for ripper-roller, conventional, broadcast-disc, and no-till tillage treatments, respectively (data derived from Figure 2). The results indicate that no-till and broadcast-disc tillage may not alleviate mechanical impedance to root growth, development, and proliferation, at least under short-term (less than a year) use. Cone index differences among tillage treatments averaged across depths in each row position for each site-year are shown in Figure 2. When measurements were taken soon after planting at Shorter-2018, Shorter-2019, and Jay-2018, ripper-roller and conventional tillage decreased soil penetration resistance across all row positions adjacent to the crop row compared to other tillage treatments (Figure 2). Despite taking measurements much later in the growing season at Jay-2019 and Quincy-2019 (Table S1), the effects of de-compaction by the ripper-roller were still evident across all row positions compared to other tillage treatments (Figure 2). Contour graphs of soil strength variation across the measurement zone for each tillage treatment during each site-year are shown in Figure 3. These results correspond with observed differences in AUC_{C.I.} values among tillage treatments (Figure 1) and with plots of cone index values averaged for each row position (Figure 2).

It is important to determine soil moisture content simultaneously with cone index measurements due to its significant influence on soil strength (Materechera & Mloza-Banda, 1997; Mathers et al., 1966; Taylor & Gardner, 1963; Taylor et al., 1964; Unger & Jones, 1998). Gravimetric soil moisture content at the 0–15 and 15–30 cm depths, determined along with cone index measurements, were not affected by tillage during any site-year, except at Shorter-2018 (15–30 cm depth, $p = 0.0034$) and Quincy-2019 (0–15 cm depth, $p = 0.0050$; Table 4). The consistency of soil moisture contents across tillage treatments and within the same depths at Shorter-2019, Jay-2018, and Jay-2019 shows that the differences observed in soil strength (Figure 1) were as a result of differences among tillage treatments alone and were not confounded by soil moisture content. Consistent soil moisture contents within

![Figure 2](image.png)

**Figure 2** Cone index measurements averaged across sampled depth in each row position. Error bars represent ±standard error of the mean (SEM)
depths ensure that cone index values are not confounded with soil moisture contents (Balkcom et al., 2016). Despite soil moisture content differences among tillage treatments at Shorter-2018 and Quincy-2019 (Table 4), they did not translate into cone index differences for those site-years (Figure 1). As expected, early season soil moisture was not influenced by seeding rate, but when soil moisture was determined 6 and 10 weeks after planting at Quincy-2019 and Jay-2019, respectively (Table S1), increasing seeding rates lowered soil moisture at the 15–30 cm depth (Table 4). This was likely due to increased evapotranspiration on a per area basis due to higher plant populations.

3.4 | Biomass, C and N uptake, and stalk diameter

Tillage had no influence on the amount of aboveground biomass, biomass C, or biomass N (Table 3). The average amount of aboveground biomass across all tillage
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The average amount of biomass C and N returned to the soil was 3302 and 80 kg ha\(^{-1}\), respectively, after desiccation and harvest. This is an average C:N ratio of 41, demonstrating N immobilization potential of carinata residue during subsequent crop production, which may have implications for N management. The ability of carinata residue to contribute to soil organic C and N in the SE US depends on its C and N mineralization rates which is unknown at this time. Neither seeding rate nor its interaction with tillage had influence on carinata biomass, biomass C, and biomass N, although means generally decreased with increasing seeding rate due to intraspecific competition for resources (Table 3).

Stalk diameter was affected by tillage (\(p = 0.0095\)), with increased stalk diameters in conservation tillage treatments (no-till, ripper-roller, broadcast-disc) compared to conventional tillage, a response likely due to increased final plant populations coupled with no difference in biomass accumulation under conventional tillage (Table 3). Similarly, stalk diameter was influenced by seeding rate (\(p < 0.0001\)), with stalk diameter decreasing with increasing seeding rate due to intraspecific competition for available resources. In a reduced plant density, individual plants are likely to share more resources such as space, sunlight, water, and nutrients than those in an increased plant density (Chai et al., 2016; Jamont et al., 2013; Li et al., 2013). No interaction between tillage and seeding rate was observed for stalk diameter (\(p = 0.3999\); Table 3).

3.5 | Seed yield and yield components, oil, and erucic acid yield

Carinata seed, oil, and erucic acid yield response to tillage were inconsistent across site-years (Table 5). At Shorter-2018, conventional tillage had the highest seed yield, and consequently greater oil and erucic acid yield, compared to other tillage treatments. Given that Shorter-2018 was a dry year (Table 1), one may expect that the soil moisture conserving benefits of conservation tillage systems would increase yield. However, it is likely that cotton residue, which was the previous crop, played a role since cotton is considered a low biomass crop (Causarano et al., 2006; Daniel et al., 1999; Siri-Prieto et al., 2007). This may have diminished the moisture conservation benefits associated with conservation tillage systems during the extremely dry conditions (total precipitation 260 mm below the long-term average) at Shorter-2018 (Table 1).

Tillage had no effect on yield at Jay-2019 and Quincy-2019 (\(p > 0.1\), Table S3). However, at Quincy-2019, pairwise mean comparisons indicated that conventional tillage yield was similar to no-till and ripper-roller tillage yield while...
broadcast-disc tillage yield was lowest (Table 5). The lack of yield differences among tillage treatments at Jay-2019 and Quincy-2019 may to a large extent be attributed to the favorably wet conditions (total precipitation 87 and 100 mm above long-term average at Jay-2019 and Quincy-2019, respectively) which were prevalent throughout the growing season (Table 1).

Seeding rate had an effect on carinata yield at all site-years (Table 5), all of which were quadratic responses, except at Quincy-2019 (Table S3). Yield was lowest at 1 kg seed ha$^{-1}$ but similar at other seeding rates (6, 10, and 15 kg seed ha$^{-1}$; Table 5). Yield optimization at 6 kg seed ha$^{-1}$ compared to higher seeding rates (10 and 15 kg seed ha$^{-1}$) may be due to phenotypic plasticity exhibited by most *Brassica* species in which vegetative growth is modulated by increased lateral branching, pod formation, and leaf area in compensation for reduced plant density. The fact that the highest plant populations (Table 3) did not result in the highest yield (Table 5) may be indicative of the phenotypic plasticity of carinata. Similar compensatory mechanism was reported in earlier studies in carinata (Hossain et al., 2018; Pan et al., 2012), canola (Angadi et al., 2003; Taylor & Smith, 1992), camelina (Agegnehu & Honermeier, 1997; Lafond et al., 2008; Urbaniak et al., 2008), and winter oilseed rape (Leach et al., 1999; Zhang et al., 2012). Hypothesis testing showed there was no significant tillage by seeding rate interaction for yield during any site-year (0.32 < $p$ < 0.96, Table 5 and Table S3).

Based on our results, the seeding rate recommendation for optimal yield in the SE US is 5.6 kg seed ha$^{-1}$ (rounded in this paper as 6 kg seed ha$^{-1}$) for all land preparation methods tested. Tillage, seeding rate, and their interaction had no influence on TSW and TW (Table 3).

### 3.6 | Seed glucosinolates, protein, and oil concentration

Tillage had more influence on seed glucosinolates concentration and oil content ($p \leq 0.0391$) than on protein content ($p = 0.0722$; Table 6). Protein and oil concentrations were similar in all tillage treatments except the ripper-roller, which produced lower seed protein and higher seed oil concentration. Soil temperature and moisture are known to influence protein and oil concentrations in *Brassica* species. High temperatures and low soil moisture during flowering and pod-filling stage reduced seed oil concentration in canola (Morrison & Stewart, 2002; Pritchard et al., 2000). The reduction in penetration resistance caused by the ripper-roller may have improved root access to soil water, and therefore increased seed oil concentration. This conjecture was supported by the negative correlation observed between penetrometer resistance values (AUC$_{C1}$) and oil content and the positive correlation between penetrometer resistance values and protein content (Table S4). Seeding rate and its interaction with tillage had no influence on these variables (Table 6). Previous studies conducted on carinata (Mulvaney et al., 2019; Pan et al., 2012) and on other crops including hemp (*Cannabis sativa* L.; Vera et al., 2006), camelina (Xie et al.,

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**Table 5** Seed, oil, and erucic acid yield as affected by the effects of tillage and seeding rates, and their interaction in each site-year

| Effect | Seed yield | Oil yield | Erucic acid yield |
|--------|------------|-----------|------------------|
|        | kg ha$^{-1}$ | L ha$^{-1}$ |                 |
| **Tillage regime (T)** | | | |
| Broadcast-disc | * | 948$^a$ | 514$^b$ |
| Conventional | 1868$^a$ | 1009$^a$ | 800$^{ab}$ |
| No-till | 1421$^b$ | 1032$^b$ | 931$^a$ |
| Ripper-roller | 1476$^b$ | 1012$^a$ | 875$^a$ |
| **Seeding rates (S)** | | | |
| 1 kg ha$^{-1}$ | 1260$^b$ | 819$^b$ | 505$^b$ |
| 6 kg ha$^{-1}$ | 1665$^a$ | 1135$^a$ | 818$^a$ |
| 10 kg ha$^{-1}$ | 1727$^a$ | 1014$^a$ | 828$^a$ |
| 15 kg ha$^{-1}$ | 1701$^a$ | 1033$^a$ | 969$^a$ |
| T × S | ns | ns | ns |

Abbreviation: ns, not significant.

*Site-2018 and site-2019 refer to site-harvest years.

*No data available for broadcast-disc because it was not part of the tillage treatment during 2017/2018 season.

‡Means followed by the same superscript letter within a column and effect were not significantly different at $p < 0.05$ (LSD).
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2020), and rapeseed (Kondra, 1975) also reported a lack of seeding rate effects on seed glucosinolates, protein, and oil concentration.

Seed oil concentration was generally higher, with lower glucosinolates and protein concentrations compared to values reported in previous carinata studies (Mulvaney et al., 2019; Pan et al., 2012; Seepaul et al., 2018). Carinata seed protein and glucosinolate contents are inversely related to seed oil content (Kumar et al., 2020; Mulvaney et al., 2019; Pan et al., 2012) due to competition for carbon skeletons between fatty acid and amino acid biosynthetic pathways (Pan et al., 2012), which explains results of the present study (Table S4).

### 3.7 Seed oil composition

Tillage, seeding rate, and their interaction had no influence on oleic (C18:1), linolenic (C18:3), or erucic (C22:1) acids (Table 6). Linoleic (C18:2) and eicosenoic (C20:1) acid concentrations were affected by tillage \( p \leq 0.0207 \), with lower concentrations produced under ripper-roller and conventional tillage, respectively, compared to other tillage treatments. Seeding rate and its interaction with tillage had no influence on these fatty acids (Table 6). Carinata is known to have a high concentration of erucic acid (C22:1) which makes it a preferred feedstock for renewable energy and bio-products for industrial applications (Kumar et al., 2020). Overall, erucic acid, the oil of primary importance, represented 44% of the total oil, which was higher than previously reported in other studies (Mulvaney et al., 2019; Pan et al., 2012; Seepaul, Small, Marois, et al., 2019).

### CONCLUSIONS

Greater plant populations were obtained under conventional tillage compared to other tillage treatments due to a finer seedbed; however, this did not translate to an increase in yield. Ripper-roller tillage produced the lowest penetration resistance and is likely to have increased early season root penetrability and water infiltration compared to other tillage treatments. No-till and broadcast-disc tillage had the greatest penetration resistance and were likely the least penetrable by roots, which may affect early stand establishment under certain conditions, such as high rainfall. During two of three site-years, land preparation methods did not influence yield under sandy loam soil conditions. Yield response to seeding rates was the same regardless of the land preparation method used. Among seeding rates tested, yield was optimized at 5.6 kg seed ha\(^{-1}\) and is thus the recommended seeding rate for commercial carinata production in the SE US.

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### CONFLICT OF INTEREST

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.
DATA AVAILABILITY STATEMENT
The data that support the findings of this study are available from the corresponding authors upon reasonable request.

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**SUPPORTING INFORMATION**

Additional supporting information may be found online in the Supporting Information section.

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