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RESEARCH ARTICLE

Effects of different timing and rate of glyphosate application on the residue level, grain quality, and processing performance of two Canadian malting barley varieties

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Abstract

Background and Objectives: A preharvest application of glyphosate on malting barley can assist in the management of perennial weed growth before harvest and serves as a harvest aid by drying down the crop. The main objective of this study was to assess the effects of preharvest glyphosate application at two glyphosate rates (900 or 1125 g ae/ha) and three application timings based on the maturity and seed moisture level (soft dough, hard dough, or mature).

Findings: The levels of glyphosate residue in barley grain were highly variable among locations and years ranging from 0 to 95 mg/kg. Glyphosate application at both rates at the soft dough stage significantly decreased the kernel weight of barley grain. Barley grain with up to 40 mg/kg of glyphosate residue exhibited adequate germination energy required for malting purposes; however, the residue negatively affected the growth of roots during the malting process. The levels of α-amylase in malt decreased with increasing levels of glyphosate residue in barley. Other malt and wort parameters were generally not affected by the glyphosate application on barley.

Conclusions: Too early application of glyphosate at the soft dough stage of barley grain development reduced the kernel weight and size, interfered with roots production, affected synthesis of α-amylase, and reduced the malt extract in several cases.

Significance and Novelty: Results indicated that in the majority of environments, when glyphosate was applied at the recommended stage and rate, neither the maximum residue limit was exceeded nor were the germination and malting quality of barley impaired. In real farming conditions, it might be hard to achieve similar results despite adherence to the recommended timing of glyphosate application because of the nonuniform level of crop maturity in the field and/or uncontrollable environmental effects.

KEYWORDS
glyphosate, malting barley, quality, residue level

1 | INTRODUCTION

Barley is a versatile crop that can be used in animal feed, human food, and in the production of malt and beer (Izydorczyk et al., 2017). Barley varieties for the production of malt for brewing are potentially the most profitable commodities for producers; however, to be selected for malting purposes, barley grain has to meet stringent quality requirements (Yin, 2021). Barley varieties specifically bred for malting and brewing have the genetic potential to produce grain with a high rate of germination, high diastatic enzyme activities, and an ample supply of fermentable sugars (Swanston et al., 2014). However, malting barley cultivars have to be grown under favorable environmental conditions to ensure the production of sound, plump, uniformly sized and bright colored grain with a specific range of grain protein concentration and germination capacity (Edney et al., 2012).

In spite of the fact that malting barley varieties account for over 50% of the barley seeded in western Canada, <25% has traditionally been deemed acceptable for malting annually due to one or more traits not meeting the quality specifications (Izydorczyk & McMillan, 2023). Improving quality and yield of barley harvested in Canada every year is, therefore, one of the priorities of the agri-food sector to increase and improve production, availability of selectable malting varieties, profitability, as well as Canada's competitiveness on the international markets. Although inclement climatic conditions can impair quality, suboptimal agronomic practices can also play a role (O'Donovan et al., 2011).

A preharvest application of glyphosate on cereal crops can assist in the management of perennial weed growth before harvest and serves as a harvest aid by drying down the crop (Darwent et al., 1994). Faster and uniform drying may mitigate the negative effects of secondary growth on seed uniformity, prevent problems with sprout damage and straw breakage, and lodging in malting barley. Glyphosate is registered for preharvest use on many crops; however, malting barley treated with glyphosate is not accepted by the Canadian maltsters (Brewing and Malting Barley Research Institute, 2017; Keep it Clean, 2022). The maltsters are reluctant to accept seed that has been treated with glyphosate due to possible negative effects on seed germination and quality and glyphosate levels in the grain that are commercially undesirable to consumers, even though they comply with established maximum residue limits (MRLs) that range from 10 to 30 mg/kg (Codex Alimentarius Commission, 2006; European Food Safety Authority, 2019; Health Canada, 2008). There have been no systematic studies up to date to assess the impact of preharvest glyphosate on malting barley quality over the variable soil and climatic conditions that can prevail in western Canada. Studies to date suggest that to prevent negative effects on cereal grains, preharvest glyphosate should not exceed the recommended rate 900 g acid equivalents (ae)/ha and should be applied at 30% moisture content or less, which is classified as the "hard dough" stage in barley (Baig et al., 2003; Cessna et al., 2002; McNaughton et al., 2015). The timing of application may be critical to avoid negative effects of the herbicide on seed germination and quality of the malt.

The objective of this study was to assess the effects of using a preharvest glyphosate application as a dry-down on the quality of grain and malting performance of barley. The studies were designed to investigate the effects of glyphosate on the quality of two Canadian malting barley varieties, AC Metcalfe and CDC Meredith, at three different timing applications (at the soft dough, hard dough, and maturity stage) and two rates of glyphosate application (900 and 1125 g ae/ha).

2 | MATERIALS AND METHODS

2.1 | Plant materials and treatments

A field experiment was conducted at five locations across western Canada over four growing seasons (2013–2016): three locations in Alberta (Beaverlodge, Lacombe, and Lethbridge) and two locations in Saskatchewan (Scott and Indian Head) (Table 1). The experimental design was a randomized complete block design with four replicates of twelve treatments and two nontreated controls. The treatments were a factorial combination of timing of glyphosate application based on seed moisture level (soft dough, hard dough, and maturity), at two application rates (900 and 1125 g ae/ha), on an early maturing (AC Metcalfe) and late maturing (CDC Meredith) malting barley cultivar (Table 2). The Zadoks scale (Zadoks et al., 1974) was used to assess seed moisture level (85 Zadoks-soft dough, 87 Zadoks-hard

TABLE 1 List of locations and number of years used for cultivating two malting barley cultivars (AC Metcalfe and CDC Meredith) and collecting data for investigating the effects of glyphosate on the quality of barley and malt.

Location	Province	Soil classification	Number of years for collecting barley quality data	Number of years for collecting malt quality data
Beaverlodge	Alberta	Grey solonetz	3	2
Lacombe	Alberta	Black chernozem	4	3
Lethbridge	Alberta	Brown chernozem	4	2
Indian Head	Saskatchewan	Black chernozem	4	1
Scott	Saskatchewan	Dark brown chernozem	4	1

TABLE 2 List of treatments (rate and timing of glyphosate application) on two different malting varieties used in this study.

Treatment	Barley variety	Glyphosate rate (g ae/ha)	Glyphosate application timing
1	AC Metcalfe	0	N/a control
2		900	Soft dough
3		900	Hard dough
4		900	Mature
5		1125	Soft dough
6		1125	Hard dough
7		1125	Nature
8	CDC Meredith	0	N/a control
9		900	Soft dough
10		900	Hard dough
11		900	Mature
12		1125	Soft dough
13		1125	Hard dough
14		1125	Mature

Abbreviations: ae, acid equivalents; N/a, not applicable.

dough, 91 Zadoks-mature) and rates of application were determined by using the recommended rate (900 g ae/ha) and a rate 25% higher (1125 g ae/ha). The RoundUp WeatherMax® (Bayer Crop Science EPA Registration No. 524-537) formulation of glyphosate was used at all sites for the preharvest treatment.

The experiment was conducted under no-till or reduced till environments. Barley was seeded at 300 seeds/m^2 and fertilizer applied at seeding. Fertilizer application was based on soil test recommendations for each location for malting barley, taking into account soil nutrient levels at each site and regionally specific yield goals. Blanket fungicide applications were made at flag leaf emergence to reduce the potential confounding effect of disease development. Plots were harvested at maturity using plot combine equipment at each location.

2.2 | Barley grain analyses

Over the course of the study, barley samples were collected from 19 environments (combination of year and location) (Table 1). Glyphosate residue on barley grain was measured on four replicates from each environment using a quantitative enzyme-linked immunosorbent assay test kit (Abraxis) (Tittlemier et al., 2017).

Barley quality assessment was performed on three replicates from each of the 19 environments (Table 1). Barley was tested for protein content (ASBC Barley-7D), 1000 kernel weight (ASBC Barley-2D), germinative energy, and water sensitivity (ASBC Barley-3C) according to the American Society of Brewing Chemists (ASBC) Methods of Analysis (2004). Germination index (GI) was calculated from germinative energy results according to Riis and Bang-Olsen (1991).

2.3 | Micromalting and malt analyses

Each year, the selection of locations from which barley samples were going to be malted was based on barley quality assessments (grain protein and germination). Barley samples from two locations in each of 2013, 2014, 2015 years and from three locations in 2016, for a total of nine environments (location/year combination), were selected for malting (Table 1). Barley samples from environments selected for malting were also tested for kernel plumpness following ASBC Barley-2C (ASBC Methods of Analysis, 2004). Plump barley (>2.38 mm slotted sieve) was malted in a Phoenix automated micromalting machine. Steeping was conducted at 13°C and consisted of two wet cycles intermitted by two dry cycles for a total of 44 h. Steep-out moisture was calculated from the difference in weight between dry-matter barley and steeped barley. To be in proximity to a target steep-out moisture of 46%, the first wet cycle was 8 h in length for all environments, but adjustments to the length of the second wet cycle were required due to differences in barley quality. The grain was germinated at 15°C for 96 h followed by kilning (12 h at 60–65°C, 6 h at 65°C, 2 h at 75°C, 5 h at 83–85°C).

Malt analysis was performed according to the procedures outlined in the ASBC Methods of Analysis (2004). Malt samples were analyzed for moisture content (ASBC Malt-3) and malt modification by friability with a Pfeuffer friabilimeter (ASBC Malt-12). Malt protein content was measured by nitrogen combustion with a LECO protein analyzer (ASBC Malt-8B). Diastatic power of malt was analyzed by segmented flow analysis (Skalar) (ASBC Malt-6C) using an automated neocuproine assay for reducing sugars, which was calibrated with malt standards analyzed by ASBC method Malt-6A. Malt extract (fine grind) was measured using ASBC method Malt-4. Wort viscosity was determined with an Anton Paar Lovis ME rolling-ball viscometer (ASBC Wort-13B). Wort-free amino nitrogen concentration was determined by segmented flow analysis (ASBC Wort-12B). The content of β-glucan in wort was determined by measuring increased fluorescence from calcofluor binding with β-glucan polymers by segmented flow analysis (Skalar) (ASBC Wort-18B). Protein in unhopped wort (soluble protein) was determined by spectrophotometry based on the differing UV absorption of protein at 215 and 225 nm (ASBC Wort-17). Wort color was determined spectrophotometrically (ASBC Wort-9, Beer-10).

2.4 | Statistical analyses

Statistical analysis and data manipulation were done using R version 4.2.2 (R Core Team, 2022) and the following packages: car (Fox & Weisberg, 2019), MASS (Venables & Ripley, 2002), lme4 (Bates et al., 2015), and emmeans (Russel, 2022). Normality was assessed using Shapiro–Wilk's test (Shapiro & Wilk, 1965) and *Q*–*Q* plots. Linear mixed models were used to assess quality factors between treatments (Pinheiro & Bates, 2005). The dependent factor was the quality factor and the independent factors were treatment and environment, modeled as interaction effects, which were all treated as fixed effects. Replicates were treated as random effects. Akaike Information Criterion was used to compare models and to assess the best fit model (Akaike, 1974). A one-way analysis of variance and posthoc Tukey's honest significance difference test were used to compare quality factors between the different treatments.

3 | RESULTS AND DISCUSSION

3.1 | Effects of timing and rate of glyphosate application on the residue level and barley quality

Detectable and quantifiable levels of glyphosate residue were found in the grains of AC Metcalfe and CDC Meredith treated with glyphosate at both application levels and at all three application times. Overall, when the results were combined from all 19 environments investigated in this study, the mean residue level reached the Canadian MRL of 10 mg/kg (Health Canada, 2008) only when glyphosate was applied at the soft dough stage at the higher rate of 1125 g ae/ha (Figure 1). This was observed for both varieties. The mean glyphosate residue level was below the MRL when glyphosate was applied at the recommended rate and stage. However, even when glyphosate was applied at the recommended level (900 g ae/ha) and time (hard dough), there were occurrences of residue levels exceeding the MRL, as shown in Figure 1. The levels of glyphosate residue remaining in the grain samples were highly variable among locations and years (environments) ranging from as low as 0 to 2.3 mg/kg in one environment to as high as 3.1 to 95 mg/kg in another. These results indicate that controlling the timing and/or rate of glyphosate application was challenging even under the small-plot experimental conditions. This is likely due to nonuniform crop maturity level in the plots as well as variable and uncontrollable atmospheric events including, wind, rain, and so forth in various environments.

The environment significantly affected all barley quality characteristics tested in this study, specifically kernel plumpness and kernel weight, protein content, germination energy (GE), and GI (Table 3). The application of glyphosate had a significant effect only on the kernel weight (Table 3). Kernel weight and plumpness are important quality parameters for malting barley. Plump kernels with a high kernel weight are associated with a high starch content in the grain and potentially high yield of extract available for brewers. Kernel weight was highly variable and dependent on the agronomic and environmental conditions. This trait is also predetermined by genetic factors, with CDC Meredith characterized by a higher kernel weight compared to AC Metcalfe (Figure 2). Glyphosate application at both rates (900 and 1125 g ae/ha) at the soft dough stage significantly decreased the kernel weight of AC Metcalfe and CDC Meredith, but no significant reduction of

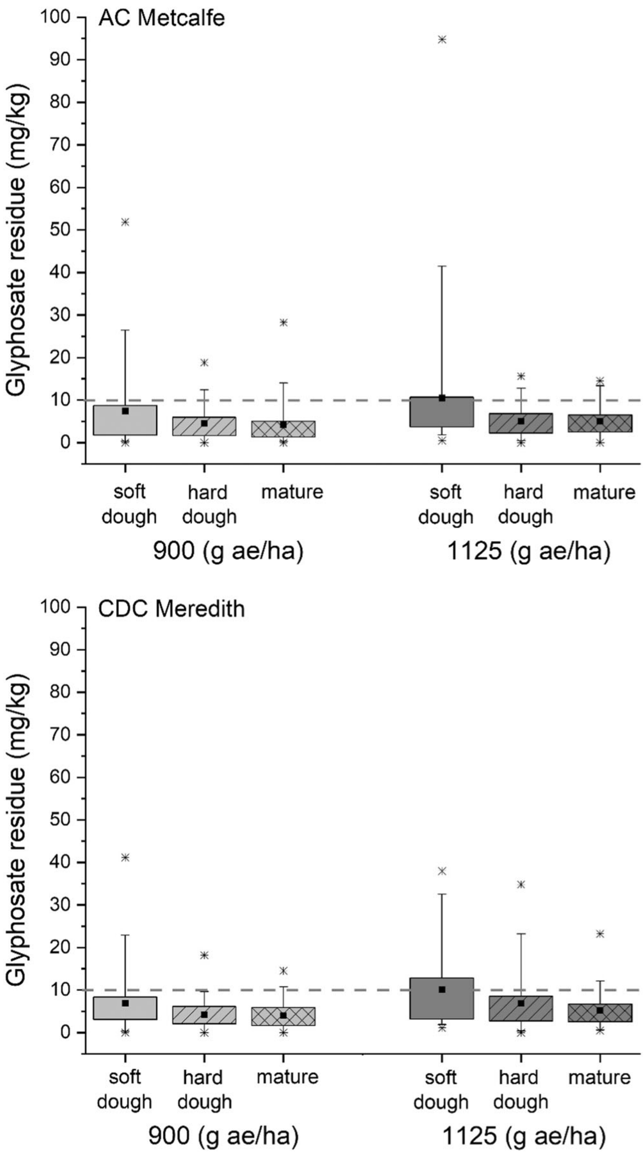

FIGURE 1 Box plots comparing the levels of glyphosate residues detected in grain treated with glyphosate at various rates (900 and 1125 g acid equivalents [ae]/ha) and timing of applications (soft dough, hard dough, mature kernel). Each box plot indicates the mean (■), 25th–75th percentile range (box), the max and min levels (x) of glyphosate residues in grain collected from 19 environments (location/year combinations).

kernel weight occurred when glyphosate was applied at the recommended stage or later. On average, the kernel weight reduction was about 2% for AC Metcalfe and 2.8% for CDC Meredith when glyphosate was applied at the soft dough stage. However, the scale of kernel weight reduction varied among different environments from no effects to the highest reduction of kernel weight reaching about 10%. Similar observations were recently reported for glyphosate-treated oats (Vegi et al., 2021). It is recognized that the application of glyphosate leads to an inability to downregulate the shikimate acid pathway, which results in an increased demand for carbon in that pathway. This leads to less carbon available for grain filling and consequently to smaller kernels, especially when glyphosate is applied too early (Zobiole et al., 2011).

Our study showed that in a few environments, the application of glyphosate resulted in a reduction of grain plumpness by 2%–3%, but in most environments, the differences were smaller and overall not statistically significant (Table 3). The application of glyphosate at any rate or timing had no effect on the concentration of proteins in the grain (Table 3).

Germination potential is the most important quality requirement for malting barley. Germination allows the embryo to grow under controlled conditions, allowing the seed to develop enzymes, which are essential both in modifying the grains and in further processing of malt in the brewery. Within the malting industry, the ability of barley to germinate is measured using standard tests called the GE and the GI. GE is determined by counting a number of germinated kernels out of 100 kernels that are placed on two pieces of filter paper in a Petri dish, followed by addition of 4 mL of water (ASBC Methods of Analysis, 2004). Kernels are considered germinated upon rupturing of the covering layer by the root and elongation of the acrospire. Barley sample with acceptable malting quality should have at least 95 out of 100 kernels germinated after 72 h. The GI is a measure of the percentage and speed of germination (Riis & Bang-Olsen, 1991). Overall, glyphosate application had no significant effect on the GI, and glyphosate-treated barley exhibited adequate GE when assessed by this standard test (Table 4). A few occurrences of substantially low germination were associated with particularly high levels of glyphosate residue detected on the grain as shown for two environments (Indian Head and Lacombe in 2013) (Figure 3). It should be noted, however, that these high levels of glyphosate residues were rare and observed only when glyphosate was applied at the soft dough stage (not a registered application timing) and at the higher than the recommended level.

3.2 | Effects of timing and rate of glyphosate application on the malting performance

The quality of barley was strongly affected by environmental conditions and, therefore, based on an adequate protein level, GE and kernel size of the control samples, grain samples from only nine out of 19 environments were selected for malting and malt

TABLE 3 The *p* values from the ANOVA for the effects (fixed) of environment (E:location/year combination), glyphosate (G), and interactions of E × G on selected barley quality parameters.

	AC Metcalfe *p*			CDC Meredith *p*		
Dependent variable	**Environment (E)**	**Glyphosate (G)**	**E × G**	**Environment (E)**	**Glyphosate (G)**	**E × G**
Plumpness (%)	**<.0001**	.734	.862	**<.0001**	.249	.705
1000-kernel weight (g)	**<.0001**	**<.0001**	**<.0001**	**<.0001**	**<.0001**	**<.0001**
Barley protein (%)	**<.0001**	.889	1.000	**<.0001**	.450	.720
Germination (4 mL, %)	**<.0001**	.064	.367	**<.0001**	.337	.367
GI	**<.0001**	.367	.361	**<.0001**	.035	.983

Note: Replicates within each environment were considered random. Significant effects ($p < .05$) are in bold.

Abbreviations: ANOVA, analysis of variance; E, environment; G, glyphosate; GI, Germination Index.

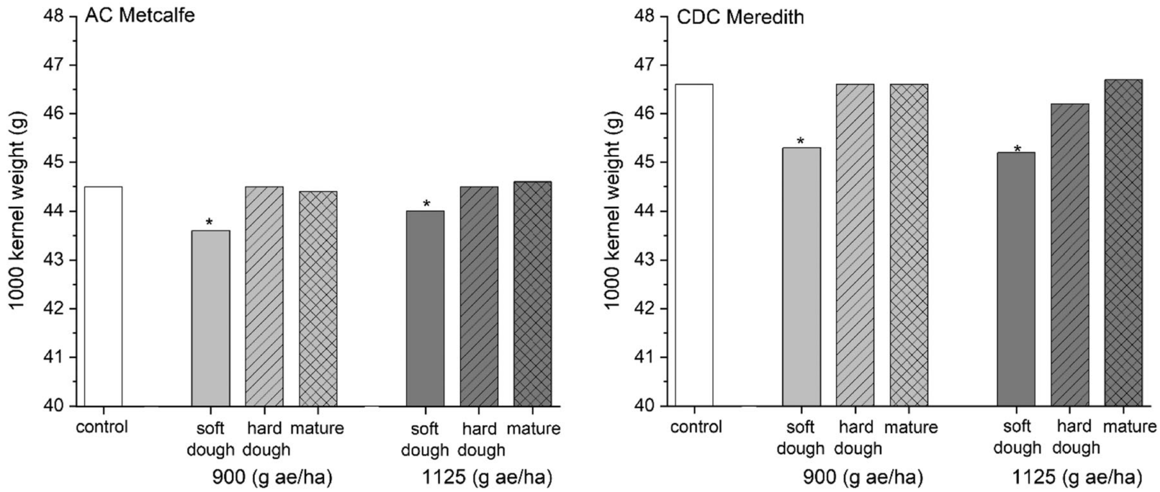

FIGURE 2 Effect of glyphosate application at different rates and times on the kernel weight of AC Metcalfe and CDC Meredith barley. The bars show average kernel weight values for barley grown in 19 environments (locations/years combinations). Asterisks (*) indicate significantly different average values for the treatments compared to the control (untreated grain) as determined by the posthoc Tukey's honest significance difference test. ae, acid equivalents.

quality evaluation. The malting schedules, especially the steeping step, for barley samples from different environments had to be slightly adjusted to take into account different kernel size and/or different protein content. However, within each environment, all glyphosate-treated barley samples were processed under the same conditions of steeping, germination, and kilning as the control, untreated barley.

Water absorption during steeping was not affected by the glyphosate application at any rate and time as shown by no differences in the moisture content of the grain at the end of the steeping stage for the glyphosate-treated and control samples for both varieties (Table 5). The average amount of roots collected after malting ranged from 3.2% to 3.9% for AC Metcalfe and 3.4% to 4.3% for CDC Meredith (Table 5). Significantly lower amounts of roots were collected from malt generated from barley treated with glyphosate at the soft dough at both application rates compared to malt generated from the control, untreated barley (Table 5). This was observed for both barley varieties. The amount of roots was inversely related to the level of glyphosate residue remaining on the grain. However, as shown in Table 5 and Figure 4, the amount of roots generated during malting of barley treated with glyphosate at the recommended stage of grain development was not different from the untreated barley. As stated above, the application of glyphosate had no significant effect on the germination potential of barley as required for malting purposes. However, when glyphosate was applied too early or at the higher than recommended rate, it undeniably affected the germination processes and hindered the production of roots.

TABLE 4 Effect of glyphosate rate and time of application on germination properties of AC Metcafe and CDC Meredith barley.

Barley variety	Glyphosate rate (g ae/ha)	Glyphosate application timing	GE[a] 4 mL (%)	GI[a]
AC Metcalfe	0	Control	98	7.50
	900	Soft dough	**98**	7.60
	900	Hard dough	98	7.65
	900	Mature	98	7.63
	1125	Soft dough	**97**	7.67
	1125	Hard dough	98	7.79
	1125	Mature	98	7.60
CDC Meredith	0	Control	98	7.51
	900	Soft dough	**98**	7.75
	900	Hard dough	98	7.77
	900	Mature	98	7.95
	1125	Soft dough	**97**	7.72
	1125	Hard dough	98	7.66
	1125	Mature	98	7.63

Abbreviations: ae, acid equivalents; GE, germination energy; GI, Germination Index.

[a]Average values for barley grown in 19 environments (locations/years combinations).

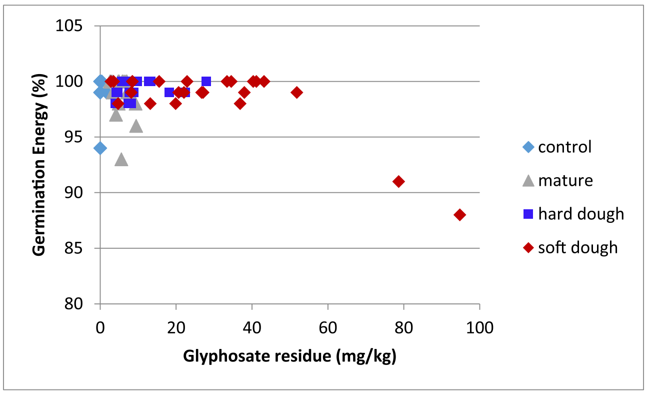

FIGURE 3 Effect of glyphosate residue remaining in barley grain after application of glyphosate at different maturity stages (soft dough, hard dough, and mature) in Indian Head and Lacombe in 2013 on the germination energy of barley. [Color figure can be viewed at wileyonlinelibrary.com]

The glyphosate application on barley had no effect on the concentration of malt proteins nor on the activity of diastatic enzymes (represented mostly by β-amylase) that are responsible for the capacity of the malt to convert starch into fermentable sugars (Table 5). In contrast, α-amylase activity, a measure of the dextrinizing capacity of the malt, was affected by the application of glyphosate on barley, although the outcomes were variable among environments. In three out of the nine environments investigated in this study, the level of α-amylase in AC Metcalfe malt was significantly reduced when glyphosate was applied at the soft dough stage at both application levels; this reduction ranged from 3% to 20%. No significant reduction of malt α-amylase was observed in the remaining six environments. Overall, when averaged across all nine environments, the reduction of α-amylase in malt produced from the glyphosate-treated barley was small but statistically significant when glyphosate was applied at the soft dough stage at the higher application level (1125 g ae/ha) (Figure 5). Compared to AC Metcalfe, CDC Meredith is a variety, which produces a lower level of α-amylase during malting as shown in Figure 5. The application of glyphosate on CDC Meredith had similar effect on the level of malt α-amylase as observed for AC Metcalfe. In three out of the nine environments investigated in this study, the level of α-amylase in AC Metcalfe malt was significantly reduced when glyphosate was applied at the soft dough stage at both application levels. The differences between the two malt enzymes (β-amylase and α-amylase) in their responses to glyphosate are not surprising. β-Amylase is an enzyme that is already present in the mature barley grain at a relatively high concentration and often related to the concentration of proteins in the grain (Izydorczyk & Edney, 2003; Yin, 2021). α-Amylase, in contrast, is an enzyme that is entirely synthesized during the germination process. Any interference with the germination processes might affect the level of this enzyme in the malt. Therefore, a too early application of glyphosate that hinders the production of roots may well be expected to also affect the level of α-amylase in the corresponding malt samples.

Malt extract is another important quality parameter used to predict the amount of beer or spirits that can be produced from malt. A high malt extract is desirable, and many grain and malt parameters, including high kernel weight, plumpness, starch content, and adequate grain modification during malting, and high concentrations of hydrolytic enzymes contribute to a high malt extract value. When averaged across all environments, the extract values of malt produced from AC Metcalfe treated with glyphosate at the soft dough stage and both application rates were lower by only 0.3% and not statistically different from the untreated control (Figure 6). However, the scale of extract reduction varied among different environments from no reduction of extract in some environments to the highest reduction of extract reaching 0.7% for malt produced from AC Metcalfe treated with glyphosate at the soft dough stage at 900 g ae/ha. For malt produced from AC Metcalfe treated with glyphosate at the soft dough stage at 1125 g ae/ha, the extract reduction ranged from 0% to 1.2% for the nine environments

TABLE 5 Effect of rate and time of application of glyphosate on processing parameters and properties of malt prepared from two barley varieties, AC Metcalfe and CDC Meredith.[a]

Barley variety	Glyphosate rate (g ae/ha)	Glyphosate application timing	Moisture after steeping (%)	Roots (%)	Malt proteins (%, db)	Malt diastatic power (°, db)
AC Metcalfe	0	Control	45.3	3.9	11.8	182
	900	Soft dough	45.5	**3.5**[b]	12.2	184
	900	Hard dough	45.3	3.9	12.1	189
	900	Mature	45.5	3.8	12.0	185
	1125	Soft dough	45.3	**3.2**[b]	12.2	186
	1125	Hard dough	45.0	3.8	12.0	181
	1125	Mature	45.4	3.9	12.0	189
CDC Meredith	0	Control	45.8	4.3	11.1	172
	900	Soft dough	46.0	**3.7**[b]	11.1	169
	900	Hard dough	46.1	4.1	11.0	167
	900	Mature	46.0	4.2	11.0	167
	1125	Soft dough	46.1	**3.4**[b]	11.1	169
	1125	Hard dough	46.1	4.2	11.3	171
	1125	Mature	46.0	4.3	11.1	171

Abbreviations: ae, acid equivalents; db, dry basis.

[a]Average values for malt prepared from barley grown in nine environments (locations/years combinations).

[b]Significantly different average values ($p < .05$) for the treatments compared to the control (untreated grain) as determined by the posthoc Tukey's honest significance difference test.

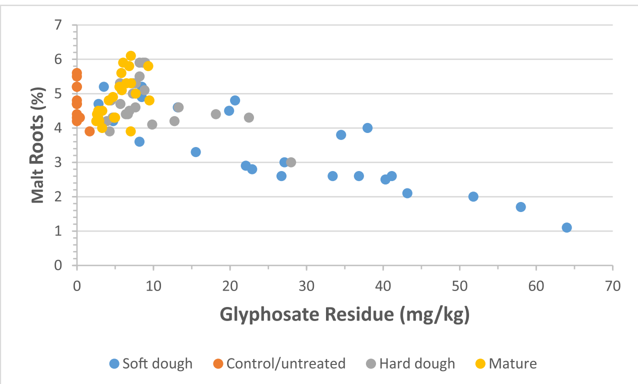

FIGURE 4 Effect of glyphosate residue remaining in barley grain after application of glyphosate at different maturity stages (soft dough, hard dough, and mature) in Indian Head and Lacombe in 2013 on the percentage of roots after malting. [Color figure can be viewed at wileyonlinelibrary.com]

investigated in this study. When glyphosate was applied at the recommended level and time, no negative effects on the malt extract were observed in the majority of environments. The malt extract values for CDC Meredith were higher than those for AC Metcalfe (Figure 6). These results were expected and are linked to the higher extract potential of CDC Meredith due to its relatively higher kernel weight compared to AC Metcalfe. The application of glyphosate on CDC Meredith had similar effects on the level of malt extract as observed for AC Metcalfe.

The concentration of β-glucans in wort and wort viscosity are quality parameters, which can predict the likelihood of processing issues or problems with the final quality of beer. Low levels of wort β-glucans and low wort viscosity are desirable to avoid problems associated with slow wort and beer filtration, development of beer haze, and formation of gels and precipitates in beer (Bamforth & Martin, 1981; Wang et al., 2004). Interestingly, our studies indicated that the concentration of β-glucans was significantly lower in wort produced from the glyphosate-treated barley compared to untreated barley. The lowest concentration of β-glucans in wort was observed for barley treated with glyphosate at the soft dough stage (Table 6); this trend was seen in both varieties. A plausible explanation for a lower concentration of β-glucans in worts prepared from glyphosate-treated barley is a lower content of these polysaccharides in the glyphosate-treated barley grain. Indeed, the content of β-glucans in AC Metcalfe treated with glyphosate at the soft dough stage at 900 and 1125 g ae/ha was 3.79% and 3.72%, respectively, and significantly lower than in

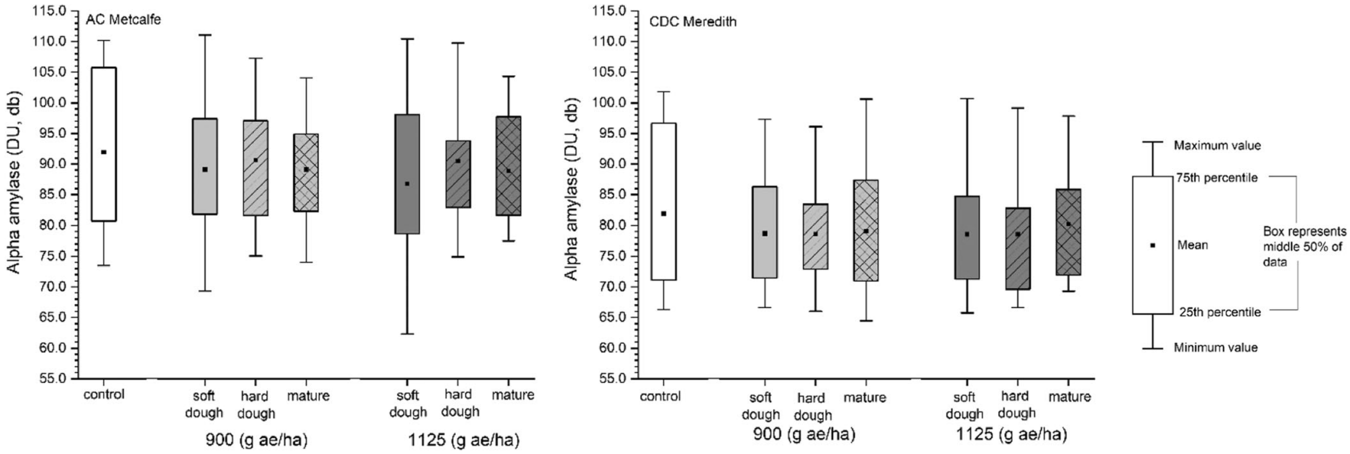

FIGURE 5 Effect of glyphosate application at different rates and times on the activity of α-amylase in malt prepared from AC Metcalfe and CDC Meredith barley. The box plots show α-amylase activity values for malt generated from barley grown in nine environments (locations/years combinations). Asterisks (*) indicate significantly different mean values for the treatments compared to the control (untreated grain) as determined by the posthoc Tukey's honest significance difference test. ae, acid equivalents. [Color figure can be viewed at wileyonlinelibrary.com]

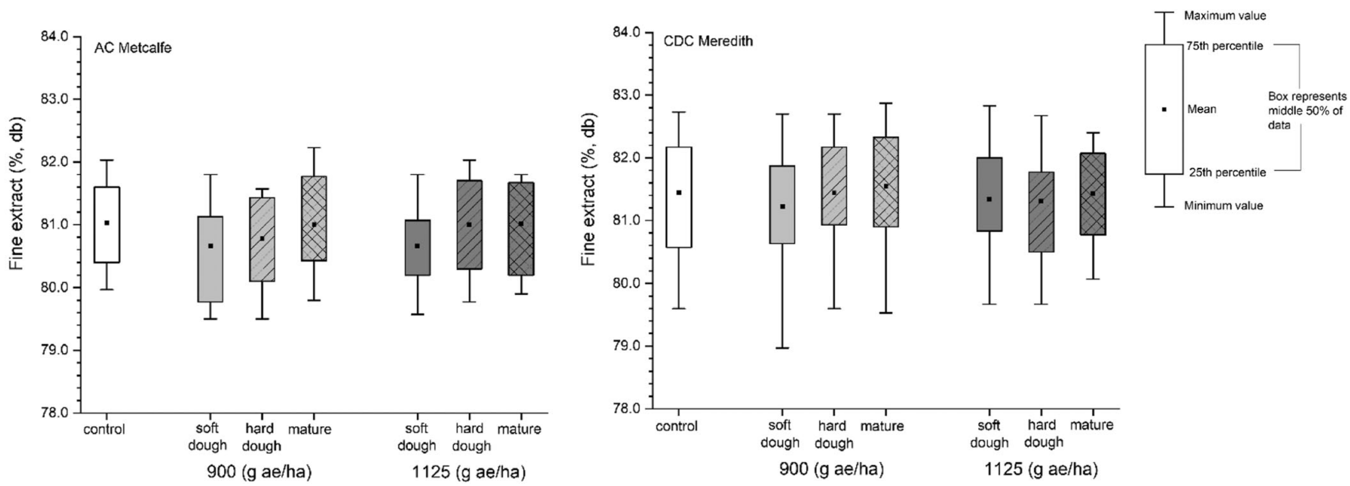

FIGURE 6 Effect of glyphosate application at different rates and times on the level of extract from malt prepared from AC Metcalfe and CDC Meredith barley. The box plots show levels of extract from malt generated from barley grown in nine environments (locations/years combinations). ae, acid equivalents.

the untreated grain (4.2%). This effect of glyphosate application, especially at the early stage, on the content of β-glucans in barley grain could be related to the previously mentioned negative consequence of glyphosate on the availability of carbon for grain filling that leads to smaller kernels and lower content of the endosperm tissue in the grain. Recently, Alahmed et al. (2020) also reported a significantly lower content of β-glucan for oats treated at soft and hard dough stages (4.35% and 4.35% β-glucans, respectively) compared with untreated oat groats (4.65% β-glucans). The lower concentration of β-glucans in wort did not result in significantly lower wort viscosity (Table 6). This is likely because the wort viscosity, although primarily affected by the concentration of β-glucans, can also be influenced by the molecular weight of these polysaccharides, as well as the concentration and molecular weight of arabinoxylans (the other major polysaccharides present in the cell walls of barley grain) (Shaluk et al., 2019; Yin, 2021).

Lastly, the application of glyphosate had no effect on the proteolytic modification of barley during malting, resulting in no differences in the levels of soluble proteins and free amino nitrogen compounds in the malt produced from glyphosate-treated and untreated barley (Table 6). The color of wort was also not affected by glyphosate application on barley (Table 6).

TABLE 6 Effect of rate and time of application of glyphosate on selective properties of wort generated from malt prepared from two barley varieties, AC Metcafe and CDC Meredith.[a]

Barley variety	Glyphosate rate (g ae/ha)	Glyphosate application timing	Wort β-glucans (mg/L)	Wort viscosity (cP)	Wort soluble proteins (%, db)	Wort FAN (mg/L)	Wort color (°SRM)
AC Metcalfe	0	Control	146	1.46	4.91	231	2.17
	900	Soft dough	**114**[b]	1.46	4.86	228	2.16
	900	Hard dough	128	1.46	4.93	232	2.13
	900	Mature	130	1.45	4.98	237	2.19
	1125	Soft dough	**96**[b]	1.46	4.71	222	2.13
	1125	Hard dough	150	1.47	4.82	229	2.10
	1125	Mature	133	1.46	4.93	237	2.18
CDC Meredith	0	Control	148	1.47	4.87	226	2.43
	900	Soft dough	**114**[b]	1.45	4.60	221	2.31
	900	Hard dough	141	1.46	4.77	229	2.51
	900	Mature	136	1.46	4.73	228	2.41
	1125	Soft dough	**102**[b]	1.45	4.61	216	2.23
	1125	Hard dough	149	1.46	4.78	231	2.39
	1125	Mature	135	1.45	4.77	231	2.36

Abbreviations: ae, acid equivalents; FAN, free amino nitrogen; SRM, standard reference method (American Society of Brewing Chemists).

[a]Average values for wort prepared from barley grown in nine environments (locations/years combinations).

[b]Significantly different average values ($p < .05$) for the treatments compared to the control (untreated grain) as determined by the posthoc Tukey's honest significance difference test.

4 | CONCLUSIONS

The results of this study clearly showed that the recommended timing (hard dough stage) and rate (900 g ae/ha) of glyphosate application on barley are critical to avoid excessive glyphosate residue levels and negative effects on the malting quality of barley. Too early application of glyphosate at the soft dough stage of barley grain development reduced the kernel weight and size, interfered with the germination processes as demonstrated by impediment of roots production, affected synthesis of α-amylase, and reduced the malt extract in several cases.

In the majority of environments, when glyphosate was applied at the recommended stage and rate, neither the MRL was exceeded nor were the germination and malting quality of barley impaired. However, even in the small research plots used in this study and when glyphosate was applied correctly, a few occurrences of quality losses were observed, demonstrating a potential risk of such preharvest practice. This was likely linked to moisture contents outside of the target range at application timing, the presence of immature tillers, or environmental conditions requiring earlier than ideal application timings.

In real farming conditions, it might be hard to achieve similar results despite adherence to the recommended timing of glyphosate application because of the nonuniform level of crop maturity in the field. In addition, many uncontrollable environmental events (wind, heat, rain) may affect the uniform and accurate rate of glyphosate application and consequently the level of glyphosate residue on the grain. This increases the risk of negative quality and residue effects of preharvest glyphosate applications. Overall, this work supports the position of the Canadian maltsters that preharvest applications of glyphosate risk the quality and residue status of malting barley and should not be a recommended practice.

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