How does plant population density affect the biomass of Ravenna grass?

Tim L. Springer

USDA, Agricultural Research Service, Southern Plains Range Research Station, Woodward, OK, USA

Correspondence
Tim L. Springer, USDA, Agricultural Research Service, Southern Plains Range Research Station, 2000 18th Street, Woodward, OK 73801, USA. Email: tim.springer@usda.gov

Abstract
Ravenna grass, *Tripidium ravennae* (L.) H. Scholz, is known to produce an abundance of biomass, but how plant density affects its biomass potential remains unknown. The objectives were to determine the effects of plant density on biomass yield; plant growth traits; biomass—carbon, nitrogen, and ash concentrations; heating value; nitrogen removal; and sucrose concentration in leaves and culms. The treatments consisted of five plant densities (1,250; 2,500; 5,000; 10,000; and 20,000 plants per hectare) in a randomized complete block design with four blocks. Plots were nonirrigated, unfertilized, and harvested once during the dormant season each year. Data were collected from 2015–2019. Dependent variables that varied with plant population density ($p < .05$) were biomass yield, number of reproductive culms per plant, reproductive culm diameter, reproductive culm sucrose concentration, and nitrogen removal with biomass. Biomass yield ranged from 5.6 to 16.3 Mg/ha for plant densities of 1,250–20,000 plants per hectare, respectively. Combined over years, nonlinear regression of the data showed the equation for biomass yield to plateau at 16.2 Mg/ha at a plant density of 10,640 plants per hectare. As plant density increased, the number of reproductive culms per plant, culm diameter, and culm sucrose concentration significantly decreased. At 1,250 plants per hectare, the number of reproductive culms per plant, culm diameter, and culm sucrose averaged 70, 10.2 mm, and 63.2 g/kg, respectively. Nitrogen removed with biomass significantly increased as biomass yield increased with plant density. At a density of 10,000 and 20,000 plants per hectare, the amount of nitrogen removed annually in the harvested biomass averaged 88 kg/ha. The data suggest that 10,000 plants per hectare would produce the greatest annual biomass yields; however, research is needed to determine the nutrient requirement for Ravenna grass to sustain biomass production at that density.

KEYWORDS
biomass, *Erianthus ravennae*, plant population density, Ravenna grass, *Saccharum ravennae*, sucrose concentration, *Tripidium ravennae*

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1 | INTRODUCTION

*Tripidium ravennae* (L.) H. Scholz, commonly known as Ravenna grass, is a perennial bunchgrass native to Eurasia and North Africa (POWO, 2019; USDA ARS, 2020a). *Saccharum ravennae* (L.) L. and *Erianthus ravennae* (L.) P. Beauv. are taxonomic synonyms of *T. ravennae* (POWO, 2019), and these binomina are predominantly used in contemporary scientific literature. Ravenna grass was logged into the USDA germplasm repository in 1929 from collections made in the Democratic Republic of Georgia (USDA ARS, 2020b), but based on an herbarium specimen collected near Fresno, California in 1918, it was brought into the USA much earlier (Thomsen & Meyer, 2007). Due to its hardiness Ravenna grass is widely cultivated as an ornamental grass (Barkworth et al., 2007; Hitchcock, 1950; Meyer, 2011), but like many plant species introduced into the USA, it has escaped cultivation (CABI, 2020; DiTomaso & Healy, 2007; Thomsen & Meyer, 2007). The chaffy spikelets of Ravenna grass are dispersed by wind (anemochory) and water (hydrochory) contributing to its success of escaping cultivation and becoming naturalized in riparian areas (CABI, 2020). For this reason, Ravenna grass has been added to noxious weed lists in several U.S. states (DiTomaso & Healy, 2007).

Springer (2018) and Palmer et al. (2014) investigated Ravenna grass for its potential as an energy crop. Springer (2018) studied the variation of biomass yield; carbon, nitrogen, and ash concentrations of harvested biomass; and sucrose concentration of leaves and culms of plants derived from open-pollinated seeds from a single plant of a naturalized population. He found that over a 4-year period the biomass yield averaged 3.9 ± 0.8 to 7.5 ± 1.8 kg per plant, that the carbon concentration was generally higher and the nitrogen and ash concentrations were generally lower than other energy crops (*Arundo donax* L., *Cynara cardunculus* L., *Miscanthus sinensis* × *giganteus* J. M. Greef, Deuter ex Hodk., *Renvoize; Panicum virgatum* L., and *Sorghum bicolor* (L.) Moench, as reported by Monti et al., 2008), that leaf sap sucrose concentration varied from 24.4 ± 4.6 to 41.6 ± 7.6 g/kg depending on the sample year, and that culm sap sucrose concentrations were approximately 1.85 times greater than those of leaves. Springer (2018) concluded that, “Ravenna grass has the potential to be developed into a viable energy crop; however, research is needed to determine optimum seeding rates and plant densities to sustain long-term biomass production.” Palmer et al. (2014) examined the biomass yield potential of six robust perennial grasses including Ravenna grass in North Carolina. They reported biomass yields of Ravenna grass varied from 3.0 to 22.1 Mg/ha, and they concluded that although Ravenna grass produced considerable biomass, it did not persist well. Palmer et al. (2014) stated that, “Ravenna grass survival decreased from 83 to 69% at the Mountain site and from 94 to 72% at the Central Plains site from 2008 to 2011.” Springer (2018) theorized that the lack of persistence observed by Palmer et al. (2014) was due to the 10 cm (1 dm) biomass cut height, a harvest cut height that could impact survival of the robust crown of Ravenna grass. Springer (2018) did not observe persistence problems of Ravenna grass when harvesting it at a height of 30 cm (3 dm).

Crop forage and grain yields are influenced by many factors, some of which are moisture, fertilization, and plant-to-plant competition. For example, the grain yields of several field crops are maximized by adjusting the sown plant population density to that of the moisture environment (Jones & Johnson, 1991; Sanderson et al., 1996). Crops sown to high population densities utilize moisture and nutrients more quickly than those same crops sown to lower population densities (Jones & Johnson, 1991). Tillering in annual grasses has been found to compensate for low plant density that resulted from drought or overgrazing (Hiernaux et al., 1994). Springer et al. (2003) reported tiller compensation for low plant density in the production of a well-managed (fertilized and irrigated) perennial bunchgrass. Studying biomass yield of Ravenna grass as well as other production traits relative to plant population density should maximize biomass and possible sustainability. The objectives of this research were to determine the effects of plant population density on biomass yield; the carbon, nitrogen, and ash concentrations of harvested biomass; nitrogen removed with biomass; number of reproductive culms per plant; reproductive culm diameter; and the sucrose concentration of leaves and reproductive culms of Ravenna grass.

2 | MATERIALS AND METHODS

2.1 | Study site, soil, weather, and plant materials

Research was conducted at the USDA-ARS, Southern Plains Range Research Station, Woodward, OK (36°25′N, 99°24′W, elevation 615 m) on a Woodward loam (coarse-silty, mixed, superactive, thermic Typic Haplustepts). The Woodward soil series is described as moderately alkaline with up to 15% calcium carbonate in the profile (USDA NRCS, 2020a, 2020b). The soil of the plot was randomly sampled to a depth of 15 cm using a T-bar soil sampling probe. The sample was dried at 60°C and ground to pass a 2-mm sieve. The sample was analyzed for pH, NO₃-N, plant available P and K, and soil organic carbon (SOC). Soil pH was measured with a glass electrode in a one-part soil to one-part water suspension and SMP buffer solution, respectively (Sims, 1996). Soil NO₃-N and NH₄-N were extracted with 1 M potassium chloride (KCl) solution and...
quantified by the cadmium reduction method on a Lachat QuikChem 8000 (Lachat Instruments). Soil available phosphorus (P) and potassium (K) were extracted using Mehlich 3 solution (Mehlich, 1984). Mehlich 3 P was quantified calorimetrically using a Lachat, while K was analyzed by a Spectro CiroS ICP (Sims, 1996). The concentration of SOC was determined using an Elementar vario MAX CN analyzer (Elementar Americas, Inc.). Weather data were provided by an automated Oklahoma Mesonet weather station (Brock et al., 1995; McPherson et al., 2007) located within 1 km of the field plot.

The open-pollinated seeds used to produce plants for the experiment were collected from a single plant located in a field at 36°25′N latitude by 99°23′W longitude at 590 m elevation and adjacent to a seasonal waterway. The chaffy seeds of a plant (designated plant 365) were harvested in October 2012 and air dried. Plant 365 was chosen because of its high sucrose concentration. The sucrose concentration of 365 averaged 70 ± 15 g/kg of leaf sap. In February 2014, caryopses were extracted from the chaffy seeds of 365 using a Woodward laboratory air–seed shucker (Ag–Renewal, Inc.). The caryopses were sown into a greenhouse flat containing a soil mixture (Sun Gro Horticulture). When seedlings were large enough for transplanting, individual seedlings were removed from the flat and each seedling was transplanted into an individual cell of cavity trays containing a soil mixture. Seedlings were maintained in a greenhouse at 25 ± 5°C under natural light and day length conditions until field planting. In April 2014, the plants were transplanted into four randomized complete blocks consisting of five variable sized plots (density treatments). Variable plot sizes were used to obtain the desired plant population densities. The treatments consisted of five plant population densities representing 1,250; 2,500; 5,000; 10,000; and 20,000 plants per hectare. Plants were spaced equal distance apart within each plot using a series of equilateral triangles. The actual plot dimensions, plant spacing within plot, number of plants per plot, harvested area, and number of plants harvested per plot are given in Table 1.

### Table 1: Actual plot dimensions, plant spacing within plot, number of plants per plot, harvested area, and number of plants harvested per plot for Ravenna grass (*Tripidium ravennae*) transplanted at five population densities

| Plant population density (number ha⁻¹) | Plot dimensions | Plant spacing (m) | Plants per plot (n) | Harvested area (m²) | Plants harvested (n) |
|---------------------------------------|-----------------|------------------|---------------------|---------------------|---------------------|
|                                       | Width (m)       | Length (m)       |                     |                     |                     |
| 1,250                                 | 12.2            | 13.1             | 2.8                 | 20                  | 40                  | 5                   |
| 2,500                                 | 8.7             | 9.2              | 2.0                 | 20                  | 20                  | 5                   |
| 5,000                                 | 6.7             | 7.2              | 1.4                 | 24                  | 12                  | 6                   |
| 10,000                                | 4.1             | 5.8              | 1.0                 | 24                  | 6                   | 6                   |
| 20,000                                | 2.8             | 5.7              | 0.7                 | 32                  | 7                   | 14                  |

### 2.2 Site management

During the establishment year (2014), plots were maintained weed-free by hoeing and dead plants were replaced to maintain population densities. In subsequent years (2015–2019), a mixture of atrazine (2-chloro-4-ethylamino-6-isopropylaminos-triazine) and 2,4-D (2,4-dichlorophenoxyacetic acid) was applied in late March. Atrazine was applied at the rate of 1.68 kg a.i./ha, and 2,4-D was applied at the rate of 1.12 kg a.i./ha². Plots were rainfed (Table 2) and were not fertilized over the course of the experiment.

### 2.3 Number, diameter, and sucrose concentration of reproductive culms and leaves

In mid-September (2015–2019), the number of reproductive culms was counted on three interior plants of each plot to determine the average number of reproductive culms per plant. The reproductive culm diameter was measured using a caliper. Each culm was measured approximately 1 m above the soil surface and three culms were measured from two interior plants of each plot. A Pocket Refractometer (Model PAL-1, ATAGO Co., Ltd.) was used to determine sucrose concentrations of leaves and culms. In mid-September of each year, three basal leaves were picked from each plant and cut into segments of approximately 3.0 cm and placed into a hand-operated juice press. Approximately 0.5 ml of sap was collected using a disposable pipet and placed onto the prism surface of the refractometer and sucrose was measured. The refractometer was cleaned and calibrated between samples using deionized water. Similarly, the culm sucrose concentration was determined. Three reproductive culms were randomly harvested from each plant, and the third internode was removed and cut into approximately 3.0 cm segments and placed into the hand-operated press. The third internode was chosen because it is adjacent to the 3.0 dm height of biomass cut (see Section 2.4). Sap collection and measurements were carried out as before. Data for sucrose concentrations of leaves and culms were collected over a 4-day period each year in early September between 18:00 DST and 21:00 UTC.
2.4 Harvest management

A single forage harvest was made each year during December (2015–2019) for biomass yield. The desired number of plants per plot (Table 1) was harvested from the center of each plot using a plot harvester with an attached row independent chopper (Wintersteiger Inc.). The height of cut was 3.0 dm for all harvests. After data collection, the remaining standing crop (plot borders and edges) was removed. The harvested material of each plot was weighed, and a 250 to 300 g subsample of forage was collected for dry matter (DM) determination. Biomass subsamples were oven dried at 60°C. The biomass DM yield of each plot was calculated by multiplying the percentage DM of the oven dried subsample by the harvested weight of the plot. Oven dried subsamples were ground using a Wiley mill (Thomas Scientific) to pass through a 1 mm screen, and the carbon and nitrogen concentration of the biomass was determined using an Elementar Vario MAX CN analyzer (Elementar Americas, Inc.).

The concentration of ash was calculated from the ground subsample of each plot. DM was determined by placing crucibles in an oven overnight at 105°C. Crucibles were cooled in a desiccator and weighed. For each plot, a 0.5 g sample of ground biomass was placed into a crucible and the weight of the crucible plus sample recorded. The crucibles with samples were dried overnight at 105°C, cooled in a desiccator, and reweighed. DM was determined by subtracting the crucible weight from the crucible plus biomass sample weight. After DM determination, samples were ashed in a muffle furnace for 6 hr at 600°C. Samples were then transferred back to the drying oven (105°C) for at least 30 min and then cooled in a desiccator and ash weights were recorded. Total ash was calculated by subtracting the crucible weight from the crucible plus sample ash weight and then dividing by the sample dry weight. All data are reported on a dry weight basis.

Heating energy values were determined for duplicate samples using a semiautomatic isoperibol calorimeter (AC600; LECO Corporation). Combustion of a 0.25 g biomass sample from each plot was aided with the addition of 0.35 g paraffin oil. The biomass sample was soaked in the oil for at least 10 min before assembling the combustion vessel and firing using a cotton fuse. Once analysis was complete, the inside of the combustion vessel and sample holder were rinsed with distilled water and rinse water collected. Rinses were titrated with an indicator and sodium carbonate solution to correct for the formation of nitric acid. The results were corrected for energy content of the cotton fuse, paraffin oil, and nitric acid formation. Heating energy values were converted to MJ/kg.

2.5 Data analysis

Data for 2015–2019 were analyzed using a general linear mixed model analysis of variance. Fixed effects were production year (Y), plant density (D), and Y × D interactions.
Random effects were block, block in Y, and block in Y×D. Statistical significance was evaluated at p ≤ .05 (F test). Mean separations for production year and plant density were made by a t test at p ≤ .05. Nonlinear regression equations were fit to the data using nonlinear least squares to obtain the best unbiased estimators of the regression parameters using plant population density as the independent variable and biomass yield as the dependent variable.

3 | RESULTS

3.1 | Number, diameter, and sucrose concentration of reproductive culms and leaves

The number of reproductive culms per plant varied with production year, plant density, and Y×D interactions (p < .01). Plant density effects accounted for approximately 68% of the total variation for the number of reproductive culms per plant, production year effects accounted for approximately 20%, and Y×D interactions accounted for approximately 6% of the total variation. The average number of reproductive culms per plant varied from 56 in production year 2015 to 19 in year 2019 and from 70 for a plant density of 1,250 plants per hectare to 9 for a density of 20,000 plants per hectare (Table 3).

The reproductive culm diameter varied with production year and plant density (p < .01), but not with Y×D interaction (p > .20). Plant density and production year effects for culm diameter each accounted for approximately 28% of the total variation. Reproductive culm diameter decreased from 10.2 to 8.5 mm as plant density increased from 1,250 to 20,000 plants per hectare, respectively (Table 3). There was a similar decrease in culm diameter related to production year (Table 3).

The reproductive culm sucrose concentration varied with production year and plant density (p < .03) but not with Y×D interaction (p > .23). Plant density effects for culm sucrose concentration accounted for approximately 10% of the total variation while production year effects accounted for approximately 15%. In general, plant densities >5,000 plants per hectare have significantly lower sucrose concentrations averaging 58.0 g/kg compared with 64.5 g/kg for plant densities ≥5,000 plants per hectare (Table 3). Production years 2015 and 2017 produced significantly greater sucrose concentration (average 67.1 g/kg) compared with production years 2016 and 2018 (average 56.9 g/kg).

The basal leaf sucrose concentration varied with production year (p < .01) but not with plant density (p > .15) or Y×D interactions (p > .23). Production year accounted for approximately 60% of the total variation. The plant density effects accounted for <3% of the total variation and the Y×D interaction accounted for <7% of the total variation. Leaf sucrose was significantly higher in production year 2015 compared with other production years (Table 3).

3.2 | Biomass yield, N concentration and removal, C and ash concentrations, and heating value of biomass

Biomass yield of Ravenna grass varied significantly with production year and plant density (p < .01), but not with Y×D interactions (p = .85). Plant density effects accounted for approximately 54% of the total variation for biomass yield, production year effects accounted for approximately

| TABLE 3 | Means ± SE for main effects for Ravenna grass (Tripidium ravennae) plant population densities and production years for dependent variables: number of reproductive culms per plant, reproductive culm diameter, reproductive culm sucrose concentration, and basal leaf sucrose concentration |
|----------|------------------------------|-----------------|--------------------------|-----------------|
| Main effects | Number of plants per hectare or year | Number of reproductive culms per plant | Reproductive culm diameter (mm) | Reproductive culm sucrose concentration (g/kg) | Basal leaf sucrose concentration (g/kg) |
| Plant density | 1,250 | 70 ± 2 a* | 10.2 ± 0.2 a | 63.2 ± 2.3 ab | 36.0 ± 1.2 a |
| | 2,500 | 51 ± 2 b | 9.5 ± 0.2 b | 63.9 ± 2.3 ab | 36.5 ± 1.2 a |
| | 5,000 | 30 ± 2 c | 9.0 ± 0.2 c | 66.5 ± 2.3 a | 35.7 ± 1.2 a |
| | 10,000 | 14 ± 2 d | 8.6 ± 0.2 cd | 60.0 ± 2.3 bc | 34.4 ± 1.2 a |
| | 20,000 | 9 ± 2 e | 8.5 ± 0.2 d | 56.1 ± 2.3 c | 32.9 ± 1.2 a |
| Production year | 2015 | 56 ± 2 a | 9.8 ± 0.2 a | 66.8 ± 2.4 a | 48.2 ± 1.7 a |
| | 2016 | 35 ± 2 b | 9.8 ± 0.2 a | 57.1 ± 2.4 b | 32.9 ± 1.7 b |
| | 2017 | 37 ± 2 b | 9.2 ± 0.2 b | 67.3 ± 2.4 a | 30.8 ± 1.7 b |
| | 2018 | 26 ± 2 c | 8.7 ± 0.2 bc | 56.8 ± 2.4 b | 32.3 ± 1.7 b |
| | 2019 | 19 ± 2 d | 8.2 ± 0.2 c | 61.4 ± 2.4 ab | 31.4 ± 1.7 b |

*Means followed by a common letter within main effect are not significantly different at p > .05 (least significant difference test).
9%, and $Y \times D$ interactions accounted for approximately 3% of the total variation. Biomass yield was not significantly different for plant densities of 10,000 and 20,000 plants per hectare and averaged 16.2 Mg/ha (Table 4). Conversely, biomass yields were significantly different from each other for plant densities of 1,250; 2,500; and 5,000 plants per hectare and yields of these densities were significantly lower than the biomass yields of plant densities of 10,000 and 20,000 plants per hectare (Table 4). In general, biomass yield began to decline in production year 2017, and by 2019, yield had declined an average 31% from its highest production years 2015 and 2016 (Table 4). If the $Y \times D$ interaction for biomass yield is split by density, the biomass yields for production years 2015 through 2019 were similar at a density of 1,250 plants per hectare. Also, for a density of 2,500 plants per hectare, the biomass yields for production years 2015 through 2019 were similar. For the 5,000 plants per hectare density, the biomass yield began to decline in production year 2018. The decline in biomass production began in production year 2017 for plant densities 10,000 and 20,000 plants per hectare.

The concentration of nitrogen in biomass varied only with production year ($p < .01$) and accounted for approximately 83% of the total variation. The nitrogen concentration was initially low in production year 2015, peaked in 2016, and declined linearly through year 2019 (Table 4). Estimates of nitrogen removed with the biomass varied with production year and plant density ($p < .01$). Plant density effects accounted for approximately 35% of the total variation for nitrogen removed with biomass yield, and production year effects accounted for approximately 28% of the total variation. The amount of nitrogen removed in biomass varied from 30 to 90 kg/ha for 1,250 to 20,000 plants per hectare, respectively (Table 4). The concentration of carbon in biomass varied only with production year ($p < .01$) and accounted for approximately 72% of the total variation. Carbon concentration varied from 454 to 469 g/kg of biomass (Table 4). Similarly, the concentration of ash varied only with production year ($p < .01$). Production year accounts for approximately 81% of the total variation for concentration of ash in biomass. Ash concentration varied from 25 g/kg for production year 2015 to an average of 60 g/kg for production years 2018 and 2019 (Table 4). The heating value of Ravenna grass biomass varied only with production year ($p < .01$) and accounted for approximately 33% of the total variation for heating value. The heating valued ranged from 18.4 to 18.7 MJ/kg for Ravenna grass biomass among production years (Table 4).

### DISCUSSION

The field plot prior to this research was in a winter wheat (*Triticum aestivum* L.) fallow system. A hay crop was produced from the wheat in the spring of each year and after forage harvest the wheat stubble was disked into the field and lay fallow until preparation for planting in the fall of each year. The soil fertility of the field was minimally maintained, fertilizing with 40 kg/ha of urea (46-0-0) once every 2 or 3 years. Wheat was used to reduce wind erosion of the soil during the fall and winter months. Prior to this research, the soil sampled to a depth of 15 cm averaged pH of 7.4 ± 0.3, NO$_3$-N of 1.5 ± 0.4 kg/ha, P of 11.5 ± 1.2 mg/kg, K of 114 ± 8 mg/kg, and SOC of 4.9 ± 0.1 mg/g. No soil amendments were made to the field plot because Ravenna grass has been reported to grow well in nutrient-poor soils (Rau et al., 2009). Moisture...
was not limited over the 5 year period and mean annual temperatures were average to slightly above average (Table 2).

4.1 | Number, diameter, and sucrose concentration of reproductive culms and leaves

The $Y \times D$ interaction for number of reproductive culms per plant was possibly due to increased competition between plants at the highest plant densities (10,000 and 20,000 plant per hectare) in production years 2016 and later compared with production year 2015 (Figure 1). Tillering has been shown to decrease at high plant densities, and tillering and reproductive development have been shown to be regulated by light quality and quantity particularly in relation to red:far-red light ratio (Skalova & Krahulec, 1992; Wan & Sosebee, 1998). Tillering has also been shown to compensate for low plant density (Hiernaux et al., 1994). As the plant crown expands, new growth takes place along its perimeter where light, nutrient-rich soil, and moisture are readily available. Tillers within the crown may be smaller in diameter and produce fewer tillers due to lower nutrient availability and shading. Reproductive and vegetative culms along the leading edge of the crown probably produce a greater number of tillers until equilibrium is reached with a limiting nutrient. This is suggested by the data and shown in Figure 1 where low plant densities have produced a greater number of reproductive culms that diminished in number over time, possibly due to a limiting nutrient. In this case, the limiting nutrient may be phosphorus, since it tested low in the soil test, or the soil may be deficient in one or more micronutrients that were not tested in the soil analysis. Springer et al. (2003) reported similar findings for the number of vegetative culms per plant for eastern gamagrass ($Tripsacum dactyloides$ (L.) L.). Ravenna grass, like eastern gamagrass, is a robust bunchgrass of the tribe Andropogoneae. Therefore, reproductive tillering of Ravenna grass is primarily driven by plant density effects and the interactions of the environment, e.g., light, moisture, and nutrients, with plant density.

The decline in reproductive culm diameter is indicative of density-related stress among plants of the same species where plants compensate their growth to accommodate their growing conditions and competition effects from their neighbors (Harper, 1977, p. 152).

Environmental extremes are known to affect the concentration of sucrose in sugarcane (Glasziou & Gayler, 1972) which may account for the variation found among production years. Ravenna grass is an open-pollinated diploid with $2n = 20$ chromosomes (Janaki-Ammal, 1936, 1941). Springer (2018) reported that the variation for sucrose concentrations found in Ravenna grass populations may make it possible to select and breed for higher sucrose concentrations, provided that the trait is heritable. Breaux (1984) reported that it is possible to enhance the sucrose content of sugarcane varieties using recurrent selection.

Springer (2018) reported that the concentration of sucrose in reproductive culms was approximately 1.6 times that of basal leaf. This is the case for this experiment as well. Since the plants used in this experiment were derived from the same population as those used in the experiment of Springer (2018), this was to be expected.

4.2 | Biomass yield, N concentration and removal, C and ash concentrations, and heating value of biomass

Plant density effects have been separated into a growth phase and an equilibrium phase (Springer et al., 2003). The growth phase is characterized by crown development and expansion of the root and shoot systems. Equilibrium occurs when growth is limited by plant competition for water, nutrients, and/or light. As shown earlier, the decline is indicative of density-related stress among plants of the same species where plants compensate their growth based on their population density and resources (Harper, 1977, p. 152). Plots with higher plant densities were expected to reach equilibrium sooner because nutrient resources are depleted more quickly, thus limiting plant growth (Jones & Johnson, 1991). Nonlinear regression of the data by year showed biomass yield to plateau between 13.2 and 19.3 Mg/ha at plant densities ranging from 8,235 to 12,820 plants per hectare (Table 5). Combined over years, the data showed the equation for biomass yield to plateau at 16.2 Mg/ha at a plant density of 10,640 plants per hectare (Table 4). The data suggest that 10,000
plants per hectare would produce the greatest biomass annually, provided that nutrient requirements of the crop were met.

From previous work, Ravenna grass grown at low plant population densities sustained high biomass yield without fertilization (Springer, 2018). However, at high plant population densities biomass yield declined. Soil available nitrogen and phosphorus were low on this site. Since biological nitrogen fixation has been reported in sugarcane (Urquiaga et al., 1992), a close relative of Ravenna grass, it is conceivable that Ravenna grass benefits from biological nitrogen fixation. If it does benefit from biological nitrogen fixation, then the yield decline may be related to the depletion of soil phosphorus or the interference of the uptake of micronutrients, e.g., iron or zinc. Nozoye et al. (2017) found that deficiencies of iron and/or zinc limited the growth of Ravenna grass on calcareous soils. The soil of the plot where this experiment was conducted is reported to contain up to 15% calcium carbonate in the soil profile (USDA ARS, 2020a, 2020b). If biological nitrogen fixation does not occur, then an annual application of nitrogen would be recommended at a rate of 60–90 kg/ha to sustain biomass yields depending on the plant population density. Regardless of these facts, research is needed to determine nutrient recommendation for Ravenna grass for optimum biomass production.

The average concentration of carbon for production years 2015–2019 was higher than that reported for other energy crops (Monti et al., 2008). A 1% increase in carbon concentration corresponds to a 0.39 MJ/kg increase in the biomass heating values (Jenkins, 1989). The heating values for Ravenna grass (18.4–18.7 MJ/kg; Table 4) were slightly higher than those reported for switchgrass (P. virgatum L.; 18.1 MJ/ha; Jenkins et al., 1998). The concentration of ash in biomass can affect the heating value. The heating value of biomass is correlated with ash concentration of biomass (Jenkins et al., 1998). It has been reported that for every 1% increase in ash concentration there is a corresponding 0.2 MJ/kg decrease in the heating value of biomass. In this experiment the heating values were correlated negatively with the concentration of ash ($r = -0.57, p < .01, n = 100$), and a 1% increase in ash corresponded to a 0.07 MJ/kg decrease in the heating value of Ravenna grass biomass. Springer (2018) suggested that it might be possible to reduce the concentration of ash in Ravenna grass biomass through selection and breeding given the variation found among individuals within a Ravenna grass population.

### 5 | CONCLUSIONS

Biomass yield of Ravenna grass is affected by plant population density in at least four ways. First, as plant population density increased biomass yield increased, especially during the growth phase. On average, biomass yield plateaued at 16.2 Mg/ha at a plant population density of 10,640 plants per hectare. For the ease of management, a plant population density of 10,000 plants per hectare would be ideal. Second, plant population density affected the diameter and number of reproductive culms per plant. Early in the life of the stand, higher plant densities have a greater number of culms per plant; however, plots with high plant densities reached equilibrium quicker than plots of lower plant densities. The number of reproductive culms per plant was greater at lower plant densities. Plots with lower plant densities produce a greater number of culms and thus compensated for their low density. Third, sucrose concentration was affected by plant density, where low plant density produced higher concentrations of sucrose, and where culms of larger diameters tended to produce higher concentrations of sucrose. It would be interesting to determine the variation among plants associated with sucrose. If significant variation exists, it might be possible to select and breed lines for greater sucrose yield. Fourth, plant density affects the amount of nitrogen removed with biomass. As biomass yield increased with plant density, the removal of nitrogen increased. As discussed earlier, Ravenna grass may benefit from biological nitrogen fixation. However, if this is not true, then supplemental nitrogen should be added annually to compensate for nitrogen removal from the site. Nutrient management experiments need to be conducted to determine nutrient recommendations for Ravenna grass under biomass production.
Lastly, production year × plant density effects were either small or did not exist, but significant effects were associated with production year. These effects showed biomass yield to decline with time. Other cultural and management practices should be studied to determine their effects on the sustainability of biomass production of Ravenna grass.

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CONFLICT OF INTEREST
The author declares that there is no conflict of interest.

DATA AVAILABILITY STATEMENT
The data that support the findings of this study are available from the corresponding author upon reasonable request.

ORCID
Tim L. Springer https://orcid.org/0000-0003-4243-5945

REFERENCES
Barkworth, M. R., Anderson, L. K., Carpels, K. M., Long, I. S., & Pip, M. B. (2007). Manual of grasses of North America. Utah State University Press.

Breaux, R. D. (1984). Breeding to enhance sucrose content of sugarcane varieties in Louisiana. Field Crops Research, 9, 59–67. https://doi.org/10.1016/0378-4290(84)90066-6

Brock, F. V., Crawford, K. C., Elliott, R. L., Cuperus, G. W., Stadler, S. J., Johnson, H. L., & Eilts, M. D. (1995). The Oklahoma Mesonet: A technical overview. Journal of Atmospheric and Oceanic Technology, 12, 5–19. https://doi.org/10.1175/1520-0426(1995)012<005:TOMATO>2.0.CO;2

CABI. (2020). Saccharum ravennae (Ravenna grass) [text by: Nick Pasiecznik]. In Invasive species compendium. CABI International. Retrieved from https://www.cabi.org/isc/datasheet/109359

DiTomaso, J. M., & Healy, E. A. (2007). Weeds of California and other western states (Vol. 2, pp. 1034–1039). University of California Press.

Glassiou, K. T., & Gayler, K. R. (1972). Storage of sugars in stalks of sugar cane. Botanical Review, 38, 471–488. https://doi.org/10.1007/BF02859248

Harper, J. L. (1977). Population biology of plants. Academic Press.

Hiernaux, P., De Leeuw, P. N., & Diarra, L. (1994). Modelling tillering of annual grasses as a function of plant density: Application to Sahelian rangelands productivity and dynamics. Agricultural Systems, 46, 121–139. https://doi.org/10.1016/0308-521X(94)90093-U

Hitchcock, A. S. (1950). Manuals of the grasses of the United States (2nd ed.). Revised by A. Chase. USDA Misc. Publ. 200. U.S. Gov. Print. Office.

Janaki-Ammal, E. K. (1936). Cytogenetics analysis of Saccharum spontaneum chromosome studies in some Indian forms. Indian Journal of Agricultural Sciences, 6, 1–8.

Janaki-Ammal, E. K. (1941). Intergeneric hybrids of Saccharum. Journal of Genetics, 41, 217–253. Retrieved from https://www.ias.ac.in/article/fulltext/jgen/041/02-03/0217-0253

Jenkins, B. M. (1989). Physical properties of biomass. In O. Kitani & C. W. Hall (Eds.), Biomass handbook (pp. 860–891). Gordon & Breach.

Jenkins, B. M., Baxter, L. L., Miles Jr., T. R., & Miles, T. R. (1998). Combustion properties of biomass. Fuel Processing Technology, 54, 17–46. Retrieved from http://gekgasifier.pbworks.com/f/biomass%2520properties%2520Miles.pdf

Jones, O. R., & Johnson, G. L. (1991). Row width and plant density effects on Texas high plains sorghum. Journal of Production Agriculture, 4, 613–621. https://doi.org/10.2134/jpa1991.0613

McPherson, R. A., Fiebrich, C., Crawford, K. C., Elliott, R. L., Kilby, J. R., Grimsley, D. L., Martinez, J. E., Basara, J. B., Illston, B. G., Morris, D. A., Kloesel, K. A., Stadler, S. J., Melvin, A. D., Sutherland, A. J., & Shrivastava, H. (2007). Statewide monitoring of the mesoscale environment: A technical update on the Oklahoma Mesonet. Journal of Atmospheric and Oceanic Technology, 24, 301–321. https://doi.org/10.1175/JTECH1976.1

Mehlich, A. (1984). Mehlich 3 soil test extractant: A modification of Mehlich 2 extractant. Communications in Soil Science and Plant Analysis, 15, 1409–1416. https://doi.org/10.1080/00103628409367568

Meyer, M. H. (2011). Ornamental grasses in the United States. Horticultural Review, 39, 121–152. https://doi.org/10.1002/978118100592.ch3

Monti, A., Virgilio, N. D., & Venturi, G. (2008). Mineral composition and ash content of six major energy crops. Biomass and Bioenergy, 32, 216–223. https://doi.org/10.1016/j.biombioe.2007.09.012

Nozoye, T., Aung, M. S., Masuda, H., Nakanishi, H., & Nishizawa, N. K. (2017). Bioenergy grass [Erianthus ravennae (L.) Beauv.] secretes two members of mugineic acid family phytosiderophores which involved in their tolerance to Fe deficiency. Soil Science and Plant Nutrition, 63, 543–552. https://doi.org/10.1007/s00380768.2017.1394168

Palmer, I. E., Gehl, R. J., Ranney, T. G., Touchel, D., & George, N. (2014). Biomass yield, nitrogen response, and nutrient uptake of perennial bioenergy grasses in North Carolina. Biomass and Bioenergy, 63, 218–228. https://doi.org/10.1016/j.biombioe.2014.02.016

POWO. (2019). Plants of the world online. Facilitated by the Royal Botanic Gardens, Kew. Retrieved from http://powo.science.kew.org/taxon/urn:lsid:ipni.org:names:77075505-1/

Rau, N., Mishra, V., Sharma, M., Das, M. K., Ahaluwalia, K., & Sharma, R. S. (2009). Evaluation of functional diversity in rhizobacterial taxa of a wild grass (Saccharum ravennae) colonizing abandoned fly ash dumps in Delhi urban ecosystem. Soil Biology and Biochemistry, 41, 813–821. https://doi.org/10.1016/j.soilbio.2009.01.022

Sanderson, M. A., Jones, R. M., & Read, J. C. (1996). Management of forage sorghum: Nitrogen, plant density and irrigation effects on yield and quality. Texas Journal of Agricultural and Natural Resources, 9, 61–78. Retrieved from https://txj jan.agnetxas.org/index.php/txjan/article/view/244

Sims, J. T. (1996). Lime requirement. In D. L. Sparks, A. I. Page, P. A. Helmke, R. H. Loeppert, P. N. Soltanpour, M. A. Tabatabai, C. T. Johnston, & M. E. Sumner (Eds.), Methods of soil analysis, part 3, Chemical methods, SSSA Book Series (Vol. 5, pp. 491–515). SSSA and ASA.

Skalova, H., & Krahulec, F. (1992). The response of three Festuca rubra clones to changes in light quality and plant density. Functional Ecology, 6, 282–290. https://doi.org/10.2307/2389518
Springer, T. L. (2018). Variation of agronomic traits of Ravenna grass and its potential as a biomass crop. *Agronomy*, 8, 70. https://doi.org/10.3390/agronomy8050070

Springer, T. L., Dewald, C. L., Sims, P. L., & Gillen, R. L. (2003). How does plant population density affect the forage yield of eastern gamagrass? *Crop Science*, 43, 2206–2211. https://doi.org/10.2135/cropsci2003.2206

Thomsen, C., & Meyer, T. (2007). Ravenna grass: A major wildland weed along Cache creek. *Cal-IPC Newsletter*, 15(4–5), 16. Retrieved from https://www.cal-ipc.org/wp-content/uploads/2017/03/Fall-2007-webnewsletter-6.pdf#page=4

Urquiaga, S., Cruz, K. H. S., & Boddey, R. M. (1992). Contribution of nitrogen fixation to sugar cane: Nitrogen-15 and Nitrogen-balance estimates. *Soil Science Society of America Journal*, 56, 105–114. https://doi.org/10.2136/sssaj1992.03615995005600010017x

USDA ARS. (2020a). Agricultural Research Service, National Plant Germplasm System. Germplasm Resources Information Network (GRIN-Taxonomy). National Germplasm Resources Laboratory. Retrieved from https://npgsweb.ars-grin.gov/gringlobal/accessiondetail.aspx?id=1742713

USDA NRCS. (2020). Soil Survey Staff, Natural Resources Conservation Service, United States Department of Agriculture. *Web soil survey*. Retrieved from https://websoilsurvey.sc.egov.usda.gov/App.HomePage.htm

USDA ARS. (2020b). Agricultural Research Service, National Plant Germplasm System. Germplasm Resources Information Network (GRIN-Taxonomy). National Germplasm Resources Laboratory. Retrieved from https://npgsweb.ars-grin.gov/gringlobal/accessiondetail.aspx?id=481904

USDA ARS. (2020b). Agricultural Research Service, National Plant Germplasm System. Germplasm Resources Information Network (GRIN-Taxonomy). National Germplasm Resources Laboratory. Retrieved from https://npgsweb.ars-grin.gov/gringlobal/accessiondetail.aspx?id=481904

Wan, C., & Sosebee, R. E. (1998). Tillering responses to red: Far-red light ratio during different phenological stages in *Eragrostis curvula*. *Environmental and Experimental Botany*, 40, 247–254. https://doi.org/10.1016/S0098-8472(98)00044-6

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