

On-going Investigation of Winter Hardiness to Advance Winter Malting Barley as a Climate Adaptation Strategy in Michigan (GG 22*1560, MCBC22-07)

2022-23 Final Report

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Abstract

In 2022-23, our team received support from Michigan Craft Beverage Council to continue investigating winter hardiness in malting barley as a climate adaptation strategy. We conducted a hybrid field and laboratory study to assess the interaction of winter barley genetics with environmental conditions across Michigan and their combined effect on winter barley hardiness. Results from our second year of research point to promising winter hardy varieties and the important role that early planting, fall hardening and insulating snow cover play in protecting the crop. Further research is needed to fully assess the physiological mechanisms driving species/variety differences in winter hardiness.

Introduction

Of the crops currently produced in Michigan as craft beverage ingredients, spring malting barley may be especially vulnerable under projected climatic changes (Niero et al., 2015). The Michigan malting barley industry has identified winter barley production as a promising alternative to spring barley, which may be better adapted to the agroecology of our state and unique opportunities/risks that climate change presents. Yet, winter barley remains susceptible to winter injury, suggesting that ongoing efforts to identify resilient winter barley varieties and environmental thresholds for winter survival should be prioritized.

Winterkill is a general term that encompasses multiple challenges for overwintering plants including insufficient fall hardening, low temperatures, ice encasement, heaving, desiccation, spring freeze-thaw episodes, and more. While low temperature or freezing stress is often front and center, many other stresses occur during Michigan fall, winter, and early spring months that may be more important in the context of climate change. Winter barley breeding programs in Virginia, Ohio and Minnesota primarily focus on cold or freezing stress and lack the ability to screen large breeding populations for other unique and important winterkill stresses. Therefore, breeders need results from physiologists to better target their breeding practices for broader winter resilience.

In 2022-23, our team received support from the Michigan Craft Beverage Council to continue investigating winter hardiness in malting barley to advance winter barley as a climate adaptation strategy. We proposed three primary objectives for the project focused on 1) an applied field study assessing the interaction of winter barley genetics with environmental conditions across locations in Michigan and their combined effect on winter barley hardiness; 2) sampling of barley tissue from the field study and laboratory analysis to measure traits associated with winter barley hardiness; and 3) development of models to predict winter barley hardiness and distribution of this new information

through extension outreach. We report significant progress on each of our stated objectives below.

Objective 1: Compare winter injury and survival of 7-10 elite winter barley cultivars and two rye check cultivars under variable temperature, precipitation and snow cover conditions at five locations across Michigan representing USDA hardiness zones 4b – 6b.

Hypothesis: With a killing temperature of -10 degrees C, winter barley will experience significant cold injury most years in zones 4b – 5b. Site-year variation in temperature, precipitation, humidity and light (sun and snow cover) will correlate with observed barley injury and survival.

Five research sites were established in the fall of 2022 at Empire (6b), KBS (6a), East Lansing (6a), UPREC (5a) and Rudyard (4b), MI. Unfortunately, loss of our 2020-21 collaborator at Stockbridge and recent updates to the USDA Hardiness Zones map meant that we had two sites in Zone 6a and no site in the critical transitional Zone 5b (-15 to -10 F). Each location included thirty-seven winter barley varieties and one hardy cereal rye check planted in a randomized complete block design with three replications. Barley varieties were sourced from the Winter Malting Barley Variety Trial (WMBT) coordinated by University of Minnesota. Sensors were installed at planting to monitor temperature at the soil surface and snow cover (light and cameras) throughout the winter (Table 1). Stand counts were taken in fall and again after spring green-up to measure stand loss over the winter, except at KBS where survival was good but unfortunately not quantified.

Location (Hardiness Zone)	Lat.	Plant Date	Avg. Fall Pop. (1 Ft ²)	Fall GDDs (40 F)	Hardening Period (days 40-32 F)	Min Crop Temp (F)	Hours Below 14F	Days With Snow	Max Snow Depth (In)
Empire (6b)	44.81	9/29	21.48	397.80	3	11.99	14	96	7
KBS (6a)	42.41	10/3	28.09	459.60	29	8.78	25	54	8
Lansing (6a)	42.69	10/20	29.89	251.61	5	13.56	1	53	5
UPREC (5a)	46.35	9/14	25.88	505.75	16	11.04	30	148	30
Rudyard (4b)	46.26	9/15	19.55	527.52	1	18.21	0	148	27

Table 1: Environmental and crop conditions at the five research sites in 2022-23

This work has provided additional evidence for variety differences and environmental factors driving winter barley hardiness. In general, 2022-23 winter survival was excellent at one location (KBS assumed near 100%), very poor at two locations (UPREC 1% and Rudyard 5%) and variable at two locations (Lansing 94% and Empire 76%). Stand loss at UPREC was likely caused by three nights of cold temperatures below the injury threshold for barley (-10 C or 14 F) in early December before sufficient snow cover had accumulated. Stand loss at Rudyard was likely caused by standing water in fall and spring due to rapid snowmelt and poorly drained soil (Figure 1). Where survival was most variable at Lansing and Empire, there were numerical differences across barley varieties, but these differences were not statistically significant. However, combining our two years of winter hardiness results has revealed moderately significant differences across commercial winter barley varieties (Figure 2, Kruskal-Wallis chi-squared = 26.424, df = 19, p = 0.1188). Barley varieties from LCS, KWS, Germany,

Idaho, and the UK generally out-performed varieties from France or Virginia, with the exception of Thoroughbred. However, these results are somewhat confounded by the fact that not all varieties were grown in both years.

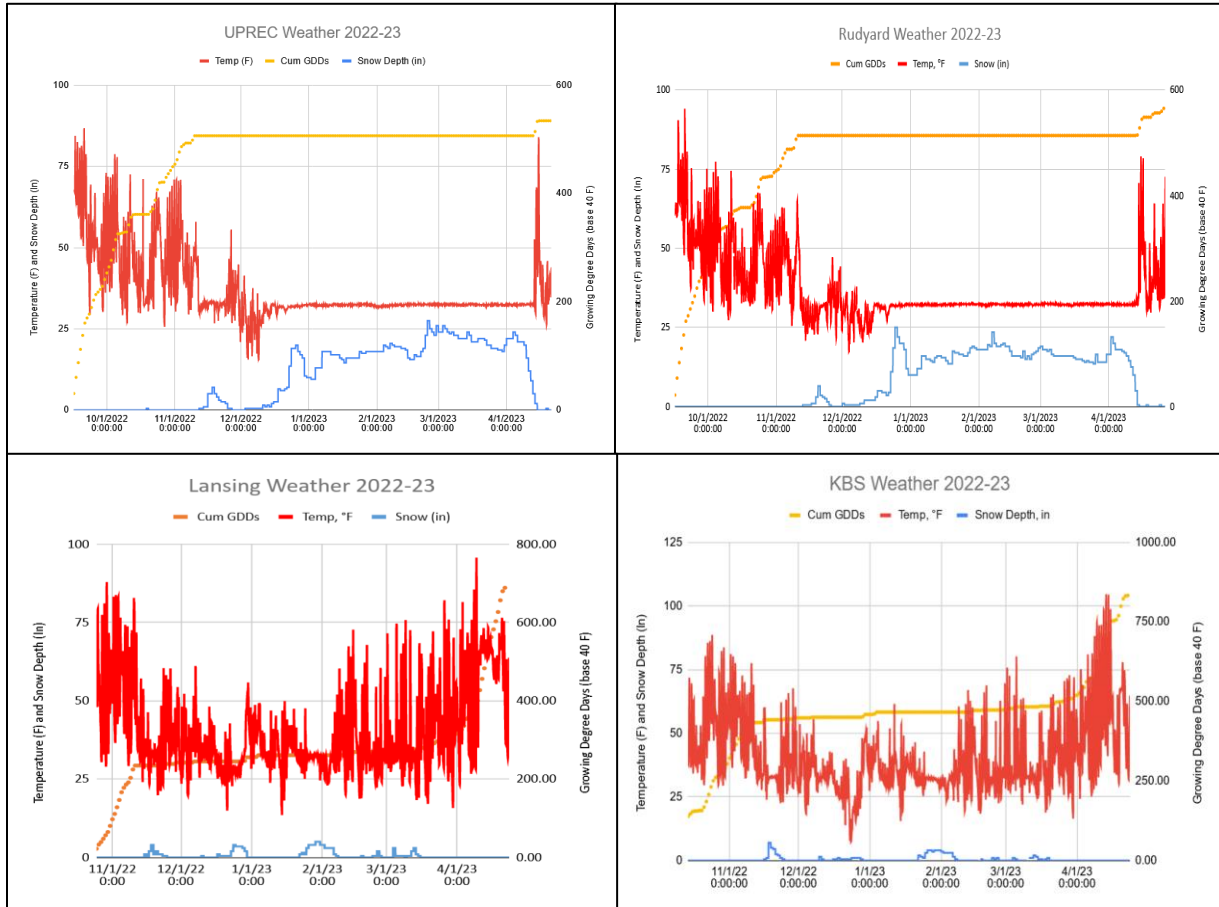
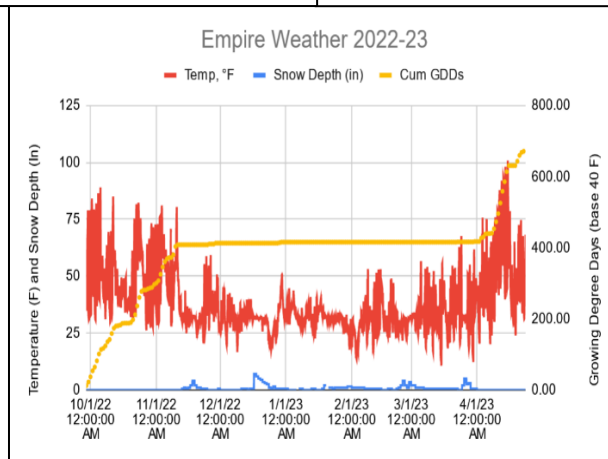
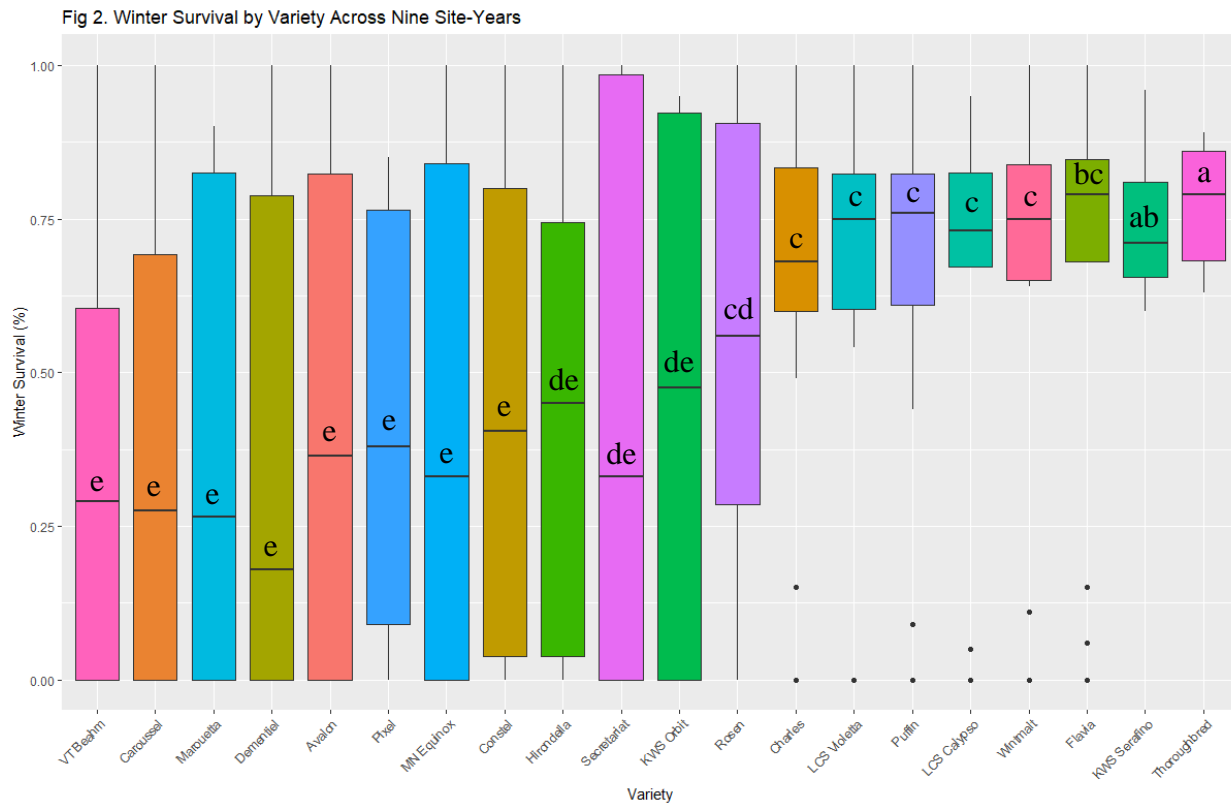


Fig 1. Weather at the Five Study Sites in 2022-23



Environmental factors found to be significantly correlated with barley survival in the second year of our study were length of the hardening period (fall days mean 40 – 32 F) ($t = 3.41$, $p = 0.1816$), and the number of hours below 14 F ($t = -5.30$, $p < 0.00001$). Length of the hardening period varied based on planting date and weather conditions at each location. Previous research in Michigan has demonstrated that winter barley is especially sensitive to planting date (Singh et al.), making timely planting more important for this crop relative to winter wheat or rye. More than ten hours of temperatures below 14 F was enough to cause significant injury to some varieties. Because temperature was measured at the soil surface, it was highly influenced by snow cover. 6-12 inches of snow was sufficient to keep the soil/crop temperature near a constant 32 F. Together, trial location and the environmental factors noted above explained 88.59% of the variation in observed winter survival at Empire, Lansing and Rudyard in 2022-23.



Objective 2: Quantify known and novel traits previously associated with winter hardiness by sampling barley tissue from the proposed field trial and analyzing it for sugar accumulation, fatty acid ratios, lipid peroxidation, antioxidant enzyme activity, and DNA repair potential.

Hypothesis: Varietal differences in winter barley cold hardiness will be observed in the field, and largely explained by the measured covariates.

Physiological processes active in barley during cold acclimation include directed desiccation to prevent freezing of important overwintering structures, sugar accumulation to act as antifreeze, shifts in enzyme temperature tolerance and activity, fatty acid changes for better functioning at low temperatures (increase fluidity by desaturation), dissipation of absorbed light energy, and maintenance of photosynthetic capacity at low temperature (Thomashow, 1998). Which mechanisms are specifically important in barley is not well understood.

In 2022-23, our team sampled barley leaf and crown tissue from the KBS and UPREC locations in the fall before and after cold hardening. Lab analysis of these samples has suggested that a lack of crown RNA protective strategies during winter may play a role in barley's susceptibility to winter injury. This was supported by our finding that Rye (*Secale cereale* 'KWS Serafino') had much less RNA damage than winter barley at most locations before and after cold hardening, especially in crown tissue (Figure 3B,D,F,H). Since leaf senescence can play a role in plants transitioning into winter dormancy, leaf RNA damage was less informative (Fig. 3A,C,E,G). It is unclear why rye had higher RNA damage compared to some barley varieties at KBS in 2020 pre-hardening. It is possible that higher RNA damage could indicate metabolic changes occurring for enhanced cold acclimation. At the UPREC location in 2020, barley varieties 'Charles' and 'Wintmalt' had the lowest RNA damage among barley varieties on most dates.

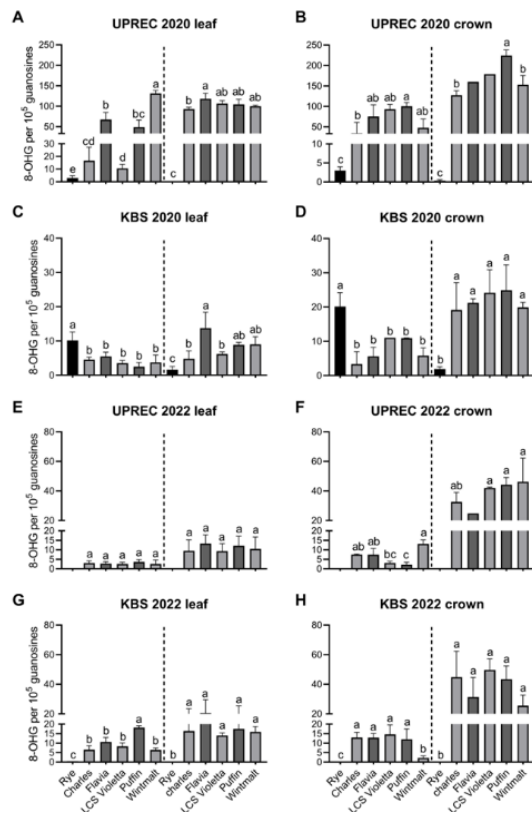


Fig 3. RNA damage (8-OHG content) during pre and post hardening in rye and barley varieties in leaf and crown tissue at various MI locations and years.

RNA damage could be a useful indicator of cold hardiness differences across species. RNA damage was also influenced by environment/location and year, which indicates a need to closely associate weather data and other field factors to these results. Our results indicate that lower RNA damage may be associated with higher cold hardiness based on our comparison of rye to barley. RNA damage creating 8-hydroxyguanosine (OHG) is primarily caused by oxidative stress. Oxidative stress (caused by buildup of reactive oxygen species) is a common feature of extreme temperature stress involving either heat or cold. Plants have a comprehensive suite of enzymes that mitigate oxidative stress by breaking down reactive oxygen species into nontoxic products. The relationship between oxidative stress and RNA damage highlights the potential value of our 2020-21 finding that cereal rye (KWS Serafino) had significantly higher Glutathione reductase activity ($P = 0.08$) than all winter barley varieties in crown tissue during cold hardening at UPREC. However, interspecies differences in metabolic rates and other factors may reduce

the power of this finding. Consistent lower levels of RNA damage could simply indicate earlier dormancy or slower metabolism of rye compared to barley on these sampling dates. Thus, additional research is needed to mechanistically understand whether these plants are truly exhibiting inherent differences in antioxidant enzyme activity and subsequent RNA damage. Additional sampling dates of a field study and controlled environment experiments proposed for the next cycle of MI CBC grants would assist with a better understanding of RNA damage and its relation to cold hardening, tissue health, and viability of winter barley.

Objective 3: Improve winter barley variety development and Michigan site selection by constructing predictive winter hardiness models and sharing this new information through extension outreach.

Hypothesis: Screening and selection of barley varieties for the most important traits identified by our work will help to improve winter hardiness long-term, while Michigan growers, maltsters and brewers will benefit from more precise recommendations regarding site selection for specific winter barley varieties.

While Michigan winters appear stark at face value (i.e. cold, dark), winter is truly a complex and dynamic phenomenon presenting multiple forms of abiotic stress at varying levels for winter cereals like barley. Our data from this second year of research suggest that 1) Winter barley varieties developed in colder areas of Europe and the US generally have greater winter hardiness; 2) Timely planting of winter barley can increase winter survival by allowing sufficient fall growth and gentle cold hardening; 3) USDA hardiness zone (5b or warmer) is likely a good, but imperfect, predictor of winter barley hardiness based on average minimum air temperature, which we found to be important for barley survival; 3) Sufficient snow cover of at least 6-12 inches can buffer barley against cold temperatures; and 4) Differences in antioxidant enzyme activity and associated RNA damage may explain some of the variation in winter barley hardiness relative to other cereals like rye or wheat. Climate change will continue to expand the adapted range of winter barley in Michigan, as evidenced by recent updates to the USDA Hardiness Zone map where parts of Michigan have gained 0.5-1 hardiness zones in the last few decades. However, climate change effects on snow cover resulting in less, or less predictable, snowfall from year to year could be detrimental to winter barley hardiness. Despite all of this progress, we have witnessed that other factors, such as rapid spring/fall temperature swings, ice and waterlogging associated with climate change, can seriously injure barley. Therefore, identifying adapted and resilient winter barley varieties and production environments will be a complex, long-term endeavor.

To-date, information on our project and findings has been shared with growers and others through several outlets including the 2020 and 2021 [Great Lakes Hop and Barley Conferences](#) (115 participants), the 2022 and 2023 [Michigan Great Beer State Conferences](#) (867 participants), the 2021 [KBS Small Grains for Brewing and Distilling field day](#) (21 participants), the 2022 KBS LTAR Field Day (80 participants), the 2023 KBS Farm Bureau Advisory Council tour (20 participants), the 2021 and 2023 UPREC legislative tours, [UP Ag Connections Newsletter](#) (1,100 recipients), and social media including the "[What's UP @ UPREC](#)" video series (60 views).