

## Article

# Variation and Correlations among European and North American Orchardgrass Germplasm for Herbage Yield and Nutritive Value

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Academic Editors: John W. Forster and Kevin F. Smith

Received: 28 September 2016; Accepted: 16 November 2016; Published: 2 December 2016

**Abstract:** Efforts to improve water-soluble carbohydrate (WSC) concentrations are common in perennial forage grass breeding. Perennial ryegrass (*Lolium perenne*) breeding has been very successful in developing new cultivars with high WSC and high agronomic performance. Breeding efforts are ongoing to improve the WSC of other perennial forage grasses, such as orchardgrass (*Dactylis glomerata*). The United States Department of Agriculture Forage and Range and Deutsche Saatveredelung orchardgrass breeding programs cooperated to characterize the expression and genotype by environment interaction (GEI) of water-soluble carbohydrates in a collection of orchardgrass populations from both breeding programs. Additionally, the effort characterized the relationship between water-soluble carbohydrates and other agronomic and nutritive value traits in these populations. Overall, the Deutsche Saatveredelung populations had higher herbage mass (15%), rust resistance (59%), and later maturity. The Forage and Range Research populations had higher water-soluble carbohydrates (4%), nutritive value, and earlier maturity. However, results were highly dependent on GEI. Differences were very pronounced at the French and German field locations, but less pronounced at the two US locations. Combining the germplasm from the Forage and Range Research and Deutsche Saatveredelung programs may be a way to develop an improved base germplasm source that could then be used separately in the EU and US for water-soluble carbohydrate and other trait improvement.

**Keywords:** cocksfoot; digestibility; genotype by environment; maturity; rust; water soluble carbohydrates

## 1. Introduction

Orchardgrass (*Dactylis glomerata*) is an auto-tetraploid, out-crossing forage grass, and is one of the main perennial grasses used for grazing and hay production in temperate climates [1]. Although *D. glomerata* is the sole species in the *Dactylis* genus, the species includes both Mediterranean and Continental ecogeographic subspecies that are diploid and tetraploid [2]. Orchardgrass is highly preferred by livestock, exhibits early season growth, and is one of the most compatible perennial forage grasses when sown with perennial legumes, e.g., alfalfa (*Medicago sativa* L.) [1,3,4]. It is less tolerant to abiotic stresses than tall fescue and, for winter injury, than timothy, but more tolerant than perennial ryegrass. The forage quality of orchardgrass is intermediate, less than perennial ryegrass (*Lolium perenne*), equal to timothy (*Phleum pratense*), but greater than tall fescue (*Lolium arundinaceum*).

In the US orchardgrass is primarily utilized under rainfed conditions of the central and eastern portions, and under irrigated conditions in the semi-arid western portions [5].

Acute and chronic drought can result in substantial decreases of forage grass biomass production [6]. Winter damage caused by freezing includes delayed spring growth, uneven growth, and mortality. Available orchardgrass germplasm has substantial genetic variation for tolerance to drought and winter injury [7–9], and cultivars have been developed with drought tolerance in mind (e.g., cv. Paiute). However, cultivar performance under different or stressed conditions has been inconsistent [9], possibly due to a lack of uniformity in orchardgrass cultivars [10,11] or the unpredictability of appropriate environmental conditions in field settings [12]. Improving forage production and quality under abiotic stresses are objectives in orchardgrass breeding and genetics programs throughout the world (e.g., [13]).

In previous breeding efforts to improve perennial forage grass quality, it was noticed that water-soluble carbohydrate (WSC) accumulation in forage grasses was correlated with abiotic stress tolerance [14–19]. The WSC in crown tissues were found to stabilize lipids in cellular membranes [19], prevent membrane leakage, and maintain osmotic potential of the cells under cold and drought stress [17,18,20–22]. In addition to their role in abiotic stress tolerance, increased WSC in leaf and stem tissue can result in improved protein digestion, decreased nitrogen waste through feces and urine, and increased meat and milk productivity in feeding animals [23]. In orchardgrass, the overwhelming majority of all WSC are fructans [14,24], which bridge the gap between resource availability and demands [17]. Increasing WSC (i.e., fructans) is a tangible method of increasing both abiotic stress tolerance and forage quality in orchardgrass.

Although published reports on variation for orchardgrass WSC concentrations are limited, more information is coming forth in this arena. Robins et al. [25] identified substantial genetic variation for WSC within orchardgrass germplasm. Sanada et al. found significant differences in crown [13] and stubble WSC concentrations, and that WSC concentrations were related to winter hardiness and spring growth. Additionally they assessed European, Asian, and North American germplasm and found that European germplasm generally contained less WSC than other location sources [26]. Casler et al. [10] also detected differences in forage productivity and quality traits between North American and European orchardgrass varieties, although WSC was not included in that study. Other evaluations reported significant variation for herbage mass (HM) and forage quality under drought conditions, and found the WSC concentration to be correlated with forage productivity [27,28]. These reports indicate substantial variation for forage production and quality traits between different breeding programs, and suggested that increases in WSC can improve both traits. An understanding of the variation within a breeding program for WSC, and the relationship with forage productivity and quality, could result in great gains in this forage grass species.

In the study described herein, we examine the effect of differing environments in North America (US) and Europe (EU) on the expression of WSC, and the relationship between WSC and associated agronomic and nutritive value traits in populations of orchardgrass from the North American United States Department of Agriculture (USDA) Forage and Range (FRR) and European Deutsche Saatveredelung Aktiengesellschaft (DSV) breeding programs. The objective was to determine the utility of these breeding pools for the improvement of WSC and to determine how to capitalize on the genotype by environment interaction (GEI) effects underlying WSC, HM, and nutritive value expression in orchardgrass.

## 2. Results

### 2.1. Overall Model Effects

The overall statistical model identified differences ( $p \leq 0.004$ ) among all main effects for each phenotype (Table 1). Year showed the largest effect (sums of squares) for WSC and acid detergent fiber

(ADF); location for digestible dry matter (DDM), crude protein (CP), neutral detergent fiber (NDF), and neutral detergent fiber digestibility (NDFD); and population for maturity, rust rating (RR), and HM.

**Table 1.** Overall mean values for years, locations (HS—Hof Steimke; LR—Les Rosiers; LO—Logan; RE—Rexburg), and orchardgrass populations. Population values include the overall mean and range for each trait and population. Numbers followed by different letters in the same row (effect) differ statistically ( $p < 0.05$ ).

	Year		Location				Entry		
	2014	2015	HS	LR	LO	RE	Checks	DSV	FRR
HM <sup>1</sup>	5.9 <sup>a</sup>	4.9 <sup>b</sup>	6.6 <sup>a</sup>	3.4 <sup>c</sup>	5.2 <sup>b</sup>	6.7 <sup>a</sup>	5.4 <sup>b</sup> (4.9–5.9)	5.8 <sup>a</sup> (5.4–6.2)	5.0 <sup>c</sup> (4.5–5.5)
DDM <sup>2</sup>	66.5 <sup>b</sup>	69.0 <sup>a</sup>	73.3 <sup>b</sup>	74.6 <sup>a</sup>	61.1 <sup>d</sup>	62.0 <sup>c</sup>	67.8 <sup>b</sup> (67.2–68.9)	67.3 <sup>c</sup> (66.7–68.1)	68.1 <sup>a</sup> (67.4–68.8)
CP <sup>3</sup>	11.3 <sup>b</sup>	14.7 <sup>a</sup>	8.1 <sup>d</sup>	14.4 <sup>b</sup>	14.0 <sup>c</sup>	15.5 <sup>a</sup>	12.9 <sup>b</sup> (12.1–13.6)	12.5 <sup>c</sup> (11.8–13.2)	13.5 <sup>a</sup> (12.7–14.4)
WSC <sup>4</sup>	7.6 <sup>b</sup>	8.4 <sup>a</sup>	1 <sup>a</sup>	8.7 <sup>b</sup>	6.4 <sup>c</sup>	6.8 <sup>c</sup>	8.3 <sup>a</sup> (7.7–1)	7.8 <sup>b</sup> (6.9–8.5)	8.1 <sup>a</sup> (7.2–9.2)
NDF <sup>5</sup>	61.5 <sup>b</sup>	56.2 <sup>a</sup>	64.6 <sup>d</sup>	58.3 <sup>c</sup>	57.1 <sup>b</sup>	55.5 <sup>a</sup>	58.8 <sup>b</sup> (56.7–6)	59.8 <sup>c</sup> (58.8–61.4)	58.0 <sup>a</sup> (57.0–59.3)
ADF <sup>6</sup>	35.6 <sup>b</sup>	31.5 <sup>a</sup>	34.6 <sup>b</sup>	29.5 <sup>a</sup>	35.7 <sup>c</sup>	34.6 <sup>b</sup>	33.5 <sup>b</sup> (32.4–34.5)	34.2 <sup>c</sup> (33.4–35.2)	33.0 <sup>a</sup> (32.2–33.8)
NDFD <sup>7</sup>	64.5 <sup>b</sup>	66.6 <sup>a</sup>	61.0 <sup>c</sup>	59.6 <sup>d</sup>	67.7 <sup>b</sup>	73.8 <sup>a</sup>	65.6 <sup>a</sup> (64.6–66.4)	64.9 <sup>b</sup> (62.9–66.1)	66.1 <sup>a</sup> (63.8–67.9)
MAT <sup>8</sup>	3.0 <sup>a</sup>	2.8 <sup>a</sup>	-	2.6 <sup>a</sup>	2.9 <sup>b</sup>	3.1 <sup>c</sup>	2.7 <sup>a</sup> (0.8–3.9)	2.6 <sup>a</sup> (2.0–3.2)	3.1 <sup>b</sup> (2.8–3.4)
RR <sup>9</sup>	5.3 <sup>b</sup>	6.2 <sup>a</sup>	5.7 <sup>b</sup>	5.9 <sup>a</sup>	-	-	5.0 <sup>b</sup> (3.1–8.0)	7.7 <sup>a</sup> (6.5–8.4)	4.2 <sup>c</sup> (2.8–5.3)

<sup>1</sup> Herbage mass; <sup>2</sup> Digestible dry matter; <sup>3</sup> Crude protein; <sup>4</sup> Water soluble carbohydrates; <sup>5</sup> Neutral detergent fiber; <sup>6</sup> Acid detergent fiber; <sup>7</sup> Neutral detergent fiber digestibility; <sup>8</sup> Maturity; <sup>9</sup> Rust rating.

Differences among the locations during the study were large (Table 2). Annual maximum temperatures were higher at each location in 2015 compared to 2014, and growing degree days were higher in 2015 for each location but Hof Steimke. The two US locations experienced lower mean and minimum annual temperatures and annual precipitation compared to the European locations (Table 1). Therefore, the supplemental irrigation at these locations was used to compensate for the effect of the low precipitation. Although the annual maximum temperatures were similar among the four locations, there were lower winter and higher summer temperatures at the US locations. Interestingly, the growing degree days were also similar between Les Rosiers and Logan and between Hof Steimke and Rexburg.

**Table 2.** Mean weather characteristics at Hof Steimke, Germany; Les Rosiers, France; Logan, USA; and Rexburg, USA, during 2014 and 2015. Characteristics include latitude (Lat), longitude (Long), June photoperiod (June), soil type (Soil), annual precipitation excluding irrigation (Prec), mean annual temperature (Mean T), maximum annual temperature (Max T), minimum annual temperature (Min T), and growing degree days (GDD).

	Hof Steimke		Les Rosiers		Logan		Rexburg	
Lat	52.77° N		47.35° N		41.74° N		43.82° N	
Long	9.00° E		0.23° W		111.83° W		111.79° W	
June (h·day <sup>−1</sup> )	18–19		17–18		16–17		16–17	
Soil	Sandy, loam		Loamy, clay		Silty, clay loam		Silt loam	
Year	2014	2015	2014	2015	2014	2015	2014	2015
Prec (mm)	883	929	739	579	319	388	428	273
Mean T (°C)	11.2	10.5	13.7	13.5	8.9	10.0	7.2	8.3
Max T (°C)	32.7	37.5	35.0	37.2	36.7	37.8	33.3	35.6
Min T (°C)	−9.2	−4.8	−6.5	−6.0	−20.0	−22.2	−28.9	−26.7
GDD (50 °C)	1172	980	1628	1656	2314	2568	1828	2039

Rust resistance ratings were higher at Hof Steimke than Les Rosiers. Hof Steimke was characterized by relatively high WSC, HM, and NDF; intermediate DDM and ADF; and low CP. Les Rosiers was characterized by relatively high DDM; intermediate HM, NDF, CP, and WSC; and low ADF and NDFD. Logan exhibited relatively high ADF; intermediate levels of HM, NDF, NDFD, and CP; and low DDM and WSC. Rexburg exhibited relatively high HM, NDFD, and CP; intermediate

DDM and ADF; and low NDF and WSC. Maturity ratings were similar at Les Rosiers and Logan, but earlier at Rexburg.

Overall, 13 populations (Muravka, Paykar and 11 FRR populations) had WSC values greater than 8.5% (Table S1). Twenty-three populations (Duero, Harvestar and 21 DSV populations) had HM greater than 5.7 kg·plot<sup>−1</sup>. Twenty-four populations (Muravka, one DSV population, and 22 FFR populations) had DDM greater than 68.0%. Twenty-five populations (Latar, Paiute, and 23 FRR populations) had CP greater than 13.5%. Twenty populations (Muravka, Paykar and 18 FRR populations) had NDF less than 58.1%. Seventeen populations (Muravka and 16 FFR populations) had ADF less than 33.0%. Twenty populations (Latar and 16 FRR populations) had NDFD greater than 66.2%. Seventeen populations (Duero and 16 DSV populations) had RR greater than 7.8. Twenty-five populations (Muravka, Paiute, and 23 FRR populations) had maturity greater than 3.9 and were the earliest populations. Seventeen populations (Paykar and 16 DSV populations) had maturity less than 2.0.

Contrasts in the overall data showed that the check cultivars showed higher WSC and NDFD than the breeding program lines (Table 1). The DSV populations had higher HM, NDF, ADF, and RR; lower DDM, CP, and NDFD; and later maturity. The FFR populations had higher WSC, DDM, CP, NDFD; lower HM, NDF, ADF, and RR; and earlier maturity.

## 2.2. Principal Component Analysis and Correlations among Phenotype

Correlation estimates indicated a strong association among many of the measured phenotypes (Table 3). Only correlations > ±0.70 will be mentioned here. Herbage mass correlations were positive for NDF, ADF, and RR, but negative for DDM and CP. Digestible dry matter correlations were positive for WSC and NDFD, but negative for NDF, ADF, and RR. Crude protein was negatively correlated with NDF, ADF, and RR. Neutral detergent fiber was positively correlated with ADF and RR. Acid detergent fiber was positively correlated with RR. Maturity was not highly correlated with any phenotype, although correlations were highly driven by origin (EU vs. US).

**Table 3.** Pearson correlation estimates among orchardgrass populations among herbage mass (HM), dry matter digestibility (DDM), water soluble carbohydrates (WSC), neutral detergent fiber (NDF), acid detergent fiber (ADF), neutral detergent fiber digestibility (NDFD), maturity (MAT), rust resistance (RR), principal component 1 (PC1), and principal component 2 (PC2) evaluated across two years and four locations. All listed correlations significantly differ from zero ( $p < 0.01$ ), unless noted NS (non-significant).

	DDM	CP	WSC	NDF	ADF	NDFD	MAT	RR
HM	−0.74	−0.74	−0.47	0.80	0.80	−0.51	−0.56	0.84
DDM		0.67	0.72	−0.92	−0.92	0.71	0.46	−0.73
CP			NS	−0.77	−0.83	0.54	0.41	−0.76
WSC				−0.66	−0.60	0.30	0.36	−0.45
NDF					0.96	−0.60	−0.51	0.83
ADF						−0.64	−0.51	0.82
NDFD							0.29	−0.53
MAT								−0.55

## 2.3. Interaction Effects between Population and Location

Significant interaction effects were identified for each phenotype. The year by location interaction was significant for all phenotypes but maturity. The year by population interaction was less consistent and differed only for maturity, RR, and HM. The location by population interaction differed for all phenotypes but maturity and ADF, although the evidence of interaction for CP was nominal ( $p = 0.08$ ). The three-way year by location by population interaction was also inconsistent, differing for RR, HM, CP, and NDFD. The sums of squares associated with the interaction effects were generally smaller than population sums of squares, with exceptions of the year by location interaction for each of the

nutritive value phenotypes; the location by population interaction for DDM, WSC, and NDFD; and the year by location by population interaction for NDFD. Due to the lack of differences for the year by population interaction, the inconsistency of the three-way interaction, and the perennial nature of orchardgrass [29], all further results are based on the location  $\times$  population interaction analysis using AMMI analysis.

The Kendall ( $\tau$ ) statistic provided evidence for the nature (magnitude vs. rank changes) of the location  $\times$  population interaction (Table 4). Population rankings between Hof Steimke and Les Rosiers were highly correlated ( $\tau \leq 0.77$ ;  $p < 0.0001$ ) for all phenotypes but WSC and NDFD. Hof Steimke rankings exhibited no to limited relationships with the Logan and Rexburg locations. Les Rosiers rankings also exhibited low to moderate relationships with the Logan and Rexburg locations, although the correlations were generally higher with the Rexburg rankings. The Logan and Rexburg rankings exhibited high correlation for DDM and WSC, moderate correlation for HM and NDFD, and low to no correlation for the remaining phenotypes. With the exception of the Hof Steimke and Les Rosiers rankings, the Kendall rankings provided strong evidence for changes in ranking leading to the underlying location by population interaction.

**Table 4.** Kendall concordance estimates of orchardgrass rankings for each phenotype across years and between locations for herbage mass (HM), dry matter digestibility (DDM), water soluble carbohydrates (WSC), neutral detergent fiber (NDF), acid detergent fiber (ADF), and neutral detergent fiber digestibility (NDFD). All listed correlations significantly differ from zero ( $p < 0.05$ ), unless noted NS (non-significant).

	Les Rosiers	Logan	Rexburg
<b>Hof Steimke</b>			
HM	0.94	NS	0.35
DDM	0.95	NS	0.24
CP	0.77	0.61	0.44
WSC	0.47	NS	NS
NDF	0.88	0.54	0.69
ADF	0.94	0.37	0.72
NDFD	0.29	0.65	0.35
<b>Les Rosiers</b>			
HM		NS	0.42
DDM		0.17	0.30
CP		0.37	0.67
WSC		0.53	0.64
NDF		0.66	0.57
ADF		0.43	0.66
NDFD		NS	−0.37
<b>Logan</b>			
HM			0.62
DDM			0.88
CP			NS
WSC			0.89
NDF			0.22
ADF			0.09
NDFD			0.70

At Hof Steimke, Les Rosiers, and Rexburg there were differences ( $p < 0.02$ ) among the populations for each phenotype (Table 5). At Logan there were pronounced population differences for maturity ( $p = < 2 \times 10^{-16}$ ), and HM ( $p = 0.007$ ); less pronounced differences for DDM, NDF, and ADF ( $p \leq 0.06$ ); and no differences for CP, WSC, and NDFD. The AMMI analysis identified the significance ( $p \leq 0.0009$ ) of the first interaction principal component (IPCA) for each phenotype. The AMMI results separated the four locations into three groups for WSC and HM based on the high performance of a single

population: (1) Hof Steimke and Les Rosiers; (2) Rexburg; and (3) Logan. All four locations grouped separately for DDM and CP. NDF resulted in two groups: (1) Hof Steimke, Les Rosiers, and Logan; and (2) Rexburg. ADF resulted in three groups: (1) Les Rosiers and Logan; (2) Hof Steimke; and (3) Rexburg. NDFD resulted in three groups: (1) Logan and Rexburg; (2) Hof Steimke; and (3) Les Rosiers. The two US locations grouped together less frequently than did the two EU locations, possibly because of the lack of differences among the Logan populations for some traits. As demonstrated by the Kendall statistic, the performance of individual populations matched the grouping of locations for each phenotype. For example, nine populations showed high HM at both Hof Steimke and Les Rosiers.

**Table 5.** Population mean values corresponding to herbage mass (HM), dry matter digestibility (DDM), water soluble carbohydrates (WSC), neutral detergent fiber (NDF), acid detergent fiber (ADF), neutral detergent fiber digestibility (NDFD), maturity (MAT), and rust resistance (RR) for the orchardgrass population sources (DSV, FFR, and check) evaluated within each of the four locations and across the two production years. Numbers followed by different letters in the same column, and within the same location, differ statistically ( $p < 0.05$ ).

	WSC	HM	DDM	CP	NDF	ADF	NDFD	MAT	RR
Hof Steimke									
Checks	10.2 <sup>a</sup>	5.6 <sup>b</sup>	75.9 <sup>a</sup>	11.3 <sup>b</sup>	60.3 <sup>a</sup>	31.3 <sup>b</sup>	64.1 <sup>a,b</sup>	-	5.7 <sup>b</sup>
DSV	8.9 <sup>b</sup>	6.0 <sup>a</sup>	75.3 <sup>b</sup>	11.3 <sup>b</sup>	61.4 <sup>b</sup>	31.8 <sup>c</sup>	63.8 <sup>b</sup>	-	7.6 <sup>a</sup>
FRR	10.3 <sup>a</sup>	4.9 <sup>c</sup>	76.3 <sup>a</sup>	11.8 <sup>a</sup>	59.8 <sup>a</sup>	30.9 <sup>a</sup>	64.3 <sup>a</sup>	-	4.1 <sup>c</sup>
Les Rosiers									
Checks	8.9 <sup>a</sup>	3.3 <sup>b</sup>	74.9 <sup>a</sup>	14.4 <sup>b</sup>	57.9 <sup>a</sup>	29.2 <sup>b</sup>	59.6 <sup>a</sup>	3.0 <sup>b</sup>	5.8 <sup>b</sup>
DSV	8.4 <sup>b</sup>	4.0 <sup>a</sup>	74.2 <sup>b</sup>	13.4 <sup>c</sup>	59.2 <sup>b</sup>	30.3 <sup>c</sup>	59.7 <sup>a</sup>	2.1 <sup>a</sup>	7.9 <sup>a</sup>
FRR	8.8 <sup>a</sup>	2.8 <sup>c</sup>	74.9 <sup>a</sup>	15.3 <sup>a</sup>	57.6 <sup>a</sup>	28.8 <sup>a</sup>	59.5 <sup>a</sup>	4.3 <sup>b</sup>	4.3 <sup>c</sup>
Logan									
Checks	6.3 <sup>a</sup>	5.5 <sup>a</sup>	61.3 <sup>a</sup>	14.2 <sup>a</sup>	56.9 <sup>a</sup>	35.4 <sup>a</sup>	67.8 <sup>a</sup>	3.0 <sup>b</sup>	-
DSV	6.4 <sup>a</sup>	5.2 <sup>b</sup>	60.8 <sup>b</sup>	13.8 <sup>b</sup>	57.7 <sup>b</sup>	36.1 <sup>b</sup>	67.0 <sup>b</sup>	1.8 <sup>a</sup>	-
FRR	6.4 <sup>a</sup>	5.1 <sup>b</sup>	61.3 <sup>a</sup>	14.1 <sup>a</sup>	56.6 <sup>a</sup>	35.4 <sup>a</sup>	68.1 <sup>a</sup>	3.7 <sup>c</sup>	-
Rexburg									
Checks	6.8 <sup>a</sup>	6.6 <sup>b</sup>	62.0 <sup>b</sup>	15.4 <sup>b</sup>	55.6 <sup>b</sup>	34.6 <sup>b</sup>	73.9 <sup>b</sup>	3.1 <sup>b</sup>	-
DSV	6.8 <sup>a</sup>	7.0 <sup>a</sup>	61.6 <sup>c</sup>	15.2 <sup>b</sup>	56.5 <sup>c</sup>	35.1 <sup>c</sup>	72.5 <sup>c</sup>	2.1 <sup>a</sup>	-
FRR	6.7 <sup>a</sup>	6.5 <sup>b</sup>	62.5 <sup>a</sup>	16.0 <sup>a</sup>	54.4 <sup>a</sup>	34.0 <sup>a</sup>	75.1 <sup>a</sup>	3.8 <sup>c</sup>	-

### 3. Discussion

Previous evaluations of North American and European orchardgrass breeding programs [10,30] noted substantial differences between the two germplasm sources for HM and some forage quality traits. The current study focused on the characterization of the WSC concentration in the germplasm pools and the relationship between WSC and the other measured phenotypes. Additionally, this study focused on the GEI effects on orchardgrass from the DSV and FRR breeding programs when grown at sites in the EU and US. The DSV populations exhibited comparatively high HM and RR, but lower nutritive value, and late maturity. In contrast, the FRR populations exhibited higher nutritive value, including WSC, but lower HM and earlier maturity. Maturity, in particular, reflects the strong preference for much later material in the EU and the preference for seed production of early flowering cultivars in the US. Because rust infection was only rated in September at the EU locations, this study did not allow the further elucidation of this relationship by correlating the response at the individual harvests.

The success of breeding perennial ryegrass for increased WSC [31] has created a new objective for many forage breeding programs, including the DSV and FRR programs. Using near infrared spectroscopy approaches, WSC is easy to measure and characterize in targeted population improvement programs; however, its inheritance is strongly affected by location and GEI effects. High WSC perennial ryegrass cultivars may fail to express the phenotype in vastly differing environments, such as New Zealand [32] and the US [33]. The prevailing hypothesis for the GEI effect is that the



higher summer temperatures limit the photosynthesis of the  $C_3$  grasses and result in lower WSC concentrations in the forage [32]. This hypothesis is consistent with the results of the current analysis. While annual maximum temperatures did not differ substantially among the locations, the mean maximum temperatures and growing degree days during the growing season were higher at the US locations. Interestingly, the EU locations are at higher latitudes and receive more sunlight for increased photosynthesis during the growing season, such that the EU locations resulted in a higher WSC concentration while the FRR populations exhibited a higher WSC than the DSV populations at those EU locations. However, there was no difference between the DSV and FRR populations for WSC at the US locations. These results are consistent with the report of Sanada et al. [26], which also found that European orchardgrass sources generally exhibited a lower WSC than orchardgrass from other regions. Further studies of the genetics and GEI of WSC will be required to determine appropriate selection targets for increased WSC in different regions and to determine limits of WSC expression in orchardgrass germplasm.

The GEI effects detected herein were also complicated by the relative RR of the two germplasm sources included in this evaluation. The relationship between the WSC and disease is not well defined, but Sanada et al. [26] found a negative relationship between rust infection and WSC. Thus, our results for WSC contradict this finding. The potential for increasing the WSC concentrations under US conditions is unclear. However, breeding for this trait is still in its infancy and substantial genetic variation exists in orchardgrass [25]. Thus, increases in WSC are likely, but overall expression levels comparable to EU conditions may not be attainable.

The higher HM of the DSV populations compared to the FRR populations, at both US and EU locations, is seemingly inconsistent with the higher HM of US cultivars at US locations in previous work [10]. At EU locations this difference may partially be due to disease pressure, where the effect of rust on the FRR populations was high and limited their productivity compared to the DSV populations. At the US locations rust was not a factor and the HM differences were not as striking, although the DSV populations as a whole had higher DM. Thus, the strong overall increased HM of the DSV populations was driven by their substantially higher HM at the EU locations.

Forage quality phenotypes included CP, cell wall components, and digestibility. At each individual location, the FRR populations had higher CP and DDM than DSV populations, but differences for NDF, ADF, NDFD were less or non-significant. In general, relationships between these phenotypes and the WSC were consistent with those identified previously in orchardgrass [25], including the negative relationship between HM and cell wall components (ADF and NDF) and the positive relationship between HM and digestibility (DDM and NDFD). The non-significant correlation between CP and WSC in this study was unexpected. Crude protein and WSC usually exhibit a negative correlation because photosynthate diverted to the production of WSC cannot be used for CP, and vice versa [23]. The non-significance in this evaluation is intriguing.

The genotype by environment (location by population) interaction was a key characteristic of this evaluation. The genotype by environment interaction is the rule rather than the exception in forage grass breeding [25,33]. However, a main objective of this study was the characterization of this interaction to allow future use of the GEI to maximize breeding goals in orchardgrass. The AMMI analysis suggested that a consistent relationship occurred between the two EU locations, but that the two US locations did not exhibit consistent phenotypic performance. Previously, the Logan and Rexburg locations exhibited substantial GEI for orchardgrass phenotypes, including WSC and HM [25], such that there were no specific populations that would perform uniformly well for the measured phenotypes across those two locations. However, the consistent performance of the DSV and FRR populations for phenotypes suggests that combining these populations through recurrent selection can result in germplasm improved for WSC, HM, and RR. These populations could then be specifically selected for improved performance in the EU and US, respectively.

## 4. Materials and Methods

### 4.1. Experimental Locations

The locations included in this evaluation were Hof Steimke, Lower Saxony, Germany; Les Rosiers-sur-Loire, Pays de la Loire, France; Logan, UT, USA; and Rexburg, ID, USA. Location descriptions, including weather data during the study, are included in Table 2.

### 4.2. Orchardgrass Populations

Eighty-four orchardgrass populations were evaluated, including 35 DSV orchardgrass breeding populations, 40 FRR orchardgrass breeding populations, and nine check cultivars from the EU and US (Table S1).

### 4.3. Experimental Design

The experimental design at Hof Steimke and Les Rosiers was a randomized complete block design with two complete blocks. Due to seed limitations only two blocks were included at each location. The experimental design at Logan and Rexburg was a double alpha-lattice with two complete blocks and 20 incomplete blocks. Plots were seeded in 2 m<sup>2</sup> plot sizes, at a rate of 17 kg·ha<sup>-1</sup> during early fall 2013 at each location, but Hof Steimke, which was seeded in 2014. The EU locations employed no supplemental irrigation while the US locations applied supplemental irrigation (beginning after the first yearly harvest) to replace approximately 75% of the evapotranspiration rate each week. Following sowing and the establishment year, the fertilization schedule during the 2014 and 2015 production years were three split applications of 57 kg·N during the year.

### 4.4. Data Collection

At Logan and Rexburg there were three forage harvests in 2014 and four in 2015. At Les Rosiers there were four harvests in 2014 and five in 2015. At Hof Steimke no biomass or forage quality data was collected in 2014, but there were five harvests in 2015. Harvest intervals were roughly each five to six weeks at each location. Each spring, maturity ratings were made from 1–5, where a score of 1 corresponded to the earliest heading while a score of 5 corresponded to the latest heading. Following maturity ratings, each harvest removed all aboveground plant material to a stubble height of approximately 60 mm. Samples were collected from each plot to determine wet weights and for nutritive value analyses. The samples were then dried at 60 °C in forced air driers and re-weighed to determine moisture percentage. Thus plot values were adjusted to a dry matter basis to determine HM in kg·plot<sup>-1</sup>. Samples were then ground to pass a 1 mm screen for nutritive value analyses. The analyses used wet chemistry and near infrared spectroscopy according to established protocols [34] to determine values of WSC, DDM, CP, NDF, ADF, and NDFD. Nutritive values were not estimated from the first harvest to avoid the confounding effects of maturity differences. Additionally, nutritive value was only taken from two harvests from Les Rosiers in 2014. Herbage mass values were summed across the growing season, and the nutritive values were derived as a weighted average from each harvest based on the contribution of the corresponding harvest to the total yearly HM. Rust resistance was rated (1—highly susceptible to 9—highly resistant) only from the two EU locations during 2014 and 2015. There was no measurable rust at the US locations. Rust ratings did not attempt to distinguish between rust type, i.e., stem vs. leaf rust, but only provided an overall rating of plant health.

### 4.5. Data Analysis

The 'lme4' package [35] of the R software [36] was used to model the phenotypic data for the overall across years and locations and individual location models. The main effects of year, location, population, and their interactions were fixed effects. The main effects of block and incomplete block were random effects. The repeated effects of years were modeled using a compound symmetry



structure. The lattice designs at the US locations were resolved to randomized complete blocks for the overall model. Contrasts between the population sources were used to determine differences in phenotypic values among the DSV, FFR, and check populations. The MATMODEL 3.0 software [37] was then used to characterize the underlying location by population effects for each genotype using the additive main effects and multiplicative interaction (AMMI) modeling. The Kendall concordance statistic was used to characterize the relative ranking of populations for each phenotype among the locations. Simple correlations among cultivar means between different phenotypes in the same environment illustrated relationships among the phenotypes.

**Supplementary Materials:** Supplementary Materials: The following are available online at [www.mdpi.com/2073-4395/6/04/61/S1](http://www.mdpi.com/2073-4395/6/04/61/S1), Table S1: Overall across year (2014 and 2015) and location (Hof Steimke, Germany; Les Rosiers, France; Logan, UT, USA; and Rexburg, ID, USA) mean values for 84 orchardgrass populations corresponding to water soluble carbohydrates (WSC), dry matter yield (DMY), crude protein (CP), neutral detergent fiber (NDF), acid detergent fiber (ADF), neutral detergent fiber digestibility (NDFD), rust resistance (RUST), and maturity (MAT).

**Acknowledgments:** Funding for this research came from internal budgets of the USDA/ARS and Deutsche Saatveredelung AG.

**Author Contributions:** J.G.R. conceived the research, conducted the US field experiments, analyzed the data, and wrote the manuscript. B.S.B. interpreted research results and wrote the manuscript. U.F. conceived the research and conducted the EU field experiments. G.B. conducted the Rexburg, ID, USA field experiments.

**Conflicts of Interest:** The authors declare no conflict of interest.

## Abbreviations

ADF	acid detergent fiber
CP	crude protein
DSV	Deutsche Saatveredelung AG
DDM	digestible dry matter
EU	European Union
FFR	Forage and Range Research
GEI	genotype by environment interaction
HM	herbage mass
NDF	neutral detergent fiber
NDFD	neutral detergent fiber digestibility
RR	rust resistance
US	United States
WSC	water soluble carbohydrates

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