

## Article

# Effect of Harvesting Corn after Frost in Alberta (Canada) on Whole-Plant Yield, Nutritive Value, and Kernel Properties

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**Abstract:** This study compares yield, nutritive value, and kernel properties of whole plant corn (WPC) harvested before and after a light frost in short growing season areas. Six corn hybrids grown in two years at three locations within Alberta (Canada) were harvested before or after the first frost. Samples of WPC were analyzed for dry matter (DM) content, neutral detergent fiber (NDF) concentration, starch concentration, and 48-h in vitro DM and NDF digestibility (DMD and NDFD, respectively). Cob samples were analyzed for DM, and kernels were analyzed for DM, hardness, particle size distribution, density, and stage of maturity. Delaying harvest to after frost increased DM content of WPC at all locations but exceeded the recommended range (32–38%) in the two warmest locations. Whatever the year and hybrid, DM yield was either not affected or decreased after frost. Postfrost harvest increased starch concentration and modified kernel characteristics only if these were less than expected before frost. Fiber concentration was not affected by harvesting time. Frost had either no impact or increased DMD or NDFD of WPC. We conclude that delaying harvest until after frost in short growing season areas can be beneficial when whole-plant DM content is low before frost.

**Keywords:** corn silage; maize; short growing season; frost; nutrient composition; yield; kernel; hybrid



**Citation:** Guyader, J.; Baron, V.S.; Beauchemin, K.A. Effect of Harvesting Corn after Frost in Alberta (Canada) on Whole-Plant Yield, Nutritive Value, and Kernel Properties. *Agronomy* **2021**, *11*, 459. <https://doi.org/10.3390/agronomy11030459>

Academic Editor: Jerome H. Cherney

Received: 18 February 2021

Accepted: 26 February 2021

Published: 1 March 2021

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## 1. Introduction

Corn silage (CS) is increasingly used in ruminant diets as a source of digestible starch and fiber [1]. Given that its average inclusion rate in dairy rations can be as much as 40% of dry matter (DM) in North America and Europe [2], maximizing CS quality by optimal cropping management is essential to promote animal performance. In locations with cooler climates and short growing seasons, harvesting time needs to be carefully determined to maximize both the quantity and quality of biomass. Harvesting immature corn results in low DM yield and starch concentration, although the digestibility of fiber is enhanced. Delaying harvest extends the grain filling period and increases DM yield, but fiber digestibility and the potential for long-term preservation [3] may be negatively affected.

In areas with a cool and short growing season, small grains such as barley, wheat, and oats are usually favored for silage production as their development is less heat-dependent than corn [4]. However, small grains usually have lower yields and digestibility compared with corn. The recent development of short-season corn hybrids makes it feasible to grow corn hybrids for silage in northern locations characterized by a short growing season. Moreover, the annual growing season for corn in the Canadian prairie provinces (Alberta, Saskatchewan, and Manitoba) has increased by 3 to 12 days over the past century as a consequence of climate change [5]. Consequently, corn production has continually increased in the Prairies even though this area is still challenged by low temperatures in spring that delay planting and the occurrence of frost early in the autumn. About 50

days are necessary from silking to harvest to reach corn maturity [6]; however, this period can be limited to 30–40 days [7] in cool environments. Early frost damages the plants through tissue breakdown, which increases plant DM content, causes leaching of nutrients (nitrogen and minerals; [8]), and negatively affects fiber digestibility [9]. Severe frost may also induce loss of plant fractions such as leaves and inhibit translocation of sugars to ears and the conversion of sugars to starch in the kernels, which are active processes, thereby preventing kernel filling [10].

In a previous study, we demonstrated that when using new hybrids, CS production is possible in Northern areas even though attainment of whole-plant maturity before frost may be difficult to achieve in some years and locations due to annual variability of corn heat unit (CHU; a temperature-based index used to measure the cumulative heat over the growing season) accumulation [7]. The impact of harvesting the corn crop for silage after the occurrence of frost in areas with a short growing season is unclear. We hypothesize that delaying harvest until after frost will not be necessary with new early-maturing hybrids as sufficient maturity and nutrient concentrations may be attained within the limited growing season. Therefore, the aim of the present work is to compare corn whole-plant characteristics, including yield, nutritive value, and kernel properties of short-season hybrids harvested before and after frost.

## 2. Materials and Methods

### 2.1. Experimental Design

Corn was grown in two years (2013 and 2015) in three different locations within Alberta: Lacombe (probability of receiving 2000 CHU between 50% and 60%), Lethbridge (probability of receiving 2000 CHU between 70% and 80%), and Vauxhall (probability of receiving 2000 CHU between 80% and 90%) [5]. Soil description, long-term climatic information, planting practices, water supply, and fertilization strategies for these sites have already been described [7]. Briefly, the texture of the soil is characterized as fine sandy loam in Lacombe and silt loam in Lethbridge and Vauxhall.

At each location, six short/medium season CS hybrids were planted in four replicates in a randomized complete block design each year (Table 1). Because of lack of seed availability, one hybrid in Lethbridge (P8622AM) could not be used in both years, and, therefore, a replacement hybrid (P8210HR) with a similar CHU rating was used in 2015. This modification was considered to have a low impact on the experimental design, and no distinction was made in further analytical treatments.

**Table 1.** Selected corn hybrids with grain maturity ratings for Lacombe, Lethbridge, and Vauxhall.

Lacombe		Vauxhall		Lethbridge	
Hybrid <sup>1</sup>	CHU Rating	Hybrid <sup>1</sup>	CHU Rating	Hybrid <sup>1</sup>	CHU Rating
P39F44	2000	P7632HR	2200	P39F44	2000
P7213R	2050	39V05	2250	P7443R	2100
P39M26	2100	P8193AM	2400	P7632HR	2200
Edge	2150	P8210HR	2475	39V05	2250
2262RR	2175	P8673AM	2550	P8673AM	2550
P7632HR	2200	P8622AM	2600	P8622AM <sup>2</sup>	2600

<sup>1</sup> Hybrids were supplied by Pioneer Hi-Bred, Johnston (IA) except for hybrids Edge (Elite, Saint-Hyacinthe, QC) and 2262RR (Pickseed, Lindsay, ON). <sup>2</sup> Hybrid P8622AM was replaced by P8210HR in 2015.

Corn was seeded between 6 May and 3 June and harvested before (between 18 and 24 September) or after (between 29 September and 7 October) light frost (Table 2). The CHU accumulated between seeding and harvesting was calculated for each year and location as the sum of daily CHU, as provided by the Government of Canada [11]. Initiation for summation of CHU at each location and year used the method outlined by Huggins-Rawlings [12]. The final day of CHU accumulation at each location and year corresponded to the harvesting date.

**Table 2.** Seeding date, first and last mean silking date, and harvesting dates for corn grown for silage at Lacombe, Lethbridge, and Vauxhall.

Year	Seeding Date	First and Last Silking Date	Early Harvest (CHU)	Autumn Frost	Late Harvest (CHU)
Lacombe					
2013	10 May	3 August–19 August	18 September (2127)	25 September	1 October (2196)
2015	5 May	23 July–3 August	15 September (1837)	17 September	23 September (1884)
Lethbridge					
2013	8 May	23 July–10 August	22 September (2631)	4 October	7 October (2692)
2015	6 May	15 July–31 July	18 September (2263)	5 October <sup>1</sup>	29 September (2418)
Vauxhall					
2013	17 May	3 August–12 August	24 September (2458)	2 October	1 October (2500)
2015	3 June	18 July–30 July	18 September (2182)	28 September	29 September (2319)

<sup>1</sup> Temperature went down to 1.3 °C on 28 September 2015.

## 2.2. Sampling and Chemical Analyses

Upon harvesting, the kernel reproductive stage was assessed according to standard scaling [13], and sampling was carried out as described earlier [7]. Briefly, five plants per replicate were separated into ear and stover and weighed. The remaining plants were harvested, weighed to determine total wet weight biomass (including the weight of the five plants), and a representative sample was retained. Frozen samples (whole plants and cobs) were shipped to the Lethbridge Research and Development Centre (LeRDC, Agriculture and Agri-Food, Alberta, Canada) after determining kernel stage maturity [14].

Whole plant samples were dried to a constant weight at 55 °C to determine DM content before being ground in a Wiley mill (A.H. Thomas, Philadelphia, PA, USA) through a 4-mm screen. One subsample was further ground through a 1-mm screen, and another subsample was ball-ground (mixer mill MM 400; Retsch Inc., Newtown, PA, USA). All samples from Year 1 were analyzed in duplicate for DM content and concentrations of organic matter (OM), neutral detergent fiber (NDF), acid detergent fiber (ADF), acid detergent lignin (ADL), crude protein (CP), and starch, as well as 48-h in vitro DM digestibility (DMD) and NDF digestibility (NDFD) [7]. All samples that had been chemically analyzed were scanned using near-infrared reflectance spectroscopy (NIRSsystems 6500 Monochromator, Foss NIRSystems Inc., Silver Spring, MD, USA). Prediction equations were developed, and the  $R^2$  and  $R^2$  of cross-validation ( $R^2$ CV) were calculated. These were greater than 0.83 for DM, OM, ADF, ADL, starch, and CP. Therefore, for samples from Year 2, these nutrients were predicted using the equations generated from Year 1 samples. Because of lower values for NDF ( $R^2$ CV = 0.75,  $n$  = 232), NDFD ( $R^2$ CV = 0.42,  $n$  = 235), and DMD ( $R^2$ CV = 0.46,  $n$  = 230), the samples from Year 2 for these components were analyzed as described for Year 1.

Silks and husks were removed from frozen cobs before they were broken in half. Ten kernels were removed from the edge of each half cob and dried at 55 °C for 3 days to determine kernel DM content. Cobs were then stored for 7 days in a ventilated room at 26 °C before removing the grains from the rachis with a cob sheller. Grain density was measured based on the test weight method [15]. Kernel hardness was determined using the Stenvert test [16] and according to the assays of Blandino et al. [17] and Ma and Dwyer [18]. Resulting ground subsamples from Year 2 were further used to assess particle size distribution by sieving on a Ro Tap particle separator (model RX-29; W.S. Tyler, Mentor, OH, USA) equipped with two screens (600 and 420  $\mu$ m) and a bottom pan [17].

### 2.3. Statistical Analyses

Statistical analyses were carried out with R software (Version 3.4.0; [19]). For each location, the fixed effects of year (2013 versus 2015), hybrid, harvest period (before versus after frost), and their interactions (harvest period  $\times$  year, harvest period  $\times$  hybrid, and year  $\times$  hybrid) on specific variables (nutrients, DM yield, cob proportion, and kernel properties) were tested with a linear model (function “lme” of the package nlme) using block (4 blocks of 6 hybrids each) as the random effect. The triple interaction (harvest period  $\times$  year  $\times$  hybrid) was not significant in most cases and was, for that reason, not reported. The “emmeans” function (package emmeans) was further applied to each model to calculate LSmeans and to conduct a pairwise comparison with a Tukey adjustment when single effects were significant. Data were considered significant at  $p < 0.05$ , and tendencies were discussed at  $0.05 \leq p \leq 0.10$ .

To illustrate the effect of the three main tested effects (year, hybrid, and harvest period) on nutritive value (whole plant DM, NDF, ADF, ADL, starch, CP, DMD, and NDFD), whole-plant DM yield, cob proportion (except for Lethbridge and Vauxhall, where cob proportion was measured in 2013 only), and kernel properties (DM content, hardness, density, and stage), principal component analyses were conducted on the data of each location (function “dudi.pca” of the package ade4), and graphical representations of individuals were obtained with the “fviz\_pca\_ind” function (package factoextra).

## 3. Results

### 3.1. Nutritive Value and DM Yield by Location

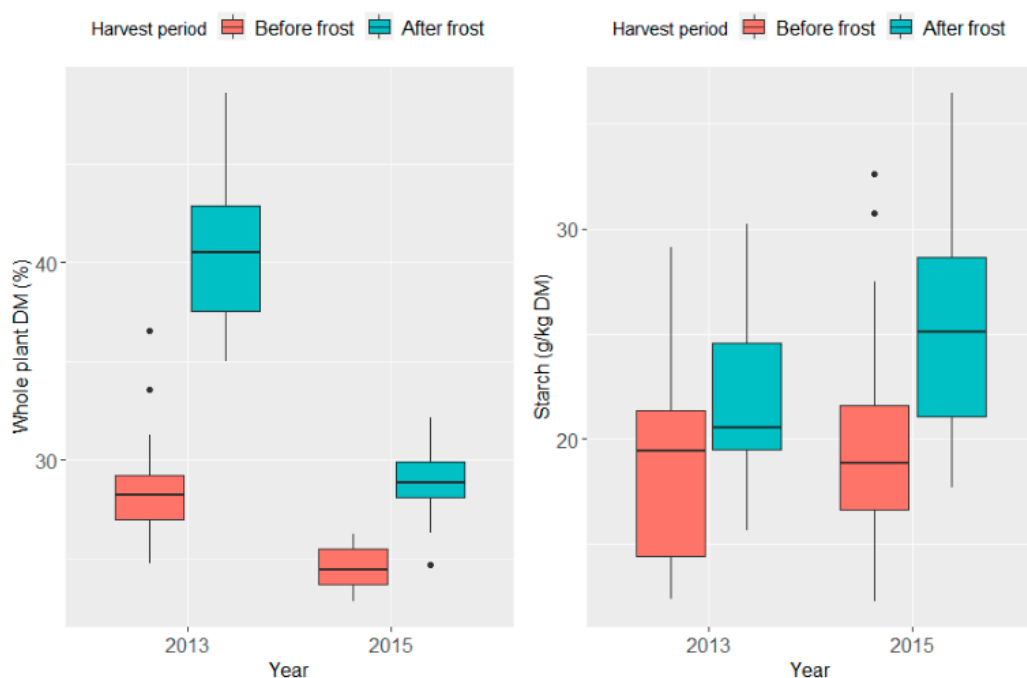
The effects of year, harvest period, hybrid, and their interactions on nutritive value and DM yield of whole-plant corn grown in Lacombe, Lethbridge, and Vauxhall are presented in Tables 3–5, respectively. For whole-plant DM content, NDF and starch concentrations, DMD, NDFD, or DM yield, boxplots are presented when a significant effect was observed for the interaction of year and harvest period.

**Table 3.** Effect of year, harvest period, hybrid, and their interactions on nutritive value and DM yield of whole-plant corn grown for silage in Lacombe.

	DM (%)	CP (g/kg)	NDF (g/kg)	ADF (g/kg)	ADL (g/kg)	Starch (g/kg)	DMD (g/kg)	NDFD (g/kg)	DM Yield (T/ha)
Year (Y)									
2013	345	79.6	566	288	21.9	204	687	569	14.7
2015	267	83.1	533	279	22.3	226	668	584	5.5
SEM	3.8	0.55	3.7	1.9	0.32	3.7	4.0	5.1	0.20
Harvest (Ha)									
Early	265	82.1	548	288	22.0	194	676	574	10.1
Late	346	80.6	550	279	22.3	236	680	579	10.1
SEM	3.8	0.55	3.7	1.9	0.32	3.7	4.0	5.1	0.20
Hybrid (Hy) <sup>1</sup>									
P39F44	326c	85.0b	537a	261a	19.5a	287c	678	584ab	8.2a
P7213R	311bc	81.1ab	551a	283bc	22.6b	225b	682	592b	11.0b
P39M26	309b	81.3ab	531a	278b	22.1b	237b	679	558a	10.2b
Edge	307b	80.6a	546a	290bcd	23.0b	192a	685	583ab	10.3b
2262RR	297ab	79.9a	552a	292cd	22.1b	174a	677	573ab	10.3b
P7632HR	285a	80.4a	579b	298d	23.5b	177a	666	570ab	10.5b
SEM	5.1	0.94	6.4	3.3	0.55	6.4	6.9	8.3	0.27
<i>p</i> -Value (Y)	<0.001	<0.001	<0.001	0.001	0.40	<0.001	0.001	0.021	<0.001
<i>p</i> -Value (Ha)	<0.001	0.087	0.71	0.002	0.52	<0.001	0.54	0.44	0.98
<i>p</i> -Value (Hy)	<0.001	0.005	<0.001	<0.001	<0.001	<0.001	0.46	0.059	<0.001
<i>p</i> -Value (Ha $\times$ Y)	<0.001	<0.001	0.27	0.25	0.26	0.038	0.13	0.091	0.34
<i>p</i> -Value (Hy $\times$ Y)	<0.001	0.87	0.20	0.11	0.25	0.18	0.19	0.30	0.15
<i>p</i> -Value (Ha $\times$ Hy)	0.28	0.14	0.021	0.33	0.37	0.92	0.26	0.52	0.11

<sup>1</sup> Within columns, means followed by the same letter (a, b, c, d) are not significantly different among hybrids ( $p < 0.05$ ).

In Lacombe (Table 3), the effect of year was significant ( $p < 0.021$ ) for all variables except ADL which averaged 22.1 g/kg ( $p = 0.40$ ). During the first year (2013), the hybrids were characterized by substantially greater DM yield (+9.2 T/ha), DM content (+7.8 percentage points), fiber concentration (+27 g/kg for NDF), and DMD (+19 g/kg) and lower CP concentration (−3.5 g/kg), starch concentration (−22 g/kg), and NDFD (−15 g/kg). Delaying harvest until after frost increased ( $p < 0.002$ ) whole-plant DM content (+8.1 percentage points) and starch concentration (+42 g/kg) while decreasing ADF concentration (−9 g/kg) and tending ( $p = 0.087$ ) to decrease CP concentration (−1.5 g/kg). Other variables were not affected. The effect of frost on nutritive value and yield was similar in both years (no harvest  $\times$  year interactions) for most variables, except for DM, CP, and starch, for which effects were not consistent between years (Figure 1). Differences ( $p < 0.005$ ) were observed among hybrids for all variables except digestibility values (a tendency for NDFD). The effect of hybrid was consistent over years (except for DM content) and harvesting time (except for NDF concentration).



**Figure 1.** Effect of year and harvest period on DM content and starch concentration of whole-plant corn grown for silage in Lacombe. Each box represents the quartiles, with the median within the box (thick middle line). The vertical lines represent the maximum and minimum values, respectively. Values greater or less than the interquartile range (third–first quartile)  $\pm 1.5$  are considered extremes and are represented with dots.

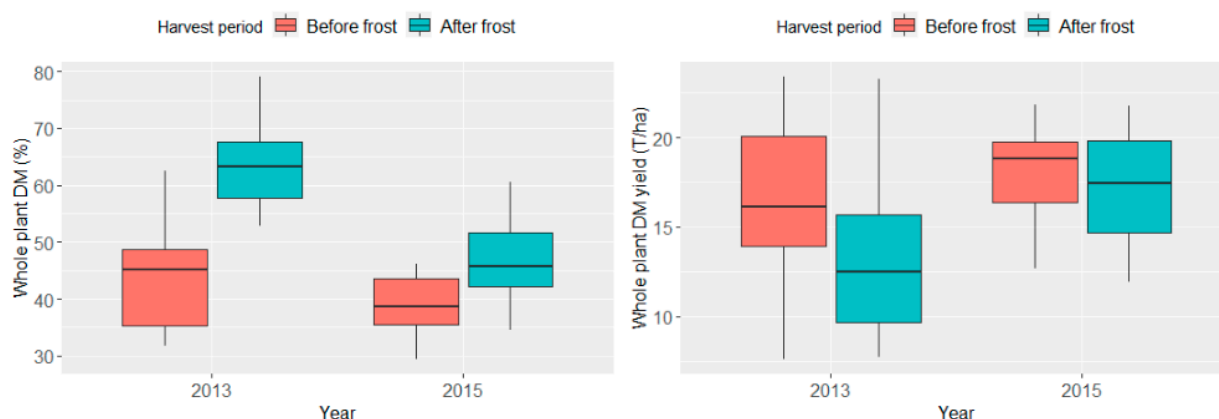
In Lethbridge (Table 4), CP and starch concentrations were similar between years (71.6 and 318 g/kg on average, respectively), whereas all other variables differed ( $p < 0.001$ ): in 2013, NDFD and DM yield were lower than in 2015 (−39 g/kg and −2.7 T/ha, respectively), whereas DM content and concentrations of NDF, ADF, and ADL, as well as DMD, were greater in 2013 (+11.2 percentage points and +42, +23, +4.1 and +19 g/kg, respectively). Harvesting time did not affect NDF, ADL, and starch concentrations or DM and NDF digestibility of whole-plant corn. However, harvesting after frost increased ( $p < 0.001$ ) DM content (+13.8 percentage points on average, with a stronger effect in 2013; Figure 2) and CP concentration (+4.8 g/kg), and decreased ( $p < 0.026$ ) ADF concentration (−8 g/kg) and DM yield (−2.2 T/ha on average, with a stronger effect in 2013; Figure 2). The effect of harvest was consistent across years for NDF, ADF, and starch concentrations as well as for DMD (tendency) and NDFD. The opposite effect of frost was observed between years for CP and ADL concentrations. The effect of hybrid was significant ( $p < 0.001$ ) for all

variables except NDFD, but the effect of hybrid was inconsistent between years ( $p < 0.047$ ) and harvesting period ( $p < 0.035$ ) for many of the variables.

**Table 4.** Effect of year, harvest period, hybrid, and their interactions on nutritive value and DM yield of whole-plant corn grown for silage in Lethbridge.

	DM (%)	CP (g/kg)	NDF (g/kg)	ADF (g/kg)	ADL (g/kg)	Starch (g/kg)	DMD (g/kg)	NDFD (g/kg)	DM Yield (T/ha)
Year (Y)									
2013	537	71.5	527	263	24.3	316	683	507	14.8
2015	425	71.7	485	240	20.2	320	664	546	17.5
SEM	8.3	0.34	6.0	2.5	0.35	4.5	3.1	7.6	0.59
Harvest (Ha)									
Early	412	69.2	502	256	22.0	320	671	522	17.2
Late	550	74.0	510	248	22.5	316	676	532	15.0
SEM	8.3	0.34	6.0	2.5	0.35	4.5	3.1	7.6	0.59
Hybrid (Hy) <sup>1</sup>									
P39F44	510b	75.2c	530bc	253abc	20.0a	327ab	654a	526	12.0a
P7443R	559c	70.4b	543c	267c	23.4cd	309ab	654a	524	13.8ab
P7632HR	501b	67.5a	489a	239a	21.6abc	340b	690b	532	16.8c
39V05	498b	71.1b	509ab	264bc	24.5d	300a	664a	520	15.2bc
P8673AM	393a	70.7b	485a	248ab	22.6bcd	298a	692b	528	19.8d
P8210HR	427a	74.7c	480a	240a	21.5ab	334b	688b	530	19.3d
SEM	11.0	0.58	9.0	4.3	0.51	7.9	5.4	9.7	0.73
<i>p</i> -Value (Y)	<0.001	0.66	<0.001	<0.001	<0.001	0.52	<0.001	<0.001	<0.001
<i>p</i> -Value (Ha)	<0.001	<0.001	0.22	0.026	0.21	0.47	0.23	0.11	<0.001
<i>p</i> -Value (Hy)	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.89	<0.001
<i>p</i> -Value (Ha × Y)	<0.001	<0.001	0.18	0.91	<0.001	0.25	0.075	0.82	0.020
<i>p</i> -Value (Hy × Y)	<0.001	0.31	0.065	0.63	0.001	0.047	0.005	0.13	0.003
<i>p</i> -Value (Ha × Hy)	0.95	<0.001	0.26	0.035	0.071	0.023	0.14	0.40	0.034

<sup>1</sup> Within columns, means followed by the same letter (a, b, c, d) are not significantly different among hybrids ( $p < 0.05$ ).

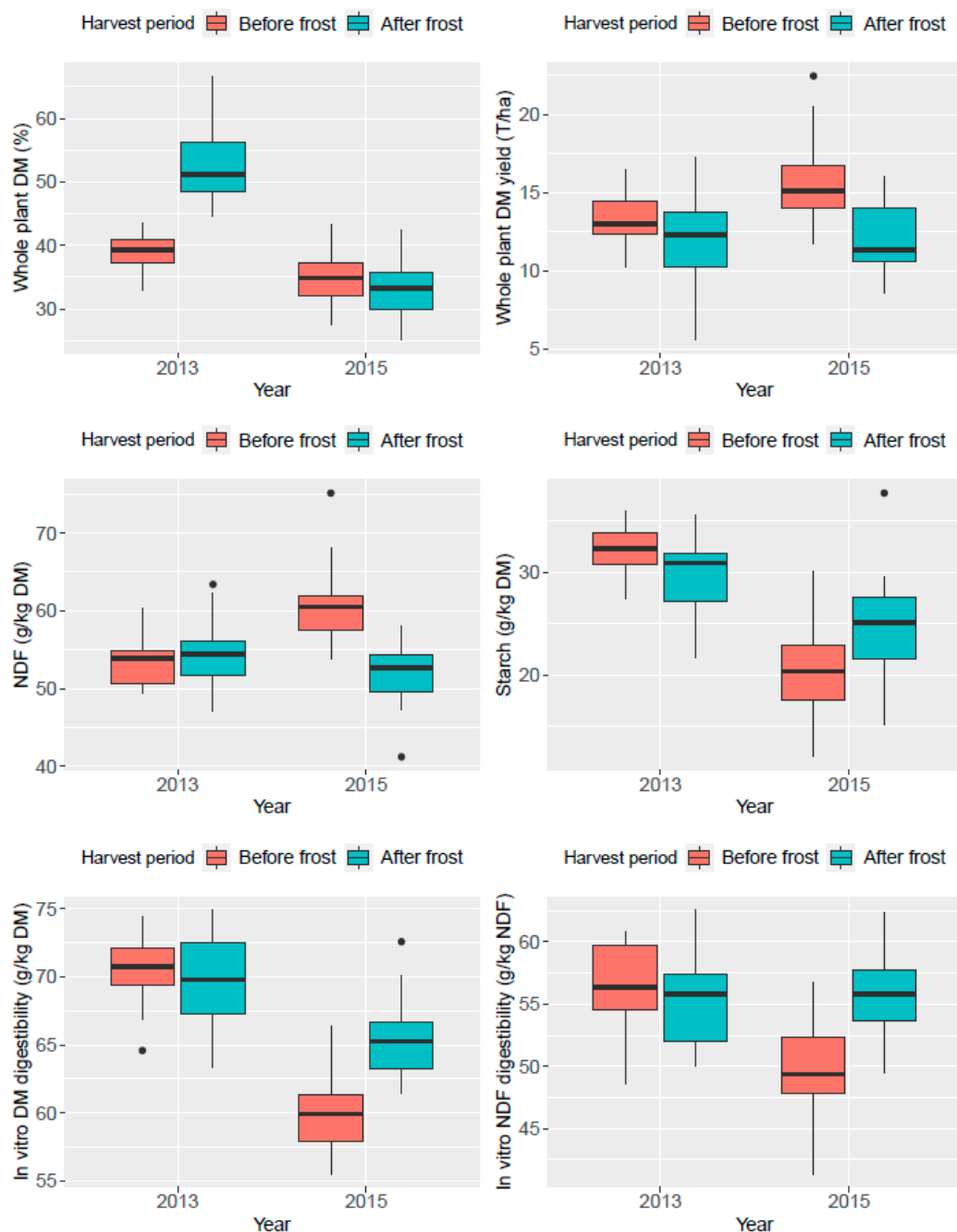


**Figure 2.** Effect of year and harvest period on DM content and yield of whole-plant corn grown for silage in Lethbridge. Each box represents the quartiles, with the median within the box (thick middle line). The vertical lines represent the maximum and minimum values, respectively. Values greater or less than the interquartile range (third–first quartile)  $\pm 1.5$  are considered extremes and are represented with dots.

In Vauxhall (Table 5), all variables were affected by year ( $p < 0.015$ ). In 2013, whole-plant corn was characterized by greater DM content (+11.8 percentage points), starch concentration (+82 g/kg), DMD (+74 g/kg), and NDFD (+33 g/kg) and lower CP (−4.9 g/kg), NDF (−24 g/kg), ADF (−27 g/kg), and ADL (−3 g/kg) concentrations and DM yield (−1.1 T/ha). Delaying harvest until after frost increased ( $p < 0.001$ ) DM content (+6.2 percentage points), DMD (+24 g/kg), and NDFD (+25 g/kg). By contrast, late harvest decreased ( $p < 0.001$ ) CP (−2.8 g/kg), NDF (−39 g/kg), ADF (−19 g/kg), and ADL (−2.6 g/kg).



concentrations, as well as DM yield ( $-2.5$  T/ha). No impact of frost was observed on starch concentration ( $268$  g/kg on average). However, the effect of harvesting time on nutrient composition was highly dependent on the year (Figure 3). Whole-plant corn DM content and CP, starch, and NDF concentrations were different ( $p < 0.047$ ) among hybrids, but other variables were not affected (except a tendency for ADL concentration). The effect of hybrid was repeatable for most variables over years (except DM content, starch concentration, and DM yield) and harvesting time (except a tendency for starch).



**Figure 3.** Effect of year and harvest period on DM content, NDF and starch concentrations, in vitro DM and NDF digestibility, and DM yield of whole-plant corn grown for silage in Vauxhall. Each box represents the quartiles, with the median within the box (thick middle line). The vertical lines represent the maximum and minimum values, respectively. Values greater or less than the interquartile range (third–first quartile)  $\pm 1.5$  are considered extremes and are represented with dots.

**Table 5.** Effect of year, harvest period, hybrid, and their interactions on nutritive value and DM yield of whole-plant corn grown for silage in Vauxhall.

	DM (%)	CP (g/kg)	NDF (g/kg)	ADF (g/kg)	ADL (g/kg)	Starch (g/kg)	DMD (g/kg)	NDFD (g/kg)	DM Yield (T/ha)
Year (Y)									
2013	457	72.4	540	266	21.3	309	701	560	12.6
2015	339	77.3	564	293	24.3	227	627	527	13.7
SEM	5.8	1.23	6.0	4.2	0.33	6.4	3.8	6.6	0.64
Harvest (Ha)									
Early	367	76.2	572	289	24.1	265	652	531	14.4
Late	429	73.4	533	270	21.5	271	676	556	11.9
SEM	5.8	1.23	6.0	4.2	0.33	6.4	3.8	6.6	0.64
Hybrid (Hy) <sup>1</sup>									
P7632HR	440d	69.9a	551	281	22.6	275b	670	555	13.2
39V05	425cd	75.0bc	560	288	24.3	264ab	658	542	13.2
P8193AM	407bc	73.9b	553	280	22.7	272b	653	526	12.5
P8210HR	401bc	77.1bc	559	279	23.0	279b	662	556	13.6
P8673AM	338a	75.0bc	546	279	21.9	234a	678	549	13.3
P8622AM	377b	78.2c	543	271	22.4	285b	663	534	13.2
SEM	8.4	1.43	10.3	6.3	0.57	9.5	6.6	9.2	0.78
<i>p</i> -Value (Y)	<0.001	<0.001	0.005	<0.001	<0.001	<0.001	<0.001	<0.001	0.015
<i>p</i> -Value (Ha)	<0.001	<0.001	<0.001	<0.001	<0.001	0.44	<0.001	<0.001	<0.001
<i>p</i> -Value (Hy)	<0.001	<0.001	0.81	0.53	0.098	0.002	0.14	0.047	0.80
<i>p</i> -Value (Ha × Y)	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.006
<i>p</i> -Value (Hy × Y)	0.039	0.15	0.49	0.86	0.95	0.031	0.40	0.36	0.047
<i>p</i> -Value (Ha × Hy)	0.63	0.25	0.82	0.19	0.39	0.075	0.21	0.45	0.39

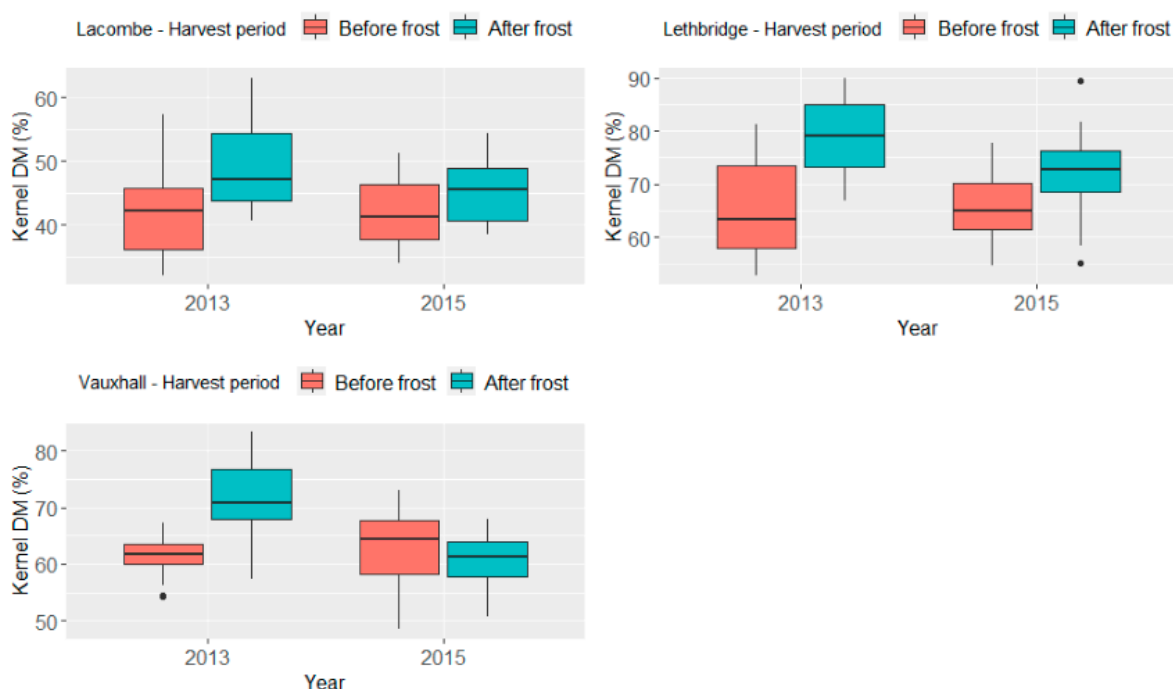
<sup>1</sup> Within columns, means followed by the same letter (a, b, c, d) are not significantly different among hybrids ( $p < 0.05$ ).

### 3.2. Cob Proportion and Kernel Properties by Location

The effects of year, harvest period, hybrid, and their interactions on cob proportion and kernel properties of whole-plant corn grown in Lacombe, Lethbridge, and Vauxhall are presented in Tables 6–8, respectively. The interactions between year and harvest period for kernel DM content at each location are shown in boxplots (Figure 4).

In Lacombe (Table 6), the year did not affect the cob proportion or the kernel hardness. Kernel DM content and stage were lower ( $p < 0.001$ ) in 2015 (−1.8 percentage points and −0.43 points), whereas kernel density was greater ( $p < 0.001$ ) that year (+7.7 points). Harvesting before frost increased ( $p < 0.001$ ) cob proportion (+4.0 percentage points in late harvest) and altered kernel properties except for the proportion of large particles. Late harvest increased kernel DM content (+5.2 percentage points), hardness (+3.4 points), small particle proportion (+1.5 percentage points), density (+1.7 points), and stage (+0.41 points). Only the proportion of medium particles decreased with late harvest (−1.1 percentage points). The effect of harvest was similar between years for cob proportion, kernel density, and stage (tendency) but different ( $p < 0.007$ ) for kernel DM content and hardness. Hybrid affected ( $p < 0.001$ ) all cob-related variables, and this effect was repeatable over years for most variables except for kernel DM content, density, and stage ( $p < 0.038$ ) and over harvesting periods except for kernel hardness (tendency), stage, and proportions of large and small particles ( $p < 0.030$ ).





**Figure 4.** Effect of year and harvest period on kernel DM of whole-plant corn grown for silage for each location. Each box represents the quartiles, with the median within the box (thick middle line). The vertical lines represent the maximum and minimum values, respectively. Values greater or less than the interquartile range (third–first quartile)  $\pm 1.5$  are considered extremes and are represented with dots.

**Table 6.** Effect of year, harvest period, hybrid, and their interactions on cob proportion and kernel properties of whole-plant corn grown for silage in Lacombe.

	Cob (% DM)	Kernel <sup>1</sup>					Density (kg/hL)	Stage
		DM (%)	Hardness (s/20 g)	Large Particles (%)	Medium Particles (%)	Small Particles (%)		
Year (Y)								
2013	49.2	45.6	28.7	-	-	-	59.0	3.83
2015	48.6	43.8	28.8	-	-	-	66.7	3.40
SEM	0.76	0.47	0.41	-	-	-	0.32	0.037
Harvest (Ha)								
Early	46.9	42.1	27.0	55.3	13.8	30.9	62.0	3.41
Late	50.9	47.3	30.4	55.0	12.7	32.4	63.7	3.82
SEM	0.76	0.47	0.41	0.41	0.16	0.44	0.32	0.037
Hybrid (Hy) <sup>2</sup>								
P39F44	53.4e	54.1e	36.6d	61.3c	10.9a	27.7a	68.2e	4.38c
P7213R	50.9de	46.0c	29.6c	53.5a	12.4b	34.1de	62.8c	3.81b
P39M26	50.5cd	48.5d	30.3c	53.0a	12.1b	34.9e	65.4d	3.86b
Edge	48.0bc	41.6b	28.2bc	56.7b	13.7c	29.6ab	62.3bc	3.27a
2262RR	44.4a	40.5b	25.7b	54.3a	14.3c	31.5bc	61.0b	3.24a
P7632HR	46.2ab	37.4a	21.9a	51.9a	16.1d	32.0cd	57.2a	3.13a
SEM	0.95	0.63	0.70	0.62	0.27	0.60	0.48	0.065
<i>p</i> -Value (Y)	0.34	<0.001	0.88	-	-	-	<0.001	<0.001
<i>p</i> -Value (Ha)	<0.001	<0.001	<0.001	0.44	<0.001	0.001	<0.001	<0.001
<i>p</i> -Value (Hy)	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
<i>p</i> -Value (Ha $\times$ Y)	0.60	<0.001	0.007	-	-	-	0.24	0.081
<i>p</i> -Value (Hy $\times$ Y)	0.31	0.002	0.59	-	-	-	0.038	0.011
<i>p</i> -Value (Ha $\times$ Hy)	0.88	0.77	0.093	0.001	0.77	<0.001	0.115	0.030

<sup>1</sup> Large, medium, and small particles are proportions of the material >600, 420–600, and <420  $\mu$ m, respectively. Kernel stage was assessed on a scale from 1 (silking) to 6 (physiological maturity). <sup>2</sup> Within columns, means followed by the same letter (a, b, c, d) are not significantly different among hybrids ( $p < 0.05$ ).

In Lethbridge (Table 7), the year affected ( $p < 0.004$ ) all variables: in 2013, cob DM content was greater than in 2015 (+4.1 percentage points), whereas kernel hardness, density, and stage were less in 2013 (−3.5, −2.0, and −1.02 points, respectively). Late harvest increased ( $p < 0.029$ ) cob and kernel DM content (+5.7 and +9.7 percentage points, respectively), proportion of small particles (+1.4 percentage points), kernel density (+1.8 points), and stage (+0.46 points). Large particle proportion decreased ( $p = 0.006$ ) when harvested after frost (−1.6 percentage points), whereas harvest did not affect kernel hardness or proportion of medium particles. The effect of frost was different between years for kernel DM content, hardness (tendency), and density. All variables were affected by hybrid ( $p < 0.029$ ). This effect was repeatable over harvesting periods for most variables, except for kernel DM content and density, and over years, except for kernel DM content and hardness.

**Table 7.** Effect of year, harvest period, hybrid, and their interactions on cob proportion and kernel properties of whole-plant corn grown for silage in Lethbridge.

	Cob (% DM) <sup>2</sup>	Kernel <sup>1</sup>						Stage
		DM (%)	Hardness (s/20 g)	Large Particles (%)	Medium Particles (%)	Small Particles (%)	Density (kg/hL)	
Year (Y)								
2013	-	72.6	38.1	-	-	-	70.9	3.56
2015	-	68.5	41.6	-	-	-	72.9	4.58
SEM	-	0.88	1.00	-	-	-	0.23	0.061
Harvest (Ha)								
Early	61.7	65.7	40.7	54.8	11.1	34.1	71.0	3.84
Late	67.4	75.4	39.0	53.2	11.3	35.5	72.8	4.30
SEM	1.85	0.88	1.00	0.45	0.13	0.48	0.23	0.061
Hybrid (Hy) <sup>3</sup>								
P39F44	70.7b	76.3cd	50.9b	61.8c	9.7a	28.4a	76.8c	3.41a
P7443R	64.3ab	77.7d	40.0a	55.7b	10.4a	33.9b	71.4b	4.09b
P7632HR	68.8ab	70.3b	37.4a	51.2a	11.6b	37.2c	71.4b	4.06b
39V05	66.3ab	72.5bc	37.8a	52.7a	11.4b	35.9bc	71.3b	4.16b
P8673AM	58.2a	61.5a	36.0a	50.6a	12.0b	37.4c	69.6a	4.28b
P8210HR	59.2ab	64.9a	36.8a	52.0a	12.1b	35.8bc	70.8ab	4.44b
SEM	3.40	1.22	1.56	0.70	0.23	0.69	0.40	0.106
<i>p</i> -Value (Y)	-	<0.001	0.004	-	-	-	<0.001	<0.001
<i>p</i> -Value (Ha)	0.029	<0.001	0.16	0.006	0.30	0.009	<0.001	<0.001
<i>p</i> -Value (Hy)	0.029	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
<i>p</i> -Value (Ha × Y)	-	<0.001	0.053	-	-	-	<0.001	0.63
<i>p</i> -Value (Hy × Y)	-	0.001	0.001	-	-	-	0.71	0.16
<i>p</i> -Value (Ha × Hy)	0.32	0.007	0.89	0.31	0.99	0.30	0.001	0.35

<sup>1</sup> Large, medium, and small particles are proportions of the material >600, 420–600, and <420  $\mu\text{m}$ , respectively. Kernel stage was assessed on a scale from 1 (silking) to 6 (physiological maturity). <sup>2</sup> 2013 data are missing <sup>3</sup> Within columns, means followed by the same letter (a, b, c, d) are not significantly different among hybrids ( $p < 0.05$ ).

In Vauxhall (Table 8), kernel characteristics were different between years ( $p < 0.002$ ), with lower DM content (−5.0 percentage points) and greater hardness (+9.7 points), density (+1.2 points), and stage (+0.64 points) in 2015. Delaying harvest to after frost increased ( $p < 0.029$ ) kernel DM content (+4.0 percentage points), proportion of large particles (+1.2 percentage points), and stage (+0.21 points) and decreased proportion of small particles (−1.1 percentage points). Harvesting time did not affect cob DM content, kernel hardness, proportion of medium particle size, or density. The effect of harvesting time was similar between years only for kernel hardness and density. Hybrid affected ( $p < 0.011$ ) all variables except kernel density (tendency). The observed effects were consistent over years, except for kernel hardness, and over harvest period, except for proportion of medium particles and kernel stage.

**Table 8.** Effect of year, harvest period, hybrid, and their interactions on cob proportion and kernel properties of whole-plant corn grown for silage in Vauxhall.

	Cob (% DM) <sup>2</sup>	Kernel <sup>1</sup>					Density (kg/hL)	Stage
		DM (%)	Hardness (s/20 g)	Large Particles (%)	Medium Particles (%)	Small Particles (%)		
Year (Y)								
2013	-	66.6	35.2	-	-	-	68.5	3.96
2015	-	61.6	44.9	-	-	-	69.7	4.60
SEM	-	0.79	0.79	-	-	-	0.32	0.077
Harvest (Ha)								
Early	50.7	62.1	40.4	54.7	12.3	33.0	69.2	4.18
Late	49.6	66.1	39.8	55.9	12.2	31.9	69.1	4.39
SEM	3.68	0.79	0.79	0.51	0.07	0.51	0.32	0.077
Hybrid (Hy) <sup>3</sup>								
P7632HR	54.7b	67.4c	39.7ab	57.1b	11.9a	30.9a	68.1a	4.72c
39V05	54.8b	66.6bc	42.3b	55.4ab	12.1a	32.5ab	68.8ab	4.34bc
P8193AM	51.5ab	67.1c	41.1b	54.6ab	12.4ab	33.0ab	69.5ab	4.16ab
P8210HR	47.7ab	64.4bc	41.8b	56.2b	12.2ab	31.6a	69.6ab	4.38bc
P8673AM	43.1a	56.3a	36.5a	53.2a	12.7b	34.1b	68.9ab	3.91a
P8622AM	48.9ab	62.9b	39.0ab	55.3ab	12.1a	32.6ab	70.1b	4.19ab
SEM	4.05	1.12	1.19	0.71	0.12	0.70	0.50	0.110
<i>p</i> -Value (Y)	-	<0.001	<0.001	-	-	-	0.002	<0.001
<i>p</i> -Value (Ha)	0.51	<0.001	0.53	0.018	0.18	0.029	0.67	0.010
<i>p</i> -Value (Hy)	0.003	<0.001	0.004	0.002	0.002	0.011	0.057	<0.001
<i>p</i> -Value (Ha × Y)	-	<0.001	0.90	-	-	-	0.88	0.002
<i>p</i> -Value (Hy × Y)	-	0.22	0.001	-	-	-	0.096	0.90
<i>p</i> -Value (Ha × Hy)	0.54	0.36	0.53	0.42	0.016	0.36	0.56	0.009

<sup>1</sup> Large, medium, and small particles are proportions of the material >600, 420–600, and <420 µm, respectively. Kernel stage was assessed on a scale from 1 (silking) to 6 (physiological maturity). <sup>2</sup> 2013 data are missing <sup>3</sup> Within columns, means followed by the same letter (a, b, c, d) are not significantly different among hybrids ( $p < 0.05$ ).

### 3.3. Overview of Pooled Results

Figure 5 depicts the relationships among variables for each location. Variables pointing in the same direction are positively correlated, whereas variables oppositely directed are negatively correlated. The proximity of the variables indicates the magnitude of the correlations, whereas the length of the arrows indicates the overall contribution to the variability of the component. A correlation matrix by location is available in the Supplementary Materials.

At all locations, strong positive correlations were observed among fiber fractions (NDF, ADF, and ADL), and these fiber fractions were negatively correlated with starch concentration. In Lacombe and Lethbridge, DMD and NDFD had a lower contribution to the overall variability compared to Vauxhall, but parameters were positively correlated at all locations. Digestibility of DM and NDF were negatively correlated with NDF at all locations except in Lethbridge, where no significant correlation was observed between NDFD and NDF. In Lacombe, DMD was also positively correlated with whole-plant DM content and yield, whereas NDFD was negatively correlated with DM yield. In Lethbridge, DMD was positively correlated with starch concentration and DM yield and negatively correlated with kernel density; NDFD was positively correlated with kernel hardness and stage of maturity. In Vauxhall, both DMD and NDFD were positively correlated with starch concentration and negatively correlated with kernel hardness, density, and stage; DMD was also positively correlated with whole-plant DM.

Figures 6–8 illustrate the effect of harvesting time, year, and CHU rating of the hybrid, respectively, on whole-plant corn nutritive value and kernel properties at each location. Samples did not clearly cluster according to harvesting period, though some groupings

can be distinguished in the Lacombe and Vauxhall samples. Clustering was very consistent for year and hybrid (CHU rating of the hybrid less or greater than 2400) effects.

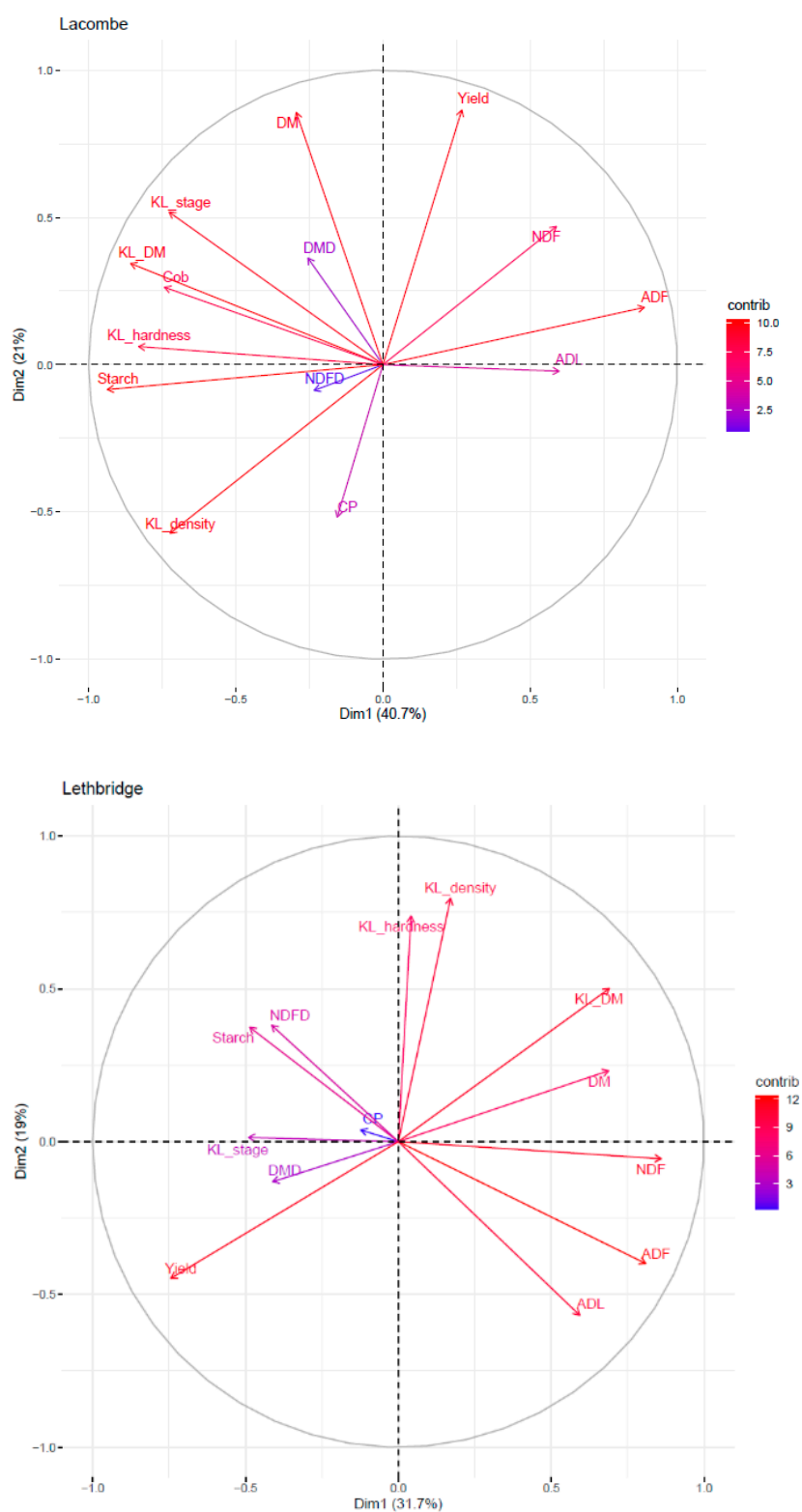
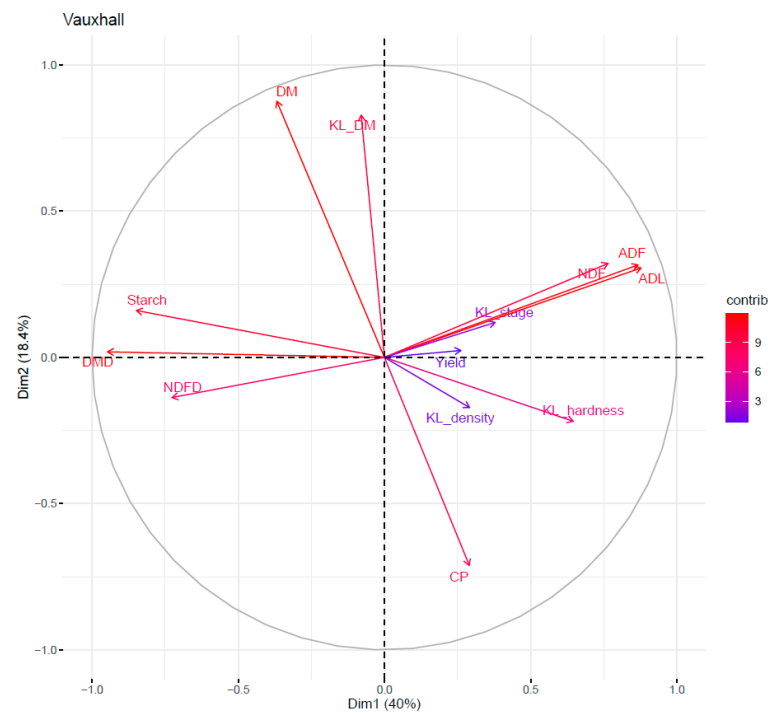
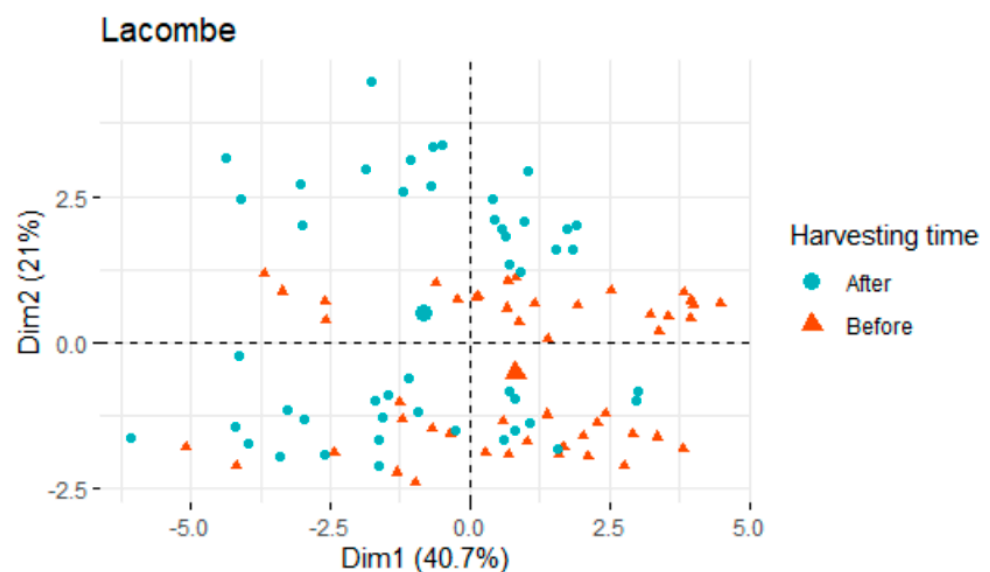


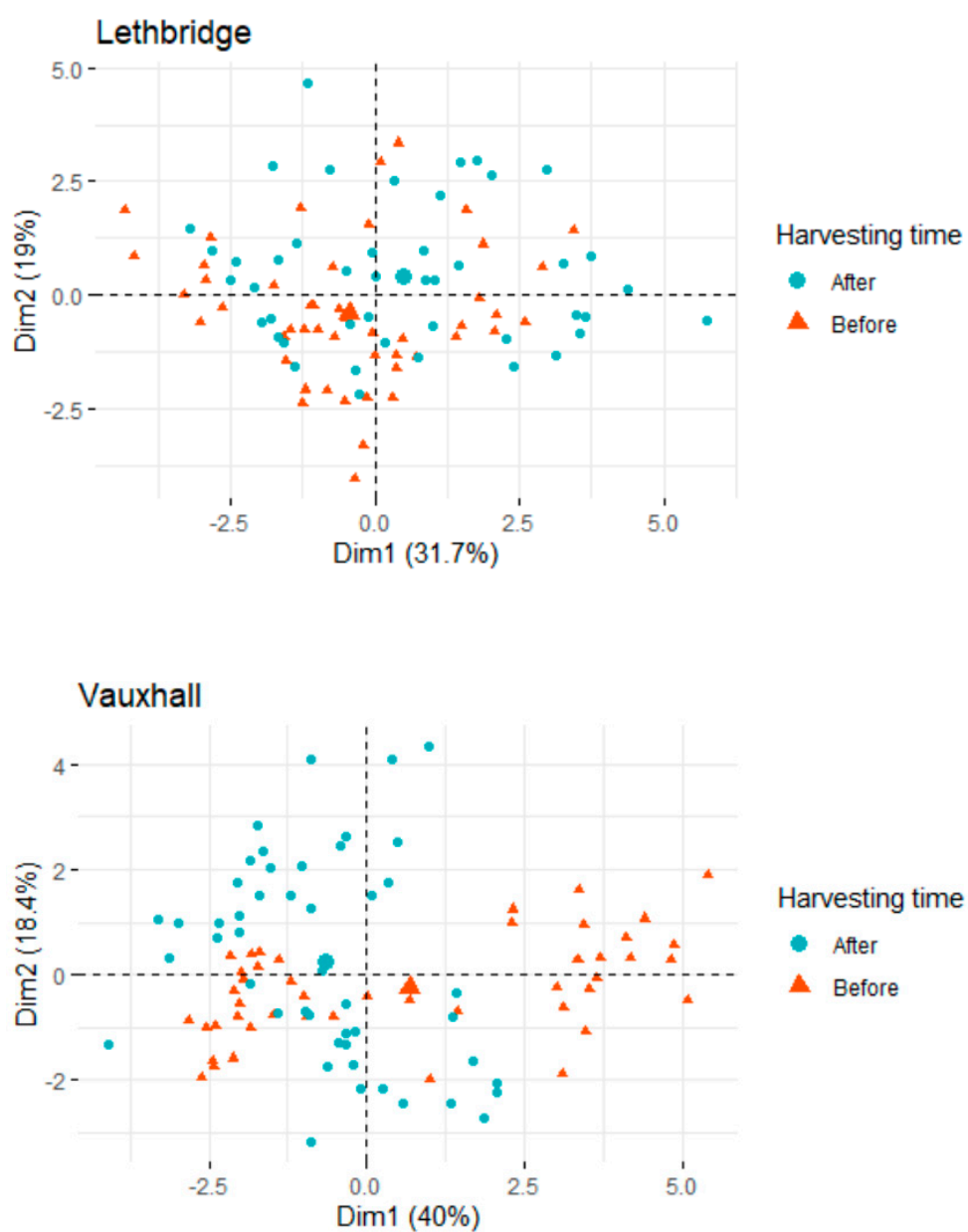
Figure 5. Cont.



**Figure 5.** Component pattern plots obtained by principal component analysis, describing the relationship among chemical analyses and kernel properties of whole-plant corn grown for silage for each location (ADF: acid detergent fiber, g/kg DM; ADL: acid detergent lignin, g/kg DM; Cob: dry cob proportion, % DM; contrib: contribution; CP: crude protein, g/kg DM; DM: dry matter, %; DMD: in vitro DM digestibility, g/kg DM; KL\_density: kernel density, kg/hL; KL\_DM: kernel DM content, %; KL\_hardness: kernel hardness, s/20 g; KL\_stage: kernel stage; NDF: neutral detergent fiber, g/kg DM; NDFD: NDF digestibility, g/kg NDF; Starch: starch concentration, g/kg DM; Yield: whole-plant DM yield, T/ha). Dim1 and Dim2 are the first two principal components, and they explained 61.7%, 50.7%, and 58.4% of the total variation of the dataset in Lacombe, Lethbridge, and Vauxhall, respectively.

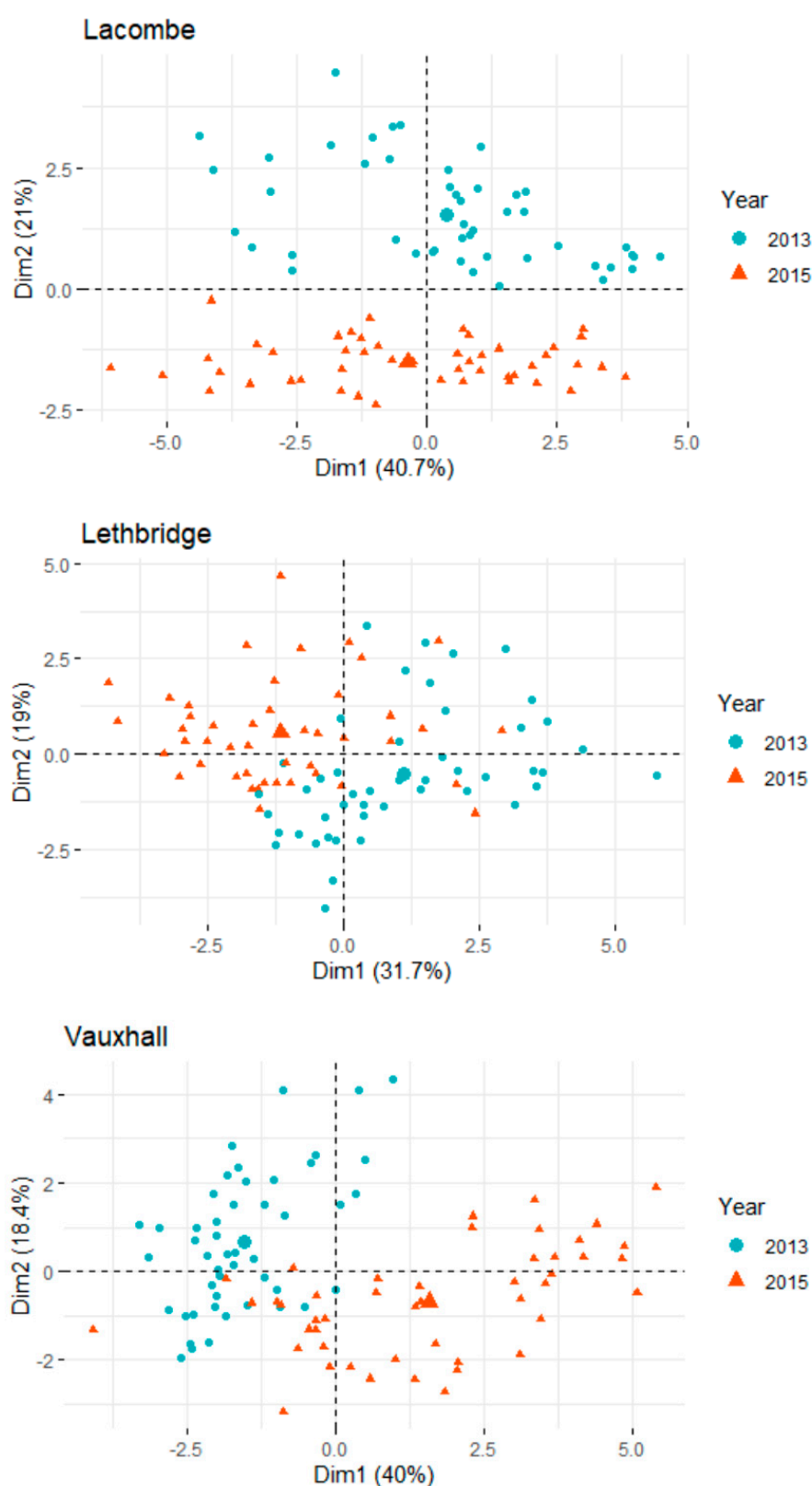


**Figure 6.** Cont.

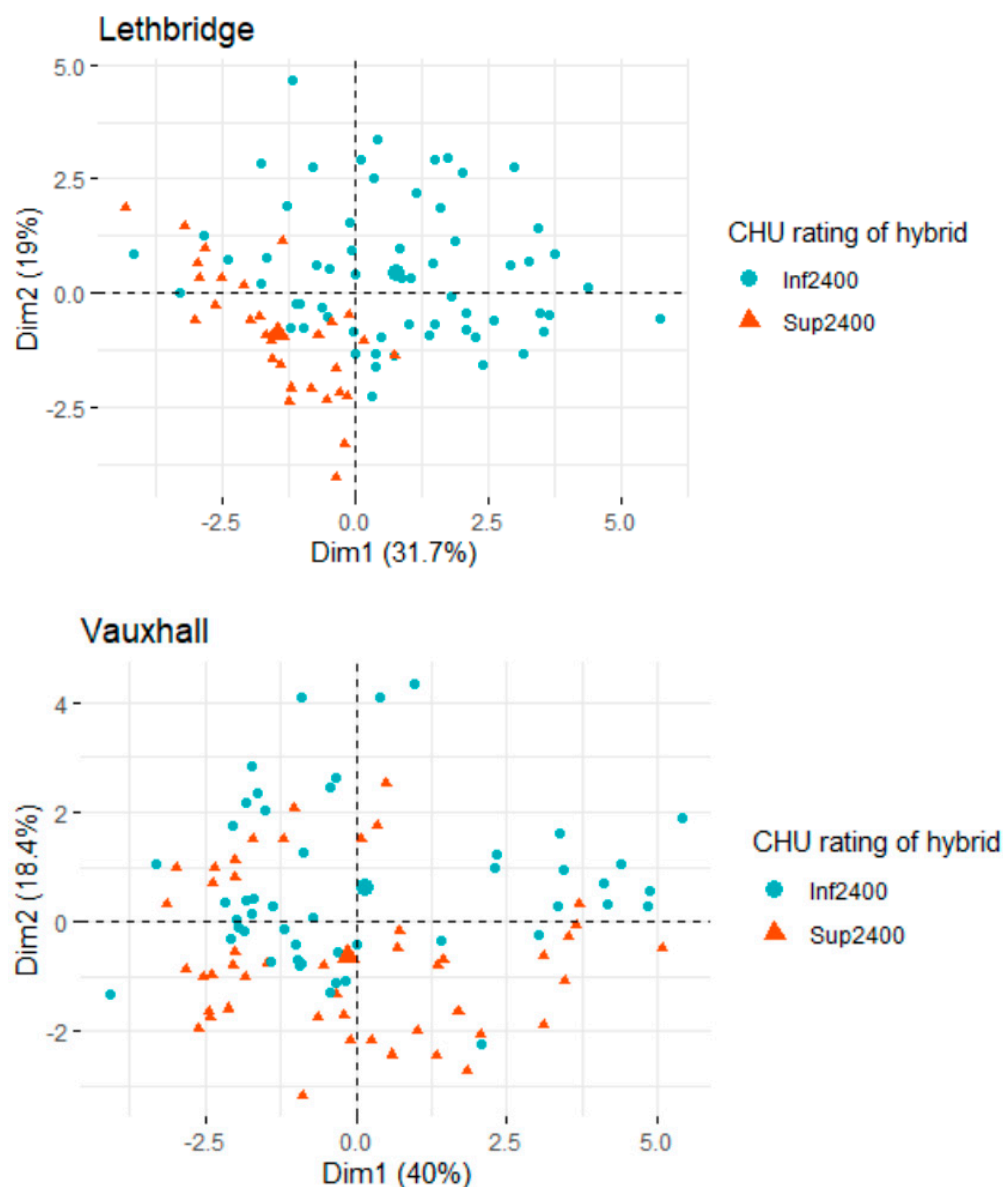


**Figure 6.** Score plots obtained by principal component analysis, describing the distribution of replicates of whole-plant corn grown for silage according to harvesting time (before or after frost) and location. Dim1 and Dim2 are the first two principal components, and they explained 61.7%, 50.7%, and 58.4% of the total variation of the dataset in Lacombe, Lethbridge, and Vauxhall, respectively.





**Figure 7.** Score plots obtained by principal component analysis, describing the distribution of replicates of whole-plant corn grown for silage according to the year and location. Dim1 and Dim2 are the first two principal components, and they explained 61.7%, 50.7%, and 58.4% of the total variation of the dataset in Lacombe, Lethbridge, and Vauxhall, respectively.



**Figure 8.** Score plots obtained by principal component analysis, describing the distribution of replicates of whole-plant corn grown for silage according to the CHU rating of the hybrid (inferior or superior to 2400) and location. Lacombe is not represented as the CHU rating of all hybrids was lower than 2400. Dim1 and Dim2 are the first two principal components, and they explained 50.7% and 58.4% of the total variation of the dataset in Lethbridge and Vauxhall, respectively.

#### 4. Discussion

Despite the development of new corn hybrids for silage production in short growing season areas such as the Canadian Prairie Provinces, the question of optimum harvesting time relative to frost has not yet been resolved. In this work, we investigated the effect of a light frost on yield, nutritive value, and kernel properties of whole-plant corn grown for silage.

##### 4.1. Frost Increased DM Content and Risk for Lower Yield

Before frost, whole-plant DM content in Lacombe (26.5%) was less than the typical range (32–38% DM) of corn grown in adapted areas, but this target was achieved in Vauxhall (36.7%) and slightly exceeded in Lethbridge (41.2%). The lower DM content observed and expected in Lacombe was attributed to delayed maturity caused by the lower

mean ambient temperature, higher accumulated precipitation, and lower accumulated CHU between seeding and harvest compared with the two other locations [7]. Delaying harvest until after frost greatly increased DM content of whole-plant corn at the three locations (+6.1, +13.8, and +6.2 percentage points on average in Lacombe, Lethbridge, and Vauxhall, respectively) due to plant tissue breakdown followed by desiccation. However, only at Lacombe was frost advantageous in bringing whole-plant DM content into the recommended range for ensilage (32 to 38% DM). Frost caused whole-plant DM content in the two other locations, especially Lethbridge, to dry excessively beyond the ideal range. Material that is too dry at harvest is difficult to pack densely and leads to weak fermentation during the ensiling process. The resulting silage has high pH and is at risk of microbial or fungal development [20,21]. Therefore, delaying harvest until after frost to increase whole-plant corn DM content in poorly adapted locations such as Lacombe (probability of receiving 2000 CHU <60%; [5]) may be successful, but this practice is not advantageous in warmer areas like Lethbridge in most years. However, it must be noted that the magnitude of the frost effect on increasing DM content depends on the year and the maturity already reached prior to frost. Indeed, the impact of harvesting time is stronger if whole-plant DM content is already high before frost, with this threshold DM value being dependent upon location.

In 2013 and 2015, DM yield of whole-plant corn for silage averaged 14.4 T/ha in Canada (13.6 T/ha in the Prairie provinces), assuming 35% DM content at harvest [22]. The overall DM yield observed in the present study was close to the national average, although slightly lower in Lacombe (10.1 T/ha). Whatever the year and hybrid, whole-plant DM yield was not affected by frost in Lacombe. In contrast, DM yield decreased with late harvest in Lethbridge and Vauxhall, confirming previous work conducted in Québec (Canada), where delaying harvest after frost decreased yield by 38 kg of DM/ha per day after the first frost [23]. In the present study, the extent of yield loss depended on the year in both locations but was not affected by the DM yield before frost. The weak interaction between hybrid and harvest period indicates fairly similar effects of harvesting after the first frost among hybrids, confirming previous work [23].

#### *4.2. Frost had only Minimal Effects on Dry Matter Digestibility*

In vitro DMD values achieved in this study (627 to 701 g/kg DM) were in the range reported in the literature (570 to 700 g/kg; [24]). Frost did not affect DMD of whole-plant corn in Lacombe and Lethbridge, whatever the year or the hybrid, supporting results obtained in 1976 in Eastern Canada [8]. However, higher DMD occurred in Vauxhall (+2.4 percentage points) when harvesting after frost, this effect being independent of hybrid but dependent on the year. These results contradict previous findings that DMD declined with progressive maturity [24]. For instance, DMD of whole-plant corn harvested after the first frost decreased by 8% in another Canadian study conducted in 1977 [25]. As the dry-down of whole-plant DMD is partially driven by corn stover digestibility, the development of new corn hybrids that maintain stover quality at advanced stages of maturity may concomitantly help to preserve whole-plant DMD after frost [24]. Interestingly, previous work showed that in vitro DMD [23] or apparent DMD measured using lactating dairy cows [26] of whole-plant corn did not change during the first month after the first frost despite multiple frost occurrences.

#### *4.3. Starch Concentration Increased in Response to Frost in the Coolest Areas of Alberta's Prairies*

Whole-plant starch concentrations observed in Lacombe (between 194 and 236 g/kg DM) were below the range usually observed in areas well suited for corn production (between 270 and 370 g/kg DM). In contrast, the higher starch concentrations (up to 316 g/kg DM) observed in the two other locations were more closely in line with this range. The lower starch concentration, combined with lower DM content, of corn grown in Lacombe indicates these plants were more immature at the time of first frost. This difference in maturity accounts for the different influence of frost on starch concentration for the three

locations, as postfrost harvesting only positively affected starch concentration in Lacombe. Therefore, delaying harvest until after frost as a means of increasing starch concentration of whole-plant corn may be a useful strategy in the coldest areas of the prairie Provinces [5], where starch concentration and DM content are in the lowest ranges. Additionally, late harvesting can also sometimes be advantageous in warmer areas, as observed in 2015 for Vauxhall, for which starch concentrations were low before frost. However, given the variability of frost in general versus maturity at harvest, as indicated by this study, harvesting after frost to enhance kernel development and starch concentration may be a risky management strategy that is hard to preplan, given the downside of over-dry silage.

Kernel characteristics were also modified with harvesting time. Being positively correlated with whole-plant DM content, kernel DM content increased after frost at all locations. One may assume that drier kernels would lead to increased kernel hardness, density, and development stage, as well as a higher proportion of small particles. However, with the exception of Lacombe, these correlations were not very prominent and possibly explained by the large differences in kernel DM content across locations, with Lethbridge and Vauxhall presenting contents up to 30% greater than in Lacombe. In other words, kernel characteristics may depend on kernel DM content until a threshold DM level is achieved, above which hardness and particle size become less predictable.

Previous research showed that increasing DM percentage of corn kernels linearly decreased starch digestibility, with the degree of vitreousness explaining 86% of the variation in ruminal starch digestibility [24]. Numerically, starch digestibility can reach 80% at 40% kernel DM, but digestibility drops to 60% when kernel DM increases to 65%. In the present study, using a kernel processor at harvest would be strongly recommended for whole-plant corn harvested after frost to ensure silage quality in terms of starch accessibility. Hybrid selection should also be considered carefully as kernel DM contents of the various hybrids differed by as much as 15%.

#### *4.4. Fiber Concentration and Digestibility Were not Negatively Affected by Frost*

Over the three locations, NDF concentration of whole-plant corn ranged between 485 and 572 g/kg, which is close to the usual range (between 405 and 635 g/kg DM; [24]). Except in Vauxhall, where frost reduced fiber concentration, delayed harvest did not affect NDF or ADL concentrations of corn harvested for silage, whatever the year. This finding supports a previous study in which delaying harvest of whole-plant corn from 34% to 41% DM did not influence fiber (NDF, ADF, and ADL) concentrations [20]. Similarly, earlier studies showed no impact of frost on cellulose [8] or fiber [26] concentrations in silage corn. However, other studies reported that delaying harvest to after frost increased fiber concentration of whole-plant corn [9] or that fiber concentration continued increasing after frost (+0.68 g/kg DM per day after the first frost, [23]; +65 g/kg DM after the fifth frost, [26]). Interestingly, ADF concentration of whole-plant corn was affected by frost in our study, unexpectedly with lower instead of higher concentrations at late harvest. Confirming a previous study in Québec [23], hybrid affected fiber concentration of whole-plant corn in Lacombe and Lethbridge, but without a clear relationship with the CHU rating of the hybrid. The impact of harvesting time was consistent among hybrids at all locations.

Fiber digestibility is an important indicator of forage quality, affecting animal performance. In a meta-analysis including different forages harvested for silage (including whole-plant corn), a one-unit increase in NDF digestibility measured in vitro or in situ was associated with a 0.17 kg increase in DM intake and a 0.25 kg increase in 4% fat-corrected milk [27]. Similarly, decreasing NDF digestibility of corn silage resulted in lower average daily gain and gain-to-feed ratios in growing cattle [28].

Frost did not affect the NDFD concentration of whole-plant corn in Lacombe and Lethbridge, whatever the year or hybrid, whereas greater NDFD was observed in Vauxhall after frost, with a stronger impact in 2015. Mertens [29] indicated that NDF concentration and its digestibility are the primary factors contributing to variation in DMD. This relationship

is corroborated by our results, especially at Vauxhall, where DMD was increased by frost and NDFD was strongly correlated with DMD.

Previously published studies generally found no effect of corn maturity on NDFD. In vitro, NDFD was similar between whole-plant corn harvested for silage at 34% or 41% DM [20]. Additionally, apparent total tract NDF digestibility measured on nonlactating Holstein cows was not different between whole-plant corn harvested at an early (<300 g/kg of DM) or late (>400 g/kg of DM) stage of maturity [30]. No specific effect of frost was reported on apparent NDF digestibility of corn silage fed to sheep [9]. In vitro fiber digestibility was also not affected by multiple frost occurrences [23,26]. However, increasing the maturity of corn silage fed to lactating dairy cows reduced the apparent ADL digestibility of the diet [31].

Fiber digestibility is mostly related to the lignification process [29,32]. In accordance with expectations, the component pattern plots clearly demonstrate the negative correlation between ADL concentration and NDFD of whole-plant corn at all locations. ADL concentrations were not affected by frost in Lacombe and Lethbridge, where frost did not affect NDFD either. By contrast, late harvest resulted in lower ADL concentrations in Vauxhall, which may partly explain the higher NDFD after frost at this location.

## 5. Conclusions

This study confirmed our hypothesis that delaying the harvest of whole-plant corn for silage until after frost should not be systematically practiced in short-season growing locations. New adapted hybrids can attain acceptable maturity for ensilaging prior to first frost, with high yield and starch concentration. However, in very short-season regions known for less-than-optimal heat unit accumulation, harvesting corn after frost may allow it to reach the targeted DM and nutrient concentrations without large detrimental impacts on yield and digestibility. Overall, the optimal harvesting time will vary yearly and by location and is a function of plant maturity (DM content) prior to the occurrence of frost. Reproducing this study at different locations may give further insights into possible interactions between the effect of harvesting time and environmental conditions, as the latter might affect the resistance of the plants to frost.

**Supplementary Materials:** The following are available online at <https://www.mdpi.com/2073-4395/11/3/459/s1>. Table S1: Correlation matrix.

**Author Contributions:** V.S.B. and K.A.B. conceived, designed, conducted the experiment, provided guidance in the laboratory analysis of samples, and helped write the final paper; J.G. analyzed the data, prepared the tables and figures, and wrote the first draft of the manuscript. All authors have read and agreed to the published version of the manuscript.

**Funding:** This research was funded by the Beef Cattle Research Council of Canada (grant number FDE.09.13) and Agriculture and Agri-Food Canada.

**Institutional Review Board Statement:** The rumen fluid collection for the in vitro studies was approved by the Lethbridge Research and Development Centre Animal Care Committee (protocol code ACC1717, 19 September 2017).

**Informed Consent Statement:** Not applicable.

**Data Availability Statement:** The data presented in this study are available on request from the corresponding author.

**Acknowledgments:** The authors thank Mary Lou Swift (Trouw Nutrition, AB, Canada) for NIR analyses and Lucia Holtshausen, Bev Farr, and David Young of Agriculture and Agri-Food Canada for laboratory assistance and data collection. In addition, the efforts of the field crews at each site are greatly appreciated for growing and harvesting the hybrids. Brian Beres (Agriculture and Agri-Food Canada) is thanked for his input on the organization of the study, and Pioneer Hybrid is acknowledged for providing hybrids for the study.



**Conflicts of Interest:** The authors declare no conflict of interest. The founding sponsors had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, and in the decision to publish the results.

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