

Article

Yield, Nutritional Composition, and In Vitro Ruminant Digestibility of Conventional and Brown Midrib (BMR) Corn for Silage as Affected by Planting Population and Harvest Maturity

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Abstract: The objective of this study was to evaluate the effects of corn-planting population, using two conventional (Conv) and two brown-midrib (BMR) hybrids, and maturity stage at harvest on forage dry-matter (DM) yield, silage quality, and in-vitro fiber digestibility. The study was conducted in two fields with contrasting production potential, where both corn hybrids were planted at a theoretical planting population of 59,000, 79,000, and 99,000 seeds/ha. Corn was harvested at the early-dent (early) or 2/3 milk-line (late) maturity stage. An interaction between planting population and field existed for biomass yield. We observed a consistent increase in forage yield with increased planting population only in the field of higher production potential. Corn hybrids that contained the BMR trait did not penalize yield but had a consistently higher digestibility of neutral detergent fiber (aNDFom) compared to conventional hybrids. Except for starch concentration, no interaction existed between planting population and maturity for forage yield, fiber digestibility, and nutritional composition. A response to increasing planting population on starch concentration was observed only when corn was harvested at the L = late maturity stage. In conclusion, increasing corn-planting population may increase forage yield, but such an effect may depend on the soil's growing potential. In addition, planting population had a negligible effect on the nutritional composition and fiber digestibility of corn silage and was minimally affected by the maturity stage at harvest.

Keywords: corn-biomass yield; fiber digestibility; brown midrib; planting density



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1. Introduction

Corn silage is usually the main forage source for dairy-farming systems. The corn crop provides an excellent combination of high dry-matter yield per hectare and quality of the biomass produced. Several agronomic and management practices are available for dairy producers when planning to maximize the yield and quality of corn silage. Farmers can select corn hybrids with the brown-midrib trait (BMR) to increase fiber digestibility [1], whereas kernel processing can improve starch availability [2]. By increasing cutting height, producers can manipulate both forage yield and quality [3]. Additionally, a major factor that affects the quality of corn silage is the maturity stage at which the forage is harvested [4].

Increasing corn-planting population can increase corn-silage yields [5–7] with negligible impact on forage quality [8,9]. However, an interesting observation reported by Ferreira et al. [9] was the interaction between corn-planting population and crop maturity at harvest. In particular, biomass yield increased linearly when planting population was increased in the second year of the two-year study but not in the first year. An important difference was the more advanced development of the kernel, which resulted in a higher concentration of dry matter (DM) in the whole plant in the second year. This difference in the physiological

stages of the crops at harvest, associated with a more advanced maturity of the plant, was related to greater cumulative growing degree-days during the second year. Another factor that might have influenced the different yield response to planting population from year to year was precipitation during kernel development. Abundant rain during the second year resulted in a greater number of kernels per plant and starch concentration. This potential interaction between planting population and crop maturity at harvest that might determine the possibility of increasing biomass yields at greater corn-planting populations warrants further evaluation under controlled conditions.

Thus, we hypothesized that increasing planting population will increase biomass yield when corn is harvested at a late but not at an early stage of maturity. Therefore, the objective of this study was to evaluate the response of planting corn for silage at three populations (59,000, 79,000, and 99,000 seeds/hectare) and harvested at two maturity stages (early dent or 2/3 milk line) on dry-matter yield, nutrient composition, and in-vitro digestibility of two conventional and two BMR corn hybrids.

2. Materials and Methods

2.1. Experimental Sites and Climate Data

This study was conducted from April to October 2020 in two fields (i.e., site replication). The low-production-potential field (LP) was located at the Simpson Research Farm, Clemson University, Pendleton, South Carolina (34°62′10.8″ N 82°73′31.5″ W), whereas the high-production-potential field (HP) was located at Clemson University Calhoun Fields, Clemson, South Carolina (34°67′36.7″ N, 82°84′39.4″ W). Soil from LP is described as Cecil sandy loam with 2 to 6% slopes (CdB) and a land-capability classification of Iie (web soil survey; www.nrcs.usda.gov (accessed on 3 March 2023)). The soil pH was 5.7, with a P and K concentration of 29 and 69 ppm, respectively. Soil from HP is described as Toccoa with a land-capability classification of IIw. The soil pH was 6.2, with a P and K concentration of 35.0 and 120 ppm, respectively. Therefore, the fertility difference between fields and, in particular, the shallow water table in the HP field, resulted in two contrasting growing environments. Weather and historic weather data (1981 to 2010) were collected from a weather station located at Sandy Springs, SC, using the National Centers for Environmental Information of the National Oceanic and Atmospheric Administration (NOAA, US Department of Commerce, www.noaa.gov (accessed on 10 November 2022)). Average monthly temperature, precipitation, and cumulative growing degree-days (GDD) for both fields are reported in Table 1.

Table 1. Average 2020 climate data and cumulative growing degree-days (GDD) for the two experimental sites ^a.

ss	April	May	June	July	August	September
Precipitation, mm						
LP	179	281	51	91	158	–
HP	183	195	78	53	134	124
30-year mean	101	99	103	108	119	100
Temperature, °C						
LP	14.3	17.9	23.5	26.6	25.7	–
HP	15.6	18.8	24.3	27.6	26.2	22.1
30-year mean	15.6	20.0	24.2	26.2	25.6	22.4
Cumulative GDD, °C ¹						
LP early		456	1173	2024	2160	–
LP late		456	1173	2024	2692	–
HP early		–	541	1421	2275	2391
HP late		–	541	1421	2275	2811

^a Data obtained from NOAA, US Department of Commerce (<https://www.nrcs.usda.gov> (accessed on 10 November 2022)). ¹ Harvest days: LP early = August 5; LP late = August 26; HP early = September 3; HP late = September 24.

2.2. Experimental Design

The trial was designed as a randomized complete block design with a split-split-plot arrangement of treatments. During the spring of 2020, each field was divided into three blocks, and within each of the three blocks, one plot (12 m wide and 7.6 m long) was randomly assigned to one of three theoretical seeding rates (hereafter, planting population), leading to nine plots in each field. Corn-planting populations were 59,000, 79,000, and 99,000 seeds/hectare (59K, 79K, and 99K, respectively). Plots on the LP field were planted on 4 May 2020, whereas plots on the HP field were planted on 9 June 2020. Within each plot, one sub-plot (1.5 m wide and 7.6 m long) was planted with one of four corn hybrids, two conventional (hereafter, Conv1 and Conv2) and two brown midrib (hereafter, BMR1 and BMR2), leading to 36 sub-plots in each field. The four hybrids were provided by Mycogen Seeds (Indianapolis, IN, USA), Augusta Seed Corporation (Verona, VA, USA), and Pioneer Hi-Bred International (Johnston, IA, USA). To avoid any form of endorsement or opposition to any material, corn hybrids are blindly addressed, and their identities will not be provided. Two rows within the same sub-plot (1 center and 1 edge row) were harvested at the early-dent stage of maturity (hereafter, early), and the other two rows were harvested at the 2/3 milk-line maturity stage (hereafter, late). In the LP field, plots were harvested on 5 and 26 August (early and late, respectively), whereas in the HP field plots were harvested on 9 and 24 September (early and late, respectively). Plots were planted with a two-row Almaco cone-type planter (Almaco, Nevada, IA, USA) mounted to John Deere planter units (Deere & Company, Moline, IL, USA) and with rows separated by 76 cm. Fertilizer was applied to each plot before planting (56 kg N/ha, 84 kg P₂O₅/ha, and 84 kg K₂O/ha). When the crop showed six visible leaves (V6), the plots were fertilized (side dress) with 84 kg N/ha as urea ammonium nitrate. In addition, at planting, the plots were sprayed with 4.7 Lts/ha of Bicep II Magnum (Syngenta, Basel, Switzerland), followed by 560 g/ha of 2,4-D + 2.3 Lts/ha of Atrazine at the time of N side-dress application.

2.3. Harvesting and Ensiling

The forage biomass of each plot was harvested using a one-row pull-type forage harvester (Sip Silo 100, Strojna Industrija, Slovenia). After weighing the harvested biomass, thoroughly mixed samples of chopped material from each plot were collected in plastic bags, immediately placed in a cooler with ice, and transferred to the laboratory. A 400 to 500 g subsample was stored at −20 °C. A second 400 to 500 g subsample of chopped material was ensiled into MR-1014 polyethylene-embossed pouches (Doug Care, Springfield, CA, USA) and double-sealed anaerobically with a FastVac vacuum sealer (Doug Care) as described by Der Bedrosian et al. [10]. No inoculants were added to enhance fermentation. The mini silos were stored in a drawer (i.e., dark) for 60 days at room temperature (25 °C).

2.4. Forage Processing and Analyses

The first subsample of fresh material was thawed and dried at 55 °C in a forced-air-drying oven (Model 89511-414, VWR International, Radnor, PA, USA) until a constant weight was reached. The resulting DM concentration was used to calculate the DM yield (kg/ha). After 60 days of fermentation, the mini silos were opened and the pH was determined by blending (Ninja Professional 1100, SharkNinja Operating LLC, Needham, MA, USA) 10 g of corn silage with 90 mL of deionized water for 5 min and immediately measuring pH with a pH meter (SympHony H10P, VWR International, Radnor, PA, USA). The remaining corn-silage samples were thawed and dried at 55 °C in a forced-air oven for 48 h. Dried samples were ground to pass a 1 mm screen of a Wiley mill (Arthur H. Thomas, Philadelphia, PA, USA). Ground samples were dried at 105 °C for 24 h to determine analytical DM. Ash concentration was determined after combusting samples in a furnace for 3 h at 600 °C (method 942.05; AOAC) [11]. Crude-protein (CP) concentration was calculated as percent N × 6.25 after combustion analysis (method 990.03; AOAC) [12] using a Vario El Cube CN analyzer (Elementar Americas, Inc., Mount Laurel, NJ, USA). Neutral detergent-fiber (aNDFom) and ADFom concentrations were determined using an Ankom200 Fiber

Analyzer (Ankom Technology, Fairport, NY, USA) and corrected for ash concentration. Sodium sulfite and α -amylase (Sigma no. A3306: Sigma Chemical Co., St. Louis, MO, USA) were included for aNDFom analysis [13]. After determining ADF, the fiber residue was incubated for 3 h in 72% sulfuric acid within 4 L jars that were placed in a Daisy II Incubator (Ankom Technology) for ADL determination. Starch concentration was determined using the acetate-buffer method by Hall [14] with α -amylase from *Bacillus licheniformis* (FAA, Ankom Technology) and amyloglucosidase from *Aspergillus niger* (E-AMGDF, Megazyme International, Wicklow, Ireland).

Care and handling of animals used for collecting rumen contents for in-vitro incubations were conducted as outlined in the guidelines of the Clemson University Committee on Animal Use (AUP2019-074). In-vitro DM digestibility (IVDMD), in-vitro true DM digestibility (IVTDMD), and in-vitro NDF digestibility (IVNDFD) were determined using a Daisy II rotating-jar in-vitro incubator (Ankom Technology). Samples were incubated for 30 h following the procedures described by Ferreira and Mertens [15]. A composite inoculum was prepared with rumen fluid and solids collected from two ruminally fistulated lactating dairy cows that were fed a diet containing 44% corn silage, 4.1% triticale, and 51.9% concentrate mix (DM basis).

2.5. Statistical Analysis

Data were analyzed with the MIXED procedure of SAS (SAS version 9.4, SAS Institute Inc., Cary, NC, USA). The statistical model included the fixed effect of the field (degrees of freedom, $df = 1$); the fixed effect of the planting population ($df = 2$); the random effect of the block ($df = 2$); the random whole-plot error ($df = 12$); the fixed effect of the corn hybrid ($df = 3$); the random sub-plot error ($df = 36$); the fixed effect of the maturity at harvest ($df = 1$); all two-, three-, and four-way possible interactions ($df = 40$); and the random split-split-plot or residual error ($df = 47$). Significant differences and tendencies to differ were declared at $p < 0.05$ and $p \leq 0.10$, respectively.

3. Results

3.1. Weather Conditions

Rainfall amounts during the 2020 growing season were above the 30-year mean during the spring (except for June) and similar to historic averages for most of the summer and early fall (Table 1). Corn planted in the LP field received more rain than in the HP field (581 vs. 389 mm, respectively). In addition, recorded temperatures during the growing season were consistent with the 30-year mean. The delayed planting date resulted in greater cumulative growing degree-days for corn planted in the HP field than in the LP field. When harvested at early dent, the cumulative growing degree-days were 2391 vs. 2160 °C for HP and LP, respectively. Similarly, when harvested at 2/3 milk line, the cumulative growing degree-days were 2811 vs. 2692 °C for HP and LP, respectively.

3.2. Forage Yield of Corn Silage

Table 2 presents the least-squares means on the effects of field, corn hybrid, planting population, and maturity at harvest on the yield of corn-silage biomass, DM concentration, and pH. Overall, the biomass yield in LP was 59% lower than in HP. However, there was a significant field-by-planting-population interaction. In the LP field, planting corn at 79K resulted in the highest yield, whereas the DM yield was similar for the highest (99K) and the lowest (59K) corn-planting populations (Figure 1). However, in the HP field, the biomass yield increased linearly and by 20% when the planting population increased from 59K to 99K. The Conv and BMR hybrids yielded similar biomass when planted in both fields. On average, the DM yield of corn harvested early was 12.8% lower compared to the corn harvested at more advanced maturity.

Table 2. Biomass yield, dry-matter (DM) concentration, and silage pH as affected by field, planting population, hybrid, and maturity.

	Yield, kg DM/ha	DM, %	pH
FIELD			
LP	9938 ^b	32.7	3.67 ^b
HP	15,580 ^a	33.2	3.74 ^a
SEM	320	0.30	0.02
POPULATION			
59K	11,664 ^b	33.3	3.71
79K	13,365 ^a	32.8	3.70
99K	13,248 ^a	32.7	3.70
SEM	393	0.36	0.02
HYBRID			
Conv1	13,239	32.1 ^b	3.70
Conv2	12,437	35.0 ^a	3.70
BMR1	12,556	32.1 ^b	3.71
BMR2	12,749	32.6 ^b	3.71
SEM	330	0.34	0.02
MATURITY			
Early	11,885 ^b	27.0 ^b	3.68 ^b
Late	13,633 ^a	38.9 ^a	3.73 ^a
SEM	255	0.26	0.02
Interactions, <i>p</i> -values			
Field × Population	0.04	0.26	0.60
Field × Hybrid	0.11	0.10	0.47
Field × Maturity	0.13	<0.01	<0.01
Population × Hybrid	0.43	0.59	0.64
Population × Maturity	0.38	0.52	0.15
Hybrid × Maturity	0.16	0.12	0.31

^{a,b} Means with different superscripts differ ($p < 0.05$).

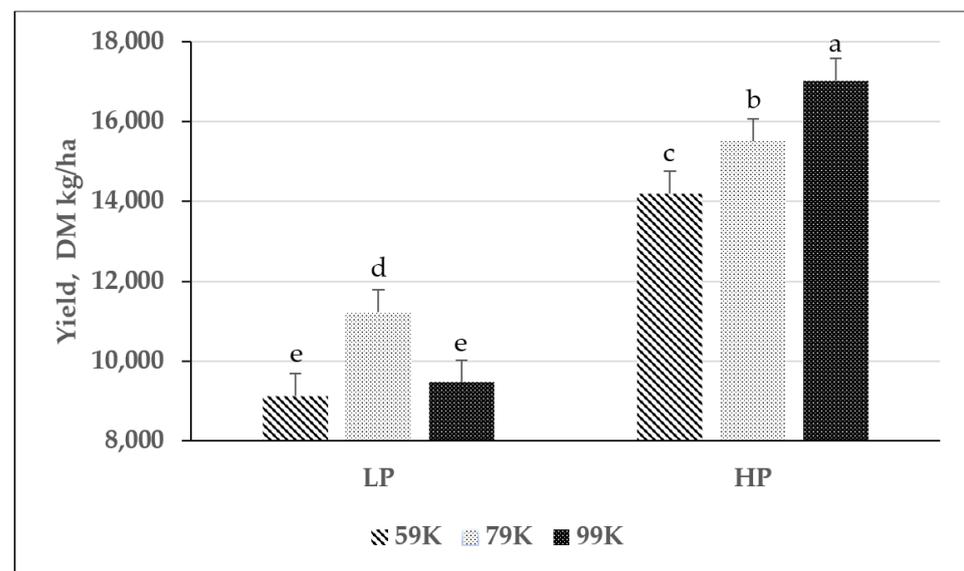


Figure 1. Interaction between field (LP or HP) and planting population (59K, 79K, and 99K plants/hectare) on corn-silage-biomass yield (kg DM per ha). ^{a-e} Means with different letters differ ($p \leq 0.05$). Vertical bars indicate standard errors of the mean.

No differences in silage-DM content existed between fields and the planting population (Table 2). The interaction between field and maturity at harvest reflected a greater difference in DM content between early- and late-maturity corn silage when harvested from the HP

field (26.3 vs. 36.6% DM) compared to corn silage harvested in the LP field (26.6 vs. 40.0% DM). The Conv2 corn silage had the greatest concentration of DM (35.0%) relative to the other three varieties, which had similar DM concentrations (32.3%). Regardless of hybrid and planting density, silage-DM concentration was highest in more mature corn silage.

We observed a higher pH in corn silage harvested from the HP field compared to the LP field (3.74 vs. 3.67, respectively), but no differences in silage pH were observed between planting populations or corn hybrids. The more mature silage had a higher pH than the less mature forage (3.73 vs. 3.68, respectively). However, there was a significant interaction between fields and maturity at harvest. There were no differences in silage pH between corn harvested early or late in the LP field or early in the HP field (3.67), but the silage pH of late corn in the HP field was 0.13 units higher (3.80). Nevertheless, the low pH observed in all treatments suggests that these differences will have little impact on fermentation characteristics and corn-silage quality.

3.3. Chemical Composition of Corn Silage

Table 3 presents the least-squares means on the effects of field, planting population, corn hybrids, and maturity at harvest on the nutritional composition of corn silage. Experimental treatments had a small effect on the concentration of ash and CP that will likely have a minor impact on the overall silage quality. The aNDFom concentration was 4% units higher when corn was planted in the LP field compared to the HP field. Additionally, the highest aNDFom was observed when corn was planted at 79K (45.8% aNDFom), and there were no differences between the 59K and 99K planting populations (44.3% aNDFom). The concentration of aNDFom was slightly lower for the Conv2 hybrid than for the Conv1 and the two BMR hybrids. The concentration of aNDFom declined as corn maturity advanced, but there was a significant field-by-maturity interaction. The concentration of aNDFom decreased from 48.9% to 44.7% as maturity advanced when corn was planted in the LP field. However, there was no difference in aNDFom concentration between the two maturity stages when corn was planted in the HP field (42.7% vs. 42.8% of DM for early and late, respectively).

Table 3. Chemical composition (% of DM unless stated otherwise) of corn silages as affected by field, planting population, hybrid, and maturity.

Item	Ash	CP	aNDFom	ADL	ADL %aNDFom	Starch
FIELD						
LP	4.48 ^b	10.0 ^a	46.8 ^a	3.05 ^b	6.56 ^b	21.2 ^b
HP	4.77 ^a	9.1 ^b	42.8 ^b	3.55 ^a	8.31 ^a	28.0 ^a
SEM	0.05	0.10	0.36	0.22	0.53	0.34
POPULATION						
59K	4.65	9.7 ^a	44.1 ^b	3.09	7.05	24.7 ^{a,b}
79K	4.71	9.8 ^a	45.8 ^a	3.46	7.63	23.5 ^c
99K	4.51	9.1 ^b	44.4 ^b	3.35	7.63	25.6 ^a
SEM	0.06	0.12	0.41	0.19	0.44	0.42
HYBRID						
Conv1	4.65 ^{b,c}	9.5	45.4 ^a	3.83 ^a	8.44 ^a	25.0 ^{b,c}
Conv2	4.15 ^d	9.4	43.0 ^b	3.50 ^{a,b}	8.25 ^a	27.0 ^a
BMR1	4.95 ^a	9.7	45.9 ^a	2.65 ^c	5.83 ^c	22.3 ^d
BMR2	4.75 ^{b,c}	9.6	44.8 ^a	3.21 ^b	7.24 ^b	24.1 ^{b,c}
SEM	0.07	0.14	0.47	0.21	0.48	0.46
MATURITY						
Early	4.79 ^a	9.9 ^a	45.8 ^a	2.94 ^b	6.47 ^b	22.5 ^b
Late	4.46 ^b	9.1 ^b	43.7 ^b	3.66 ^a	8.41 ^a	26.7 ^a
SEM	0.05	0.10	0.34	0.17	0.41	0.33

Table 3. Cont.

Item	Ash	CP	aNDFom	ADL	ADL %aNDFom	Starch
Interactions, <i>p</i> -values						
Field × Population	0.44	0.32	0.60	0.49	0.58	0.27
Field × Hybrid	<0.01	0.02	0.23	0.59	0.70	0.01
Field × Maturity	0.22	0.26	<0.01	<0.01	0.02	<0.01
Population × Hybrid	0.74	0.37	0.68	0.05	0.03	0.09
Population × Maturity	0.95	0.24	0.17	0.26	0.20	0.01
Hybrid × Maturity	0.35	0.54	0.42	0.41	0.40	0.11

^{a-d} Means with different superscripts differ ($p < 0.05$).

Compared to corn planted in the LP field, the concentration of ADL (DM basis) and ADL on an aNDFom basis were higher in the corn silage harvested in the HP field. In addition, we observed a field-by-maturity interaction. The more mature corn silage had a higher concentration of ADL (% DM) and ADL as a percentage of aNDFom than the early-harvest corn when planted in the HP field but not the LP field (Figure 2a,b). Increasing plant population from 59K to 99K had no effect on ADL concentration. Compared to BMR hybrids, Conv corn-silage hybrids had a higher ADL and ADL as a percentage of aNDFom concentration. However, there was an interaction between the planting population and the corn hybrids. The concentration of ADL (%DM and % aNDFom) was relatively consistent between planting populations for both Conv hybrids. However, the ADL concentration for both BMR hybrids, although lower than for both Conv corns hybrids, increased when the planted population increased from 59K to 99K (Figure 2c,d). Surprisingly, the BMR2 hybrid had one of the highest ADL concentrations when planted at 99K. Late-harvest corn silage had a higher concentration of ADL on a DM basis and on an aNDFom basis.

The starch concentration was 6.8% units higher when corn was planted in the HP field compared to the LP field. Planting corn at the intermediate population (79K) resulted in the lowest starch concentration (23.5%), whereas the starch concentration was similar for the lowest (59K) and highest (99K) planting populations (25.2%). The BMR hybrids had a 12% lower starch concentration than the two Conv, but the interaction between field and hybrid reflected a greater variation in starch concentration between Conv and BMR corn silage when harvested from the LP field compared to the corn silage harvested in the HP field (Figure 3a). In the LP field, the BMR1 hybrid had the lowest starch concentration (18.3%), followed by Conv1 and BMR2 (20.8%), whereas Conv2 had the highest concentration (25%). In the HP field, there were no differences in starch concentration between Conv1, Conv2, and BMR2 (28.5%), whereas BMR1 had the lowest starch concentration (26.3%). The starch concentration increased as maturity progressed from the early-dent to the 2/3 milk-line stage, but there was a difference in the magnitude between the two fields (field-by-maturity interaction, Figure 3b). Compared to the early-harvested forage, the more mature corn silage had 6.9 units more starch in the LP field (24.7% vs 17.8% DM). However, the difference between maturities was significantly reduced in the HP field (27.2% vs. 28.8% DM for early and late harvest, respectively). We observed a planting-population-by-maturity interaction (Figure 3c). Planting population had no effect on starch concentration when harvested at an early maturity stage. However, the highest starch concentration was observed when corn was planted at 99K and harvested at the 2/3 milk-line stage (28.6%), followed by corn planted at 59K (26.6%) and 79K (25.0%) planting populations.

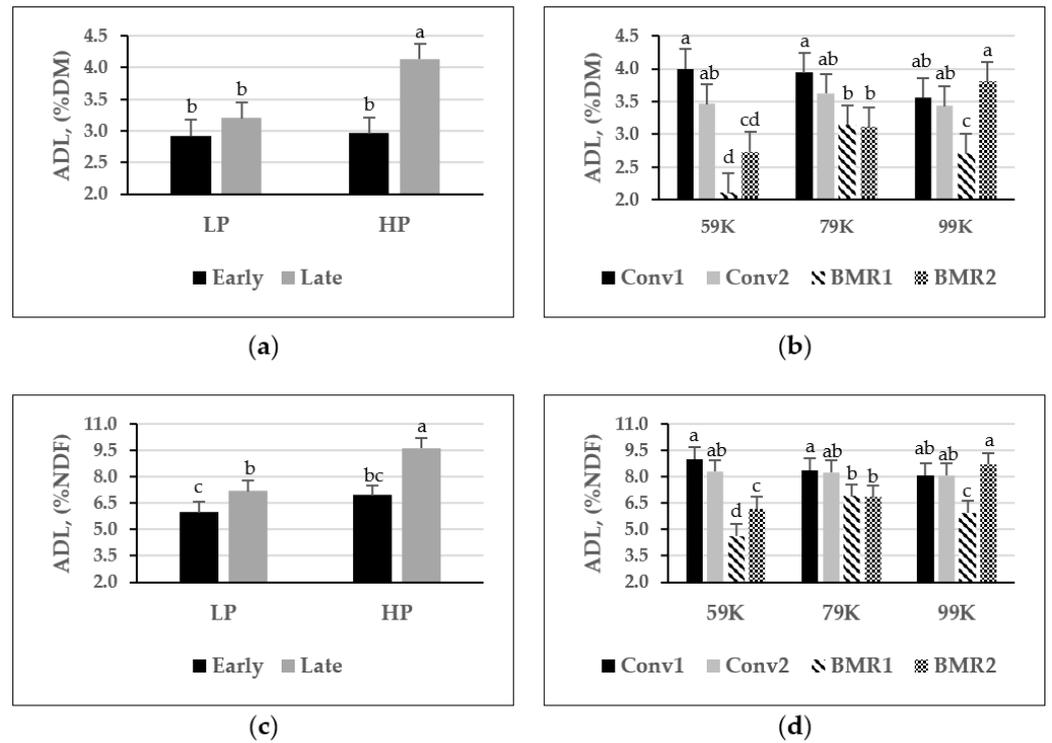


Figure 2. Interactions between field (LP or HP) and maturity at harvest (early or late) (a) and hybrid (Conv1, Conv2, BMR1, and BMR2) and planting population (59K, 79K, and 99K plants/hectare) (b) on ADL concentration (% DM). Interactions between field (LP or HP) and maturity at harvest (early or late) (c), and hybrid (Conv or BMR) and planting population (59K, 79K, and 99K plants/hectare) (d) on ADL concentration as a percentage of aNDFom. ^{a-d} Means with different letters differ ($p \leq 0.05$). Vertical bars indicate standard errors of the mean.

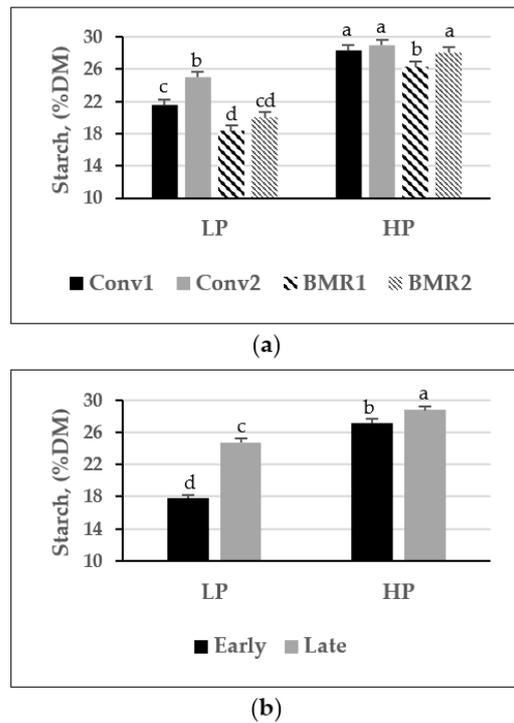
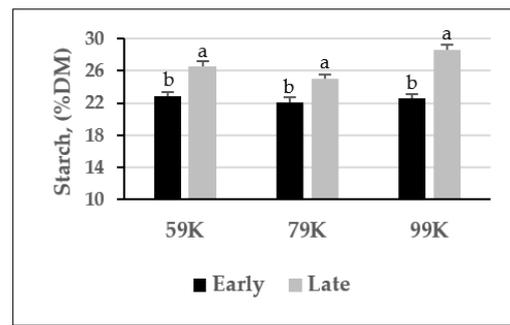


Figure 3. Cont.



(c)

Figure 3. Interactions between field (LP or HP) and hybrid (Conv1, Conv2, BMR1, and BMR2) (a); field and maturity at harvest (early or late) (b) and maturity at harvest and planting population (59K, 79K, and 99K plants/hectare) (c) on starch concentration (% DM) of corn silage. ^{a-d} Means with different letters differ ($p \leq 0.05$). Vertical bars indicate standard errors of the mean.

3.4. In-Vitro Digestibility of Corn Silage

Table 4 presents the least-squares means on the effects of field, corn hybrid, planting population, and maturity at harvest on corn-silage in-vitro digestibility. Compared to corn planted in the LP field, the IVNDFD tended ($p = 0.08$) to be lower in silage harvest in the HP field. However, there was an interaction between field and maturity at harvest for IVNDFD. The early-harvest corn had a higher IVNDFD than the late-harvest corn in the HP field (41.8% vs. 37.3%, respectively) but not in the LP field (45.0% vs. 45.8%, respectively). Increasing planting population had no impact on IVDMD, IVTDMD, or IVNDFD digestibility. The BMR hybrids had greater IVDMD, IVTDMD, and IVNDFD digestibility than the conventional hybrids. Even though statistical differences existed for IVDMD and IVNDFD among different corn maturities at harvest, these differences were small (<2.0% units).

Table 4. In-vitro digestibility of corn silage as affected by field, planting population, hybrid, and maturity.

Item	IVDMD ¹	IVTDMD ²	IVNDFD ³
FIELD			
LP	67.3	74.0	45.4 ^A
HP	65.3	74.5	39.5 ^B
SEM	0.92	0.85	2.07
POPULATION			
60K	66.5	74.3	42.6
75K	65.5	74.0	42.3
90K	66.9	74.9	42.5
SEM	0.75	1.05	1.64
HYBRID			
Conv1	63.8 ^c	70.9 ^c	38.6 ^c
Conv2	65.1 ^{b,c}	74.6 ^b	37.5 ^c
BMR1	70.5 ^a	78.3 ^a	51.8 ^a
BMR2	65.6 ^b	73.5 ^{b,c}	41.8 ^b
SEM	0.80	1.10	1.63
MATURITY			
Early	66.7	74.8	43.4 ^a
Late	65.9	74.0	41.5 ^b
SEM	0.69	0.80	1.52

Table 4. Cont.

Item	IVDMD ¹	IVTDMD ²	IVNDFD ³
Interactions, <i>p</i> -values			
Field × Population	0.44	0.40	0.15
Field × Hybrid	0.45	0.24	0.75
Field × Maturity	<0.01	0.07	<0.01
Population × Hybrid	0.99	0.22	0.78
Population × Maturity	0.35	0.43	0.51
Hybrid × Maturity	0.11	0.33	0.78

¹ IVDMD = in-vitro 30 h dry-matter digestibility (% DM). ² IVTDMD = in-vitro 30 h true dry-matter digestibility (% DM). ³ IVNDFD, in-vitro 30 h neutral detergent-fiber digestibility (% aNDFom). ^{a-c} Means with different superscripts differ ($p < 0.05$). ^{A-B} Means with different superscripts tend to differ ($p < 0.10$).

4. Discussion

4.1. Forage Yield of Corn Silage

As a result of the contrasting growing environments, clear differences in forage yield were observed between the LP and HP fields. Rainfall and, in particular, the presence of a shallow water table during the critical stages of kernel development, supported the greater biomass yield observed for the HP field [16]. In addition, even though the growing periods differed by only a few days between the two fields, the planting delay resulted in greater cumulative GDD for the HP field compared to the LP field. For example, early-harvest corn from the HP field had 231 °C more cumulative GDD than early-harvest corn harvested from the LP field (Table 1). This difference in GDD between fields probably resulted in a more advanced physiological stage at harvest for corn planted in the HP field.

A consistent increase in DM yield with increased planting population has been previously documented [5,6,17]. On the contrary, in a two-year study, Ferreira et al. [9] reported an increase in forage-biomass yield when the corn-planting population increased from 60,000 to 90,000 plants per ha only in the year with abundant precipitation and with higher cumulative GDD during the growing period, resulting in an increased number of kernels per plant and higher starch concentration. In the current study, the significant interaction between planting population and field indicates that biomass yield increased when planting population was increased in the field with less limiting growing conditions. However, when corn was planted in the field with the lowest growth potential (LP), the highest DM yield was obtained when the corn-planting population increased to approximately 79,000 plants/ha, but no differences were observed between the highest and lowest planting populations. Although at greater planting populations individual plant biomass can be penalized [8], the larger number of plants usually increases the DM yield per hectare. Thus, although not measured, we speculate that in this study more plants per hectare were not able to compensate for the potential reduction in plant weight. Consequently, the observations from this study suggest that under limiting fertility and water conditions, increasing corn-planting population will not always result in a higher biomass yield per hectare. Furthermore, contrary to what we originally hypothesized, the likelihood of obtaining greater biomass yields at greater corn-planting populations was not affected by the corn maturity at harvest.

Previous studies have reported a higher DM yield for conventional corn hybrids compared to BMR hybrids [18,19]. However, under the conditions of this study, DM yield was similar between the four corn hybrids, indicating that the BMR trait in the corn-silage hybrids evaluated in this study did not penalize the biomass yield when planted in contrasting growing conditions and at different population densities.

4.2. Chemical Composition of Corn Silage

The small reduction in CP concentration with increasing planting population and maturity is consistent with previous studies [4,9,20]. Furthermore, the magnitude of change in aNDFom concentration with different planting populations and corn hybrids

probably has a minor impact on the overall quality of the silage. Differences in aNDFom concentrations between BMR and non-BMR forages are not consistent in the literature. For example, several studies reported similar aNDFom concentrations for BMR and non-BMR corn silage [1,3]. However, other authors [21,22] reported greater aNDFom concentrations for non-BMR than for BMR corn hybrids, whereas Holt et al. [23] and Ferraretto et al. [24] reported greater aNDFom concentrations for BMR than for non-BMR corn hybrids. Thus, data from this study and several previous studies suggest that differences in aNDFom concentrations between BMR and non-BMR hybrids are small. The decrease in aNDFom content as the maturity of the plant advanced was related to the increase in the proportion of grains in the whole corn plant as it matured [4]. However, this response was only observed in corn silage planted in the LP field. Interestingly, starch concentration increased almost 7% units as maturity progressed in the LP field, but only 1.6% units in the HP field (Figure 3b). Taken together, these results suggest that in the HP field, the increased fiber concentration in the stover as the corn matured was able to offset any increase in the proportion of grain in the whole corn plant.

The increased starch concentration in corn harvested in the HP field further supports that corn in the HP field was at a more advanced physiological stage at harvest compared to corn from the LP field. Although the differences in starch concentration between the four corn hybrids followed a similar pattern in both fields, there was a clear difference in the magnitude of these differences when corn was planted in the LP field (Figure 3a). These data suggest that some of the corn hybrids were more affected than others by the poor growing conditions. Interestingly, the starch concentration was similar between planting populations when the corn was harvested at an early stage (Figure 3c), but when harvested at a late stage there was a 2.8% difference between corn planted at 99K compared with 59K and 79K. However, the higher concentration of starch at 99K was not reflected in a higher biomass yield.

In this study, ADL concentration increased when corn was planted in the HP field (Table 3). In addition, CP and aNDFom concentrations were lower in the HP field compared to those in the LP field. Adding to the higher starch concentration and more cumulative GDD (Table 1) observed in the corn harvested in the HP field, there is substantial evidence to state that the corn planted in the HP field was more mature at harvest than in the LP field. As expected, and consistent with the literature [25], the BMR hybrids had a lower ADL concentration (DM and aNDFom basis) than conventional hybrids. Interestingly, the largest difference between hybrids was observed at the lowest planting populations. As illustrated in Figure 2a, the ADL concentration for the two conventional corn hybrids remained relatively constant when the plant population increased, whereas the ADL concentration in the two BMR hybrids increased. Ferreira et al. [9] also observed a significant but small increase in ADL (% DM) when the planting population increased from 60K to 90K. The increases in ADL concentrations in higher planting populations could be explained by a higher surface-to-mass ratio observed when corn stems become thinner. The stem pith of corn and other warm-season grasses is filled with thin cell walls (parenchymal tissue) with a much lower concentration of aNDFom than the cortex of the same corn-stem internodes [25]. When the planting population increases, the width of the stem decreases [8,9], likely increasing the proportion of more lignified material (cortex) compared to the stem pith in the stem internodes. However, it remains unclear why these changes in plant-tissue proportions were only observed in the BMR hybrids.

4.3. *In-Vitro* Digestibility of Corn Silage

The differences observed in ADL (%DM) and ADL per unit of aNDFom between locations, hybrids, and maturity were clearly reflected in the observed IVNDFD. For example, corn silage from the HP field had a 16% higher ADL (%aNDFom) and a concomitant 13% lower IVNDFD than corn silage from the LP field. Similarly, a lower ADL concentration of an average of 20.7% (DM basis) for the two hybrids with the BMR trait increased fiber digestibility by 23%. These results are consistent with the 22.4 to 23.9% increase in NDFD

between conventional and BMR corn silages reported by Oba and Allen [1,26] and Ferreira et al. [9]. Interestingly, the ranking of corn hybrids according to ADL concentration consistently followed the same pattern of corn hybrids ranked by IVNDFD. In line with Bal et al. [4,27], ADL concentration was higher, whereas fiber digestibility was lower, when the forage was harvested at a more advanced stage of maturity. Furthermore, the difference in ADL concentration between early- and late-harvest corn observed only in the HP field was mirrored by a 11% lower IVNDFD in the late-harvest corn from the HP field. However, the significant interaction between population and maturity observed for ADL concentration was not reflected in IVNDFD. Taken together, these results indicate that differences in ADL and IVNDFD were detectable between locations, hybrids, and maturity at harvest but not between planting populations. Furthermore, no interaction was detected between the planting population and maturity. Thus, regardless of maturity stage at harvest, increasing the corn-planting population does not affect or minimally affects corn-silage-fiber digestibility.

5. Conclusions

Results from this study suggest that increasing corn-planting population may increase forage yield, but this effect may depend on the soil's growing potential and, in particular, the soil's water availability. Thus, high planting populations may lead to increased plant water stress during periods of water deficit. However, the results of this study do not support our hypothesis that increasing planting population will increase biomass yield when corn is harvested at a late but not at an early stage of maturity. In addition, planting population had a negligible effect on the nutritional composition and fiber digestibility of corn silage and was minimally affected by the maturity stage at harvest.

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