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Effect of drought stress on *in vitro* neutral detergent fiber digestibility of corn for silage

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ABSTRACT

The objective of this study was to determine the effect of drought stress on neutral detergent fiber (NDF) and lignin (LIG) concentrations and on in vitro dry matter digestibility (IVDMD) and in vitro neutral detergent fiber digestibility (IVNDFD) of leaf blades and stem internodes of corn for silage. Eight plots were blocked (i.e., 4 blocks) and randomly subjected to a watered (W) or non-watered (NW) treatment. Within each block, plots were split into 7 sub-plots, to which 1 of 7 corn hybrids were randomly assigned. Before planting, all plots were irrigated with 150 mm of water to ensure a consistent emergence of corn seedlings. After this pre-planting irrigation, NW plots were not irrigated ever again. After planting, W plots were irrigated with 225 and 360 mm of water pre-tasseling and post-tasseling, respectively. Stem internodes and leaf blades from the second phytomer below (LOWER) and the second phytomer above (UPPER) the ear insertion were collected to determine tissue composition and digestibility. Drought stress increased the concentration of NDF in both leaf blades (628 vs. 613 mg NDF/g DM) and stem internodes (625 vs. 572 mg NDF/g DM). Drought stress decreased IVDMD in stem internodes (0.575 vs. 0.525 IVDMD) but had no effect on IVDMD of leaf blades (0.561 IVDMD). Similarly, drought stress decreased IVNDFD in stem internodes (0.422 vs. 0.391 IVNDFD) but had no effect on IVNDFD of leaf blades (0.536 IVNDFD). Drought stress increased the concentration of lignin in the cell wall of leaf blades (161 vs. 141 mg/g CW) but had no effect on stem internodes (266 mg/g CW). Under the conditions of this study, water supply had a minimal effect on lignin concentration in the cell wall and did not increase the in vitro digestibility of fiber in corn for silage. The latter observation is contrary to the general industry belief that water stress increases fiber digestibility in forages.

Abbreviations: ARA, arabinose; CW, cell wall; DM, dry matter; GLU, glucose; IVDMD, *in vitro* dry matter digestibility; IVNDFD, *in vitro* neutral detergent fiber digestibility; IVTDMD, *in vitro* true dry matter digestibility; LIG, lignin; LOWER, second phytomer below the ear insertion; LSD, least significant difference; NDF, neutral detergent fiber; NW, non-watered or non-irrigated; UPPER, second phytomer above the ear insertion; W, watered or irrigated; XYL, xylose.

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1. Introduction

Drought stress has a tremendous impact on the agricultural economy and global food security (Brown and Funk, 2008; Farooq et al., 2009). According to the National Center of Environmental Information (National Oceanic and Atmospheric Administration, USA), the droughts from 1988 and 2012 resulted in agricultural losses ranging from $30 to $40 billion per event (Rippey, 2015). The major and most direct effect of drought stress is on the yield reduction of crops for grains and forages and on the yield reduction of pastures and rangelands. At a plant tissue level, however, scarce and confusing information exists regarding the impact of drought stress on cell wall **(CW)** digestibility.

There is a belief among farmers, nutrition consultants, and extension educators that water stress increases the digestibility of the CW (Mahana and Thomas, 2011; Ferreira, 2020). However, there are at least two reasons for which we think there is no scientific evidence to support such belief. First, most of the old studies evaluating the effects of water stress on dry matter **(DM)** digestibility (Van Soest et al., 1978; Wilson, 1983; Dias Filho et al., 1991) did not describe whether the effect is on the concentration of CW in the whole plant, on the digestibility of the CW, or on a combination of both. The second reason is that studies evaluating the effects of drought stress on CW composition at a transcriptional or metabolic level (Lee et al., 2007; Hura et al., 2012; Cabane et al., 2012) suggest that CW digestibility would likely decrease with drought stress, although a few other studies may challenge this possibility (Vincent et al., 2005).

The relationship between lignin and forage digestibility has been extensively studied by ruminant nutritionists (Van Soest, 1994; Grant et al., 1995; Jung et al., 1997; Oba and Allen, 1999; Jung and Lamb, 2004), and it is generally accepted that, in addition to being indigestible, lignin limits the digestibility of other components of the CW, such as cellulose and hemicellulose (Jung and Lamb, 2004; Penning et al., 2014; Carpita and McCann, 2008). An agronomy report (Lauer, 2016) stated that drought stress generally increases CW concentration and increases CW digestibility due to reduced lignin production in corn. Such a statement implies two non-trivial postulates. First, that lignin synthesis in plant tissues is quite sensitive to water stress. Second, that CW digestibility is quite responsive to lignin concentration in the CW. However, such postulates do not clarify whether more lignification in a forage imply having greater proportions of more lignified tissues in the whole plant, or whether more lignification implies having a greater concentration of lignin within the same plant tissue as a consequence of a greater lignification rate. Distinguishing these two possibilities is quite relevant when evaluating the effects of drought stress on CW digestion. While some evidence exists to support that lignin synthesis in plant tissues is quite sensitive to water stress (Jung et al., 1997), very limited and confusing data support that water stress reduces lignin concentration in the CW.

In this study, we hypothesized that drought stress decreases CW digestibility at a tissue level, and that this decrease in CW digestibility is related to greater lignin concentrations in the CW. Therefore, the objectives of this study were to determine the effects of drought stress on CW composition and in vitro digestibility of corn stem internodes and leaf blades.

2. Materials and methods

2.1. Location and corn materials

This experiment was performed at the Kimberly Research and Extension Center located in Kimberly, Idaho, USA (42°32′55″ N; 114°20′30″ W). Soil from the experimental field is described as Bahem silt loam with a land capability classification of IIIe (web soil survey; www.nrcs.usda.gov). Average and year-specific climate information are presented in Table 1.

Seven commercial corn hybrids recommended for silage were donated by seed companies. Other than using a diverse set of genotypes with a wide range of relative maturity (96–115 days of relative maturity), the research team had no interests in specific corn materials. Therefore, seed companies selected the hybrids to use, which included: 1069114 (Dyna-Gro Seed, Geneseo, IL), 1720349

Table 1
30-year average and 2018 climate data for the experimental site.

	30-year Average			2018		
	Minimum Temperature, °C	Maximum Temperature, °C	Rainfall, mm	Minimum Temperature, °C	Maximum Temperature, °C	Rainfall, mm
January	−5.3	3.0	31	−2.1	3.7	113
February	−3.6	6.0	19	−4.6	3.3	11
March	−0.5	11.7	26	−0.6	10.4	31
April	1.9	15.2	29	2.2	16.0	13
May	6.1	20.4	35	7.8	19.2	52
June	9.6	25.4	17	10.0	25.1	15
July	13.1	30.9	5	13.5	30.0	0
August	11.9	29.9	11	10.9	30.5	0
September	7.8	24.7	12	7.0	23.4	0
October	3.0	17.8	19	0.1	16.0	37
November	−2.3	9.0	23	−0.2	8.8	41
December	−5.8	2.9	31	−7.2	1.7	8

Data obtained from Twin Falls (Kimberly) Idaho AgriMet Weather Station 7E (https://www.usbr.gov/pn/agrimet/wyreport.html).

(Dyna-Gro Seed), CF664 (Seed Consultant, Caverndale Farms, Danville, KY), CF753 (Seed Consultant, Caverndale Farms), MCT4632 (Masters Choice, Anna, IL), MCT4934 (Masters Choice), and P1449AMX (Corteva Agriscience, Johnston, IA). To avoid any sort of endorsement or opposition towards any material, hereafter, corn hybrids are blindly addressed, and their identities are not provided. Also, due to an infestation of wire worm that severely affected 2 corn hybrids, data from only 5 of the 7 corn hybrids were utilized.

2.2. Treatments, experimental design, and plot setting

The experimental field was subjected to furrow irrigation using 1.5-m siphon tubes with a 2.54-cm diameter. Fig. 1 depicts a sketch of the experimental setting and design. Eight plots, or experimental units, were blocked (i.e., 4 blocks) and randomly subjected to a watered **(W)** or non-watered **(NW)** treatment. Within each block, plots were split into 7 sub-plots, to which 1 of the 7 corn hybrids were randomly assigned. Hence, this experiment was designed as a split-plot in a randomized complete block design with 4 replicates to evaluate treatments and hybrids.

Each plot, or experimental unit, consisted of 4 rows separated by 76 cm of distance. Before planting, all plots were fertilized (280 kg N ha^{-1}, 68 kg P_2O_5 ha^{-1}, and 45 kg K_2O ha^{-1}) and irrigated with 150 mm of water to ensure a consistent emergence of corn seedlings. After this pre-planting irrigation, NW plots were not irrigated ever again. Each sub-plot had a length of 9 m and was planted with a theoretical population of 99,200 seeds ha^{-1} on May 12, 2018. Inducing drought stress was paramount to the objective of this study. Therefore, to avoid roots from NW plots reaching water from W plots, only 2 of the 4 rows from each W plot were irrigated (Fig. 1). After planting, W plots were irrigated with 75 mm of water when the crop showed 2 visible leaves (V2), 6 visible leaves (V6), and pre-tasseling (VT) and with 45 mm every week, thereafter, for a total of 8 weeks (360 mm post-tasseling).

2.3. Sample collection

All plots and subplots were harvested on September 9, 2018, when corn crops were between 1/4 and 3/4 milk-line stage of maturity. For harvesting, 10 consecutive plants from the 2nd row of each plot within each subplot (Fig. 1) were cut by hand at 15 cm above ground. Whole plants were weighed and chopped with a woodchipper (Ferreira, 2016; Ferreira and Teets, 2017). After mixing thoroughly, a subsample of chopped material was collected and dried at 55 °C in a forced-air drying oven (Freas 645, Thermo Electron Corporation, Marietta, OH) for 48 h to determine DM concentration of the whole plant. In addition, 5 plants from each subplot were cut by hand, and stem internodes and leaf blades from the second phytomer below **(LOWER)** and the second phytomer above **(UPPER)** the ear insertion were dissected and frozen to determine tissue composition and digestibility.

2.4. In vitro neutral detergent fiber digestibility

After drying the stem internodes and leaf blades at 55 °C in a forced-air oven (Memmert UL 83; Wisconsin Oven Corporation; East Troy, WI) for 48 h, tissue samples were ground to pass through a 1-mm screen of a Wiley mill (Thomas Scientific, Swedesboro, NJ). Ash-free neutral detergent fiber (NDF) concentration was determined using the Ankom200 Fiber Analyzer (Ankom Technology,

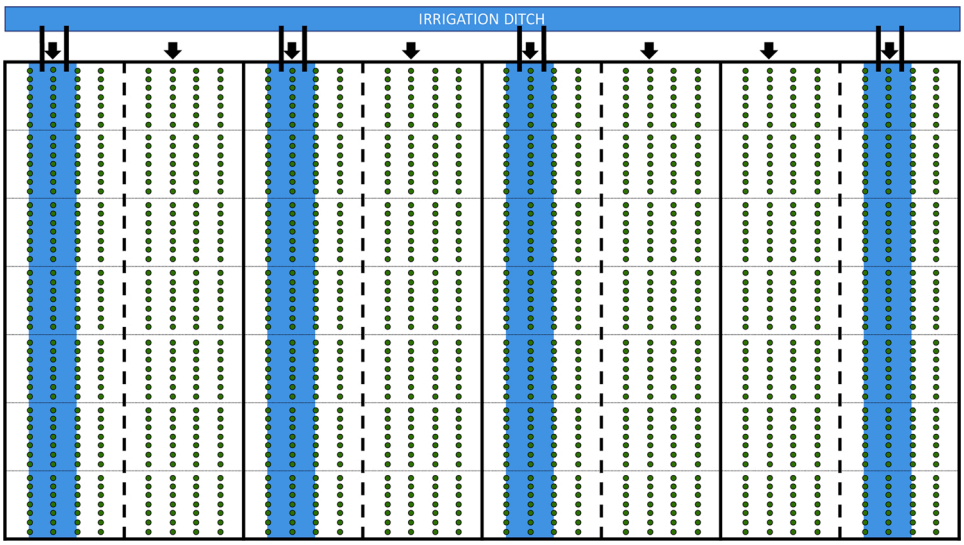

Fig. 1. Sketch of the experimental design. Each of the 4 blocks is delimited by thick and solid lines. Each of the 2 plots, or experimental units, within a block is separated by a thick and broken line. Each plot was split into 7 subplots. Paired subplots within a block were planted with 1 of 7 corn hybrids. Subplots consisted of 4 rows of corn separated by 76 cm between rows and had 9 m of length. Colored furrows represent irrigated furrows. Black arrows represent the corn rows from which samples were obtained. Corn plants are represented by green circles (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

Macedon, NY) using sodium sulfite and α-amylase (Ankom Technology) as described by Ferreira and Mertens (2007).

The Institutional Animal Care and Use Committee of Virginia Tech approved all procedures involving dairy cows for collecting rumen contents (Protocol DASC 15-234). To determine the 30-h in vitro disappearance, 0.25 g of ground sample was placed into F57 filter bags (Ankom Technology, Macedon, NY) that were previously soaked in acetone and air-dried. For each tissue sample, 2 bags were prepared for in vitro incubation. The 30-h ruminal in vitro apparent DM digestibility **(IVDMD)**, 30-h ruminal in vitro true DM digestibility **(IVTDMD)**, and 30-h ruminal in vitro NDF digestibility **(IVNDFD)** was determined using the Daisy^{II} rotating jar in vitro incubator (Ankom Technology) following the procedures described by Ferreira and Mertens (2005). A composited inoculum was prepared with rumen fluid and rumen solids collected from 3 rumen cannulated lactating cows that were fed a diet containing (DM basis) 43 % corn silage, 6% triticale silage, 4% alfalfa hay, and 47 % concentrate mix. The concentrate mix contained corn grain, soybean meal, brewer's grains, soy hulls, and a mineral plus vitamin mix.

2.5. Cell wall extraction

Cell walls were extracted from each plant tissue after modifying a procedure generously provided by Dr. Ronald D. Hatfield (Rancour et al., 2012). After securing enough material to determine NDF concentration and IVNDFD, tissue samples were reground to pass through a 0.5-mm screen of a cyclone mill (UDY Corporation, Inc., Fort Collins, CO), and 1 g of sample was inserted into a 35-mL Oak Ridge high-speed centrifuge tube (ThermoFisher Scientific; Waltham, MA) that was previously dried and weighed. After adding 20 mL of 50 mM Tris-acetate buffer (pH = 6.2), the soaking samples were incubated overnight at 4 °C. The following morning (named Day 1), after centrifuging at 20,000×g for 20 min at 20 °C, the supernatant was aspirated and discarded. Samples were subjected to 2 more cycles of soaking in 10 mL of 50 mM Tris-acetate buffer but with immediate centrifugation (20,000×g for 20 min at 20 °C) and aspiration and discard of the supernatant. For a fourth time, samples were soaked in 10 mL 50 mM Tris-acetate buffer and incubated for 2 h in a water bath at 100 °C. After cooling to approximately 55−60 °C, centrifuging (20,000×g for 20 min at 20 °C), and aspirating the supernatant, 20 U of amylase and 40 U of amyloglucosidase diluted in 10 mL of 50 mM Tris-acetate buffer were added into each tube, and tubes were then incubated for 2 h in a water bath at 55 °C. Samples were then centrifuged at 1000×g for 20 min at 20 °C, and the supernatant was aspirated and discarded. After adding 20 mL of 80 % ethanol to each tube, samples were mixed for 20 min using a reciprocal shaker (Eberbach Model 6000; Belleville, MI) at 200 bouts min^{-1}. After centrifuging at 2000×g for 15 min at 20 °C, the supernatant was aspirated and discarded. The soaking in 80 % ethanol, the shaking, the centrifugation, and the aspiration steps were repeated 2 more times for stem internodes and 4 more times for leaf blades. At the end of Day 1, samples were soaked in 20 mL of acetone and incubated overnight at 4 °C. The following morning (named Day 2), after centrifuging at 2000×g for 15 min at 20 °C, the supernatant was aspirated and discarded. The pellet was soaked for a second time with 20 mL of acetone, shaken, and centrifuged at 2000×g for 15 min at 20 °C. The supernatant was aspirated and discarded. Then, the pellet was rinsed with 20 mL of a chloroform: methanol (2:1) mix and shaken. After centrifuging at 2000×g for 15 min at 20 °C, the supernatant was aspirated and discarded. The pellet, which contained the CW extracts, was soaked for a third time with 20 mL of acetone, shaken, and incubated for 30 min at 20 °C. Then, CW extracts were centrifuged at 2000×g for 15 min at 20 °C, and the supernatant was aspirated and discarded. The CW extracts were soaked for a fourth time with 20 mL of acetone, shaken, and incubated for 30 min at 20 °C. Then CW extracts were centrifuged at 2000×g for 15 min at 20 °C, and the supernatant was aspirated and discarded. Finally, the resulting CW extracts were allowed to air dry overnight under the hood. Periodic breaking of CW extract clumps with a spatula was necessary to help the evaporation of acetone. After acetone was apparently evaporated, the CW extracts were dried in an oven at 55 °C. The concentration of CW in plant tissues was determined gravimetrically after reaching constant weight of the CW extracts. Crude protein concentration in CW was calculated as percent N × 6.25 after combustion analysis (method 990.03; AOAC, 2019) using a Vario El Cube CN analyzer (Elementar Americas, Inc., Mount Laurel, NJ). The resulting concentration of crude protein was subtracted from the CW to yield data on a protein-free CW-basis.

2.6. Acetyl-bromide lignin

Lignin **(LIG)** concentrations in CW were determined following a procedure generously provided by Dr. Ronald D. Hatfield (Fukushima and Hatfield, 2004; Rancour et al., 2012). Cell wall extracts were dried overnight at 55 °C. Then, 20 mg of CW extract was weighed into capped (Teflon liner) glass tubes, and 2.5 mL of 25 % (v/v) acetyl bromide in glacial acetic acid was added into each tube. Tubes were capped tightly and gently swirled, avoiding getting too much of the sample stuck to the glass above the acetyl bromide solution. Capped tubes were then incubated in a dry bath at 50 °C for 2 h, with gentle swirling every 30 min. Then, tubes were cooled in an iced water bath, and approximately 1.5 mL of the reaction mixture was transferred into microcentrifuge tubes. After centrifuging at 12,000×g for 3 min at 20 °C, 0.5 mL of the supernatant was transferred into a scintillation vial containing 9.5 mL of a freshly degassed (N_2 stream) matrix solution composed of 2.0 mL of 2 M NaOH, 7.15 mL of glacial acetic acid, and 0.35 mL of 0.5 M hydroxylamine. The resulting solution was transferred into a 1-cm quartz cuvette (Millipore-Sigma, St. Louis, MO), and UV absorbance at a 280-nm wavelength was determined by spectrophotometry. The concentration of lignin in the resulting solution was determined dividing the absorbance by an extinction coefficient for corn tissues equal to 17.747 L·g^{-1} cm^{-1} (Rancour et al., 2012) in a pathway length equal to 1 cm, as described in Eq. (1).

$$Lignin_{(g/L)} = \frac{Abs_{280}}{17.747\ \frac{L}{g.cm} \times 1\ cm} \quad (1)$$

The concentration of lignin within the CW was determined using the dilution factor and the initial CW sample weight as described in Eq. (2).

$$Lignin_{(mg/g\ CW)} = \frac{Lignin_{(g/L)} \times 0.050\ L}{0.020\ g} \times 1,000 \quad (2)$$

Lignin concentrations were reported on a protein-free CW basis.

2.7. Cell wall monosaccharides

The concentrations of arabinose **(ARA)**, xylose **(XYL)**, and glucose **(GLU)** in CW were determined following procedures of Blakeney et al. (1983) and Hoebler (1989) with modifications. Cell wall extracts were dried overnight at 55 °C. After weighing 20 mg of CW extract into capped glass tubes and adding 250 µL of 12 M H_2SO_4, CW extracts were incubated for 2 h at room temperature. Then, 1.7 mL of distilled water and 1 mL of 11.1 M inositol solution (internal standard) were added to each tube, and the samples were incubated in an oven for 3 h at 100 °C followed by an overnight incubation at 4 °C. On the following morning, 0.6 mL of 25 % NH_4OH (w/w) was added to each tube, and these were mixed gently. After transferring 200 µL of particulate-free supernatant into 35-mL Oak Ridge centrifuge tubes, 2 mL of a 2% (w/v) $NaBH_4$ in DMSO solution was added to each tube. After incubating in a water bath at 40 °C for 90 min, 200 µL of glacial acetic acid was added, and tubes were vortexed. After cooling, 400 µL of 1-methylimidazole followed by 4 1-mL aliquots of acetic anhydride were gently added. After a 10-min incubation at room temperature, 20 mL of distilled water followed by 8 mL of dichloromethane were added to each tube. Tubes were capped, vortexed, and centrifuged at 1000×g for 5 min at 20 °C. After removing the aqueous upper layer, and after repeating 3 more washes with water, 1 mL of each sample was placed into GC vials and dried under N_2 stream. The dried extracts were then dissolved in 500 µL of dichloromethane, capped, and vortexed. The sugars were identified and quantified by GC on an Agilent Technologies 6890 N GC system (Agilent Technologies, Santa Clara, CA) using a DB-225 column (30 m x0.25 mm with 0.15 µm film thickness, Agilent J & W, Santa Clara, CA). The injector temperature was 225 °C, and the detector temperature was 275 °C. A temperature program was used with initial oven temperature of 150 °C, ramped 10 °C /min to 175 °C before holding for 10 min then to 220 °C at 10 °C /min and holding for 5 min at constant linear velocity of 56 cm/s and split ratio of 10:1. Concentrations ARA, XYL, and GLU were reported on a protein-free CW basis.

2.8. Statistical analysis

All variables were analyzed using the MIXED procedure of SAS (SAS version 9.4, SAS Institute Inc., Cary, NC). The statistical model included the effects of block [random; degrees of freedom (df) = 5], irrigation (fixed; df = 1), block by irrigation (random; df = 3), hybrid (fixed; df = 4), irrigation by hybrid (fixed; df = 4), block by irrigation by hybrid (random; df = 24), phytomer (fixed; df = 1), phytomer by irrigation (fixed; df = 1), phytomer by hybrid (fixed; df = 4), phytomer by irrigation by hybrid (fixed, df = 4), and the random residual error (df = 30). To contrast irrigation treatments, the 10 % least significant difference (LSD) between means was calculated as the product of the standard error of the difference by the t value for a probability of 0.10 with 3 df (Snedecor and Cochran, 1989). To contrast hybrids and phytomers, the 5% LSD between means was calculated as the product of the standard error of the difference by the t value for a probability of 0.05 with 24 and 30 df, respectively (Snedecor and Cochran, 1989).

Table 2
Fiber concentration (mg/g), in vitro digestibility, and cell wall composition (mg/g) of leaf blades from bottom or upper phytomers and watered or non-watered corn hybrids.[†‡].

	NDF	IVDMD	IVTDMD	IVNDFD	LIG	ARA	XYL	GLU
Irrigation								
Watered	613 b	0.555	0.714	0.531	141 b	38 b	235 b	323 b
Non-watered	628 a	0.567	0.715	0.541	161 a	41 a	259 a	346 a
LSD[§] (P < 0.10)	6	0.021	0.010	0.012	8	1	6	5
Hybrid								
A	627 bc	0.585 a	0.729 a	0.568 a	148 a	39 ab	263 a	354 a
B	593 d	0.557 b	0.717 ab	0.524 b	149 a	39 ab	244 b	337 b
C	611 c	0.558 b	0.703 b	0.498 c	145 b	39 ab	241 b	316 c
D	628 b	0.544 b	0.705 b	0.530 b	152 a	38 b	242 b	322 c
E	642 a	0.563 ab	0.716 ab	0.559 a	160 a	40 a	243 b	342 ab
LSD (P < 0.05)	13	0.024	0.017	0.019	12	2	10	15
Phytomer								
Bottom	648 a	0.560	0.701 b	0.535	159 a	40 a	253 a	349 a
Upper	593 b	0.562	0.728 a	0.537	142 b	38 b	241 b	320 b
LSD (P < 0.05)	6	0.013	0.008	0.010	6	1	6	8

[†] NDF = neutral detergent fiber (% DM); IVDMD = in vitro dry matter digestibility (% DM); IVTDMD = in vitro true dry matter digestibility (% DM); IVNDFD = in vitro NDF digestibility (% NDF); LIG = lignin (% CW); ARA = arabinose (% CW); XYL = xylose (% CW); GLU = glucose (% CW).
[‡] Letters with different superscripts within a column differ.
[§] Least significant difference.

3. Results

3.1. Leaf blades

No 2-way or 3-way interactions existed ($P > 0.10$) for any of the variables evaluated. Therefore, we reported only main effects for leaf blades (Table 2). Leaf blades from NW corn had similar IVDMD (0.561 IVDMD; $P = 0.42$), IVTDMD (0.715; $P = 0.81$), or IVNDFD (0.536 IVNDFD; $P = 0.25$) than leaf blades from W corn. Water restriction increased the concentrations of NDF in leaf blades (628 vs. 613 mg/g; DM basis). The greater concentration of NDF corresponded with a greater degree of senescence for the drought stressed leaf blades (Fig. 2). Water restriction slightly increased the concentrations of lignin (161 vs. 141 mg/g; $P < 0.03$), arabinose (41 vs. 38 mg/g; $P < 0.03$), xylose (259 vs. 235 mg/g; $P < 0.01$), and glucose (346 vs. 323 mg/g; $P < 0.03$) in the CW of leaf blades.

Lower leaf blades had a greater concentration of NDF than upper leaf blades (647 vs. 593 mg/g; $P < 0.01$; Table 2). Although no differences existed for IVDMD between lower and upper blades (0.561 IVDMD; $P = 0.79$), upper leaf blades had greater IVTDMD than lower leaf blades (0.728 vs. 0.701 IVTDMD; $P < 0.01$). In vitro NDF digestibility did not differ between lower and upper leaf blades (0.536 IVNDFD; $P = 0.64$). Cell walls from lower leaf blades contained slightly greater concentrations of lignin (159 vs. 142 mg/g; $P < 0.01$), arabinose (40 vs. 38 mg/g; $P < 0.05$), xylose (253 vs. 241 mg/g; $P < 0.01$), and glucose (349 vs. 320 mg/g; $P < 0.01$) than CW from upper leaf blades.

3.2. Stem internodes

Stem internodes from NW corn had greater concentrations of NDF than stem internodes from W corn (625 vs. 572 mg/g; $P < 0.02$; Table 3), and this was consistent among most of the hybrids (Fig. 3). Water restriction decreased the IVDMD of stem internodes (0.575 vs. 0.525 IVDMD; $P < 0.02$; Table 3), although the significant interaction between hybrid and irrigation ($P < 0.08$) indicates that water restriction affected each hybrid differently (Fig. 4). Water restriction also decreased IVTDMD (0.667 vs. 0.617 IVTDMD; $P < 0.01$) and IVNDFD (0.422 vs. 0.391 IVNDFD; $P < 0.07$) of stem internodes (Table 3). Water restriction did not affect the concentrations of lignin (266 mg/g; $P = 0.38$), arabinose (23 mg/g; $P = 0.99$), xylose (252 mg/g; $P = 0.62$), and glucose (376 mg/g; $P = 0.94$) in the CW of stem internodes (Table 3).

Upper stem internodes had a greater concentration of NDF than lower stem internodes (615 vs. 582 mg/g; $P < 0.01$; Table 3). However, an interaction existed between phytomer and irrigation for NDF concentration ($P < 0.01$). According to this interaction, the difference in NDF concentration between the upper and the lower stems (Fig. 5) was greater for W corn (49-mg/g difference) than for NW corn (17-mg/g difference). Water restriction decreased IVDMD and IVTDMD in both stem internodes, but these coefficients decreased more in lower stem internodes than in upper internodes (Fig. 6). Water restriction decreased IVNDFD only in the lower stem internodes (Fig. 6). Cell walls of lower and upper stem internodes had similar lignin concentrations (260 mg/g; $P = 0.38$), and CW from lower stem internodes contained lower concentrations of arabinose (22 vs. 24 mg/g; $P < 0.01$) and xylose (244 vs. 260 mg/g; $P < 0.01$) but greater concentrations of glucose (390 vs. 363 mg/g; $P < 0.01$) than upper stem internodes.

Fig. 2. Drought stress in non-watered (NW) plots was evidenced by senescent leaf blades (right) that were not observed in watered plots (left).

Table 3
Fiber concentration (mg/g), in vitro digestibility, and cell wall composition (mg/g) of stem internodes from bottom or upper phytomers and watered or non-watered corn hybrids.[†‡].

	NDF	IVDMD	IVTDMD	IVNDFD	LIG	ARA	XYL	GLU
Irrigation								
Watered	572 b	0.575 a	0.667 a	0.422 a	260	23	253	376
Non-watered	625 a	0.525 b	0.617 b	0.391 b	271	23	251	377
LSD[§] (P < 0.10)	15	0.015	0.012	0.018	17	1	6	10
Hybrid								
A	506 c	0.666 a	0.750 a	0.506 a	238 c	25 a	263 a	384
B	625 ab	0.529 b	0.626 b	0.404 b	282 ab	22 b	249 b	369
C	605 b	0.532 b	0.622 b	0.376 c	263 bc	23 b	248 b	371
D	613 b	0.532 b	0.622 b	0.383 bc	257 bc	22 b	251 b	385
E	642 a	0.491 c	0.590 c	0.362 c	289 a	22 b	249 b	372
LSD (P < 0.05)	25	0.024	0.020	0.030	26	2	7	17
Phytomer								
Bottom	582 b	0.563 a	0.648 a	0.400	269	22 b	244 b	390 a
Upper	615 a	0.537 b	0.637 b	0.413	263	24 a	260 a	363 b
LSD (P < 0.05)	7	0.010	0.010	0.014	14	1	4	6

[†] NDF = neutral detergent fiber (% DM); IVDMD = in vitro dry matter digestibility (% DM); IVTDMD = in vitro true dry matter digestibility (% DM); IVNDFD = in vitro NDF digestibility (% NDF); LIG = lignin (% CW); ARA = arabinose (% CW); XYL = xylose (% CW); GLU = glucose (% CW).
[‡] Letters with different superscripts within a column differ.
[§] Least significant difference.

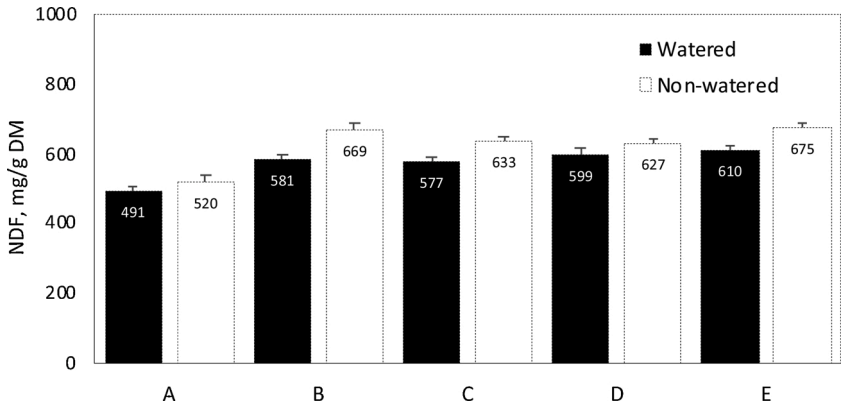

Fig. 3. Drought stress increased the NDF concentration in stem internodes from 3 of 5 hybrids (i.e., hybrids B, C, and E) of corn for silage.

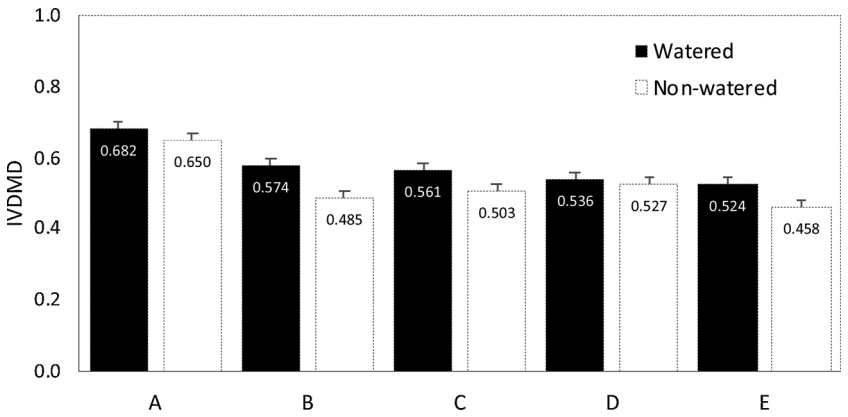

Fig. 4. Drought stress decreased the in vitro dry matter digestibility (IVDMD) of stem internodes from 4 of 5 hybrids (i.e., hybrids A, B, C, and E) of corn for silage.

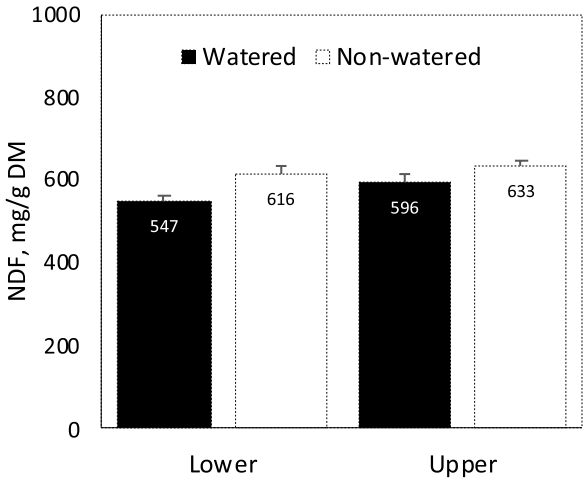

Fig. 5. Effect of drought stress on the concentration of neutral detergent fiber (NDF) of upper and lower stem internodes of corn for silage.

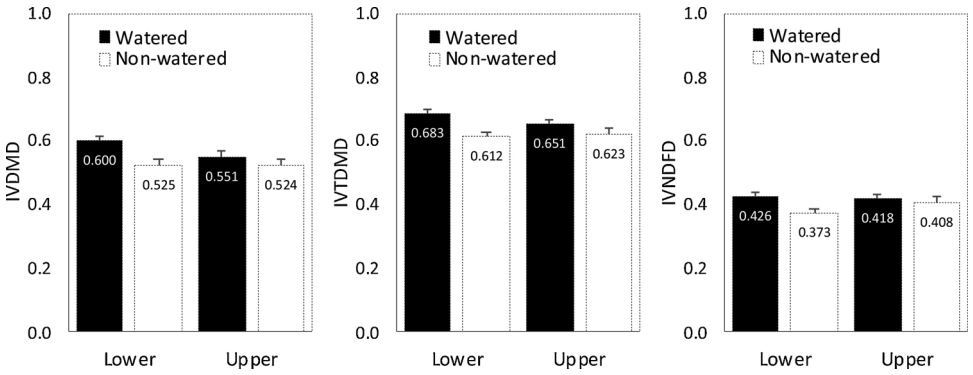

Fig. 6. Effect of drought stress on in vitro dry matter digestibility (IVDMD), in vitro true dry matter digestibility (IVTDMD), and in vitro neutral detergent fiber digestibility (IVNDFD) of upper and lower stem internodes of corn for silage.

4. Discussion

Drought stress, as other abiotic stresses related to climate change, is a threat to global food security, mainly through reductions of grain and cereal crop yields (Brown and Funk, 2008; Farooq et al., 2009). From a nutritional perspective, the effects of drought stress on fiber utilization by ruminants are less clear (Ferreira and Brown, 2016), and studies evaluating the effects of drought stress on corn CW composition or fiber utilization are limited (Vincent et al., 2005). The main reasons for this void in knowledge are the challenges to accomplish and reproduce drought stress treatments in controlled studies (Farooq et al., 2009).

In this study, to control the environmental conditions (Farooq et al., 2009) and to evaluate the effects of drought stress on CW composition and fiber digestibility, we induced drought stress by limiting irrigation in corn plots within a single season in a semi-arid region (Table 1). The greater DM concentration in the whole-plant (Fig. 7) and the evident senescence of leaf blades (Fig. 2) suggest we succeeded affecting the water status of the NW corn plots. The greater senescence of leaf blades can explain the greater concentration of NDF in leaf blades of NW corn relative to W corn. Interestingly, stem internodes from NW corn also had greater NDF concentrations than stem internodes from W corn. Even more, the magnitude of this difference was greater for stem internodes than for leaf blades. The greater concentration of NDF in stem internodes might be related to cells of less volume and greater surface area caused by the reduced turgor pressure within cells (Farooq et al., 2009; Haswell and Verslues, 2015).

Increased NDF concentrations in vegetative tissues under drought stress makes biological sense. This concept deserves special attention when discussing the effect of water stress on nutrient digestibility. Partially based on a study of Dias Filho et al. (1991), Van Soest (1994) stated that restrictions of water increase digestibility and that irrigation tends to decrease it. Interestingly, the mentioned study (Dias Filho et al., 1991) reported greater protein concentrations with water stress, which makes little biological sense when considering tissue senescence. However, it is worth mentioning that the referenced study separated and discarded dead tissues before sample analysis. As tissue senescence is likely to increase under drought conditions, the study of Dias Filho et al. (1991) has little merit to arrive to the conclusion that restrictions of water increase digestibility (Van Soest, 1994).

Contrary to the accepted belief that drought stress increases NDF digestibility (Mahana and Thomas, 2011; Van Soest, 1994), this

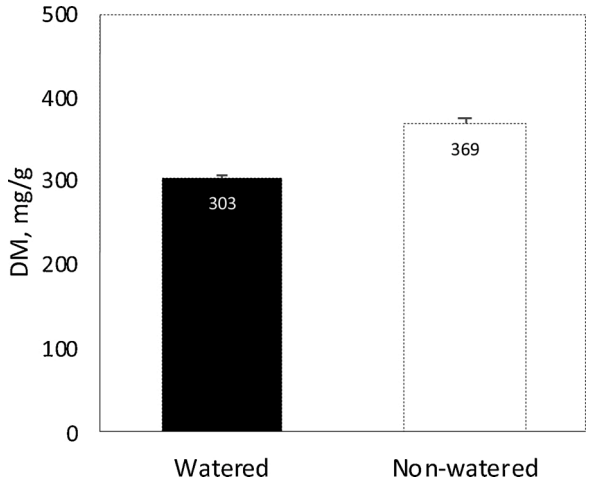

Fig. 7. Effect of drought stress on DM concentration of corn whole-plants.

study shows that drought stress decreased NDFD in stem internodes (Table 3 and Fig. 6), although not in leaf blades. Van Soest et al. (1978) stated that water stress induces a more digestible crop of lower yield. However, the authors did not specify whether they referred to a more digestible DM, a more digestible CW, or both. As DM digestibility of forages is determined by both the concentration of CW in the forage and the digestibility of the CW (Smith et al., 1971; Van Soest, 1994; Ferreira and Mertens, 2005), an incomplete description of the results is not trivial. Based on their report (Van Soest et al., 1978), water stress would have decreased CW concentrations in the forage, so likely an increase in (presumably) DM digestibility due to water stress would be related to greater concentrations of highly digestible cell contents (Evans and Wilson, 1984; Buxton and Casler, 1993; Liu et al., 2018) and not to a greater digestibility of the plant CW, the latter being contrary to our observations.

Van Soest et al. (1978) implied that water stress decreases lignin concentrations. Similarly, an agronomy report (Lauer, 2016) stated that drought stress generally increases CW digestibility due to reduced lignin production in corn. These statements suggest that lignin synthesis in plant tissues is quite sensitive to drought stress. Contrary to these statements, in our study drought stress slightly increased the concentration of lignin in the CW of leaf blades (Table 2) but had no effect on stem internodes (Table 3). More in line with our observations, studies at a molecular level have shown that drought stress increased lignin concentration (Lee et al., 2007; Le Gall et al., 2015). In rice (*Oryza sativa*), water stress increased the expression and activity of the enzyme phenylalanine lyase (PAL) with a consequent increase in the concentration of lignin (Le Gall et al., 2015). The enzyme PAL catalyzes the formation of cinnamic acid from phenylalanine, which is the first step in the synthesis of monolignols for lignin polymerization (Penning et al., 2014). In white clover (*Trifolium repens*), drought stress also increased PAL activity and lignin concentrations (Lee et al., 2007). A study with triticale (*Triticosecale*) showed that water stress increased the concentration of CW-bound phenolic compounds (Hura et al., 2012), which could decrease CW digestibility (Grabber et al., 2000; Rancour et al., 2012; Hatfield et al., 2017). Therefore, simply stating that plants have more or less lignification is ambiguous, as greater concentrations of lignin can mean that there are greater proportions of more lignified tissues in the whole plant (e.g., greater stem to leaf ratio in alfalfa) or that there are greater concentrations of lignin within the same tissues. Hence, when evaluating the effects of drought stress on forage quality, it is paramount to distinguish whether the effect of drought stress on lignification is at a plant level or at a tissue level. The latter is the reason for analyzing CW composition and NDFD at the tissue level.

In this study, we also evaluated the composition and digestibility of corn tissues across hybrids and within plants (i.e., bottom and upper phytomers). We observed that IVNDFD of leaf blades (Table 2) and stem internodes (Table 3) varies much more across hybrids (0.498 to 0.568 IVNDFD and 0.362 to 0.506 IVNDFD, respectively) than due to the effect of drought stress. Even more, given the inexistence of a significant interaction, the effect of drought stress did not depend on hybrid (e.g., presence of the brown midrib mutation). These observations imply that drought stress might have a much less impact on CW digestibility than anticipated. From the comparison between phytomers, we observed that IVNDFD did not differ within the plant for either leaf blades (Table 2) or stem internodes (Table 3). However, IVTDMD differed in different ways, depending on the tissue. For leaf blades (Table 2), the greater IVTDMD of upper phytomers corresponded with a lower NDF concentration, likely explained through differences in senescence. This observation highlights the interrelationship between NDF concentration and DM digestibility (Smith et al., 1971). In stem internodes, this relationship also existed (Table 3), although in this case IVTDMD was greater in bottom internodes than in upper internodes. We speculate this is due to the thicker stems with a greater proportion of parenchymal cells, although data is needed to support such speculation.

In regard to sugar concentrations, given the structure and the spatial organization of cellulose microfibrils, we speculated that lower IVNDFD would be associated with increased concentrations of GLU and reduced concentrations of ARA and XYL. However, at the irrigation level, IVNDFD did not change when the concentrations of GLU, ARA, and XYL changed in leaf blades, and IVNDFD changed when the concentrations of GLU, ARA, and XYL did not changed in stem internodes. Whether sugar concentrations in the CW affect or not IVNDFD still needs to be evaluated.

5. Conclusions

Performing controlled studies to evaluate the effects of drought stress on forage composition and digestibility is very difficult to accomplish. In this study, we subjected different corn hybrids to drought stress by limiting irrigation. From visual appraisal and tissue composition and digestibility, we concluded that non-watered corn was affected by drought stress. Overall, depending on the tissue analyzed, drought stress decreased or had no effect on fiber digestibility. Also depending on the tissues analyzed, drought stress increased or had no effect on lignification of the CW. These observations are contrary to what is generally accepted in the industry and, therefore, more research evaluating the effects of drought stress on CW composition and digestibility is warranted.

Declaration of Conflicts Interest

All authors declare no conflicts of interest.

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