**Final Report for:** Influence of Ice Encasement & Winter Desiccation of Winter Wheat Physiology & Yield
**MWP Tracking Number:** MWP 22-08-01-AS

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**Project goals and value for Michigan Wheat Growers**:
Climate predictions indicate winter conditions will include reduced protective snow cover, increased severe storms, and more fluctuating temperatures in areas like Michigan, which will leave winter wheat more exposed to winterkill. Winterkill can be caused by ice encasement, winter and/or spring desiccation, freeze thaw cycles, and other stresses. In a 2021 survey, Michigan winter wheat industry members cited ice encasement and spring freeze thaw cycles as primary causes of crop loss. Thus, ice encasement and winter desiccation are important and on-going problems that may escalate due to climate change. The results presented below represent highlights of the findings from this project to date since additional objectives and data collection and analysis are ongoing.

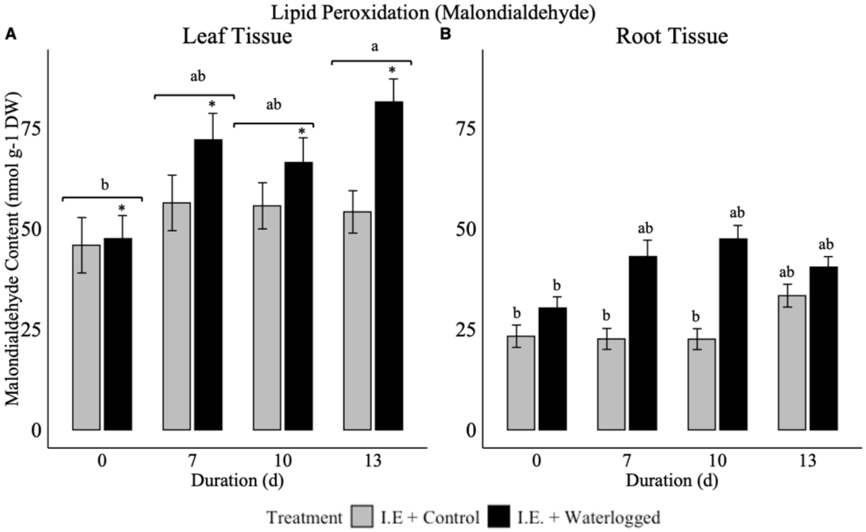
The first objective aimed to evaluate the impact of varying levels of ice encasement followed by spring waterlogging on yield, tillering, membrane health, and root production of winter wheat. The second objective aimed to determine if winter and spring soil moisture conditions impact winter wheat plant health and productivity traits and to develop experimental methods for dry soil condition experiments.

We expected that wheat plants exposed to increasing levels of ice encasement followed by waterlogging will have reduced physiological health, tillering, root production, and yield attributes. We additionally anticipated that dry soil during dormancy coupled with dry soil and during spring recovery may be most detrimental to plant growth and productivity compared to other treatments.

**Methods**
To accomplish objective 1, we evaluated two winter wheat varieties contrasting in winterkill tolerance. These were determined from previous experiments and the source of the germplasm was the MSU breeding program panel selections and will be called MSU and OSU for brevity. Plant treatments were subjected to 0, 7, 10 or 13 days of soil surface ice encasement (IE), which was approximately 1 inch deep. These plants were then exposed to 3 days of waterlogging or control water conditions. Half of the plants of each treatment underwent destructive sampling while the other half remained in the greenhouse for yield assessment. We measured malondialdehyde content (MDA) of plant organs which is an indicator of lipid peroxidation. A higher level of MDA indicates greater damage to cellular membranes or stress damage in the sampled plants.

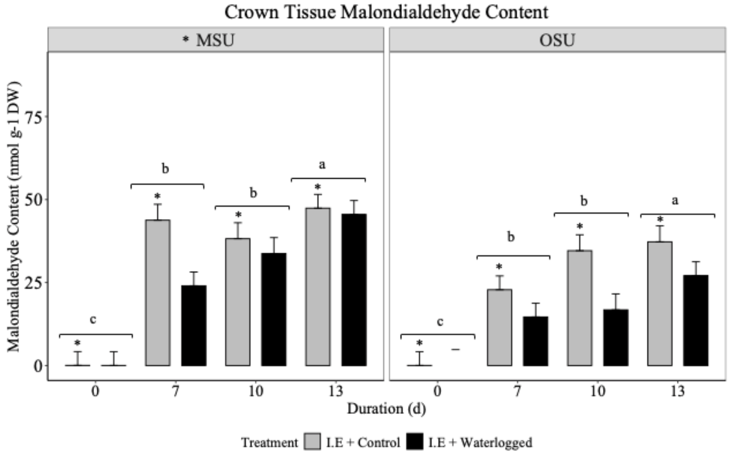
**Results of Objective 1**

Figure 1. Lipid peroxidation as expressed by malondialdehyde content showing leaf tissue content across duration of treatment (A), and root tissue between treatment and duration (B). Letters indicate fishers least significant differences between duration for leaf tissue and the interaction between duration and treatment for root tissue. Asterisk represents significant difference between treatments in leaf tissues (P<0.1). Error bars represent standard error.



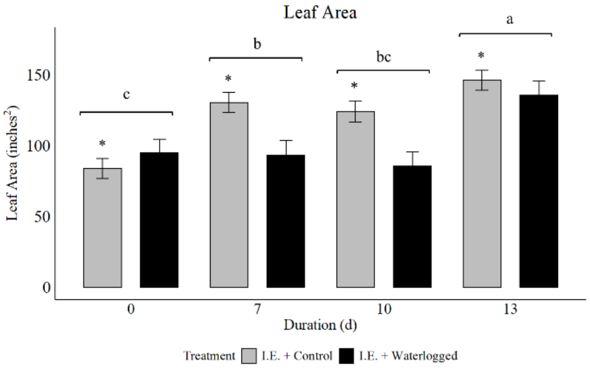
In leaf tissue, the duration of treatment significantly influenced the MDA content, with day 13 exhibiting the highest damage (Figure 1A). Leaf tissue had higher MDA content in waterlogged plants compared to controls (Figure 1A). These findings underscore the detrimental impact of short spring episodes of waterlogging on plant health (Figure 1B). Like in leaves, in wheat roots the MDA level increased due to longer periods of ice encasement and the waterlogged treated plants had a more apparent and earlier increase in MDA content. This indicates that both leaves and roots were damaged by ice encasement, waterlogging, and the combined stress.

Figure 2. Lipid peroxidation as expressed by malondialdehyde content in crown tissue in MSU (left) and OSU (right) varieties in two different treatments: I.E. plus control (grey) and I.E. plus waterlogged (black) across duration of treatment of 0, 7, 10, or 13 days. Letters represent fishers least significant differences between variety and duration of treatment, asterisk represent significant differences between treatments and variety. Errors bars represent standard error.



In crown tissues, which are the major overwinter structure in winter wheat during severe winters, significant differences in MDA content were observed between winter wheat varieties (Figure 2). The MSU variety displayed higher MDA content than OSU. Additionally, treatment duration had a notable effect, with day 7,10, and 13 having higher levels compared to the control. Longer stress durations corresponded to increased oxidative stress, causing higher MDA levels. Lastly, treatment influenced MDA content, with control plants exhibiting higher levels compared to waterlogged plants (Figure 2). The crown tissue results are unexpected for both genotype and water treatment response, which means more investigation may be warranted.

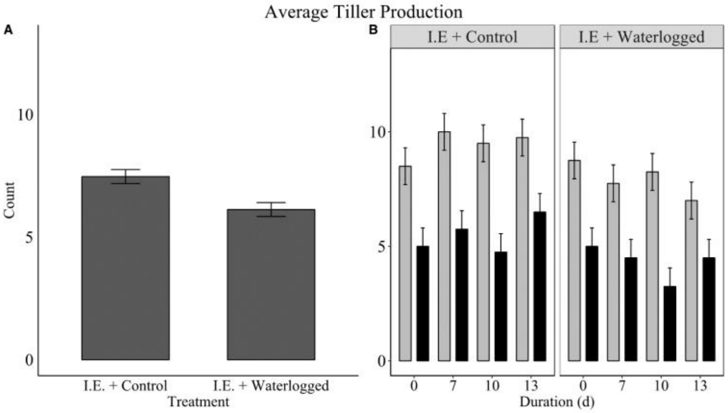
Figure 3. Total leaf area in MSU and OSU variety with I.E plus control optimal water (grey) and I.E. plus waterlogging treatment (black) over duration of treatment (days). Letters indicate fishers least significant difference between duration of treatment within a variety (P<0.1).



Leaf area measurements were conducted on plants remaining in the greenhouse immediately following winter treatment. MSU, which has exhibited higher tolerance to ice encasement in our previous studies, exhibited significantly greater leaf area averaged across all samples compared to OSU (Figure 3). Ice encasement for 13 d caused higher level of leaf area compared to day 0 plants, which is an unexpected result. Following ice encasement, waterlogging caused less leaf area on several dates compared to optimal levels of water during spring recovery.

The impact of ice encasement followed by waterlogging on total and reproductive tiller numbers was investigated. Analysis of total tiller numbers (Figure 4B) revealed significant variation between genotypes, with MSU exhibiting consistently higher tiller numbers compared to OSU (Figure 4A). This observation is consistent with MSU also having higher average leaf area across treatments (means not shown). Waterlogged plants exhibited a significantly lower number of tillers compared to the control treatments (Figure 4B). This reduction in tiller numbers among waterlogged plants indicates a negative effect of the treatment on overall tiller count.

Figure 4. Average tiller production in response to (A) watering treatment (B) all experimental factors with MSU (grey) and OSU (black) varieties over 0, 7, 10, or 13 days of I.E. plus control (left) and I.E. plus waterlogged (right). (C) Change in tiller numbers due to ice encasement is shown between MSU (grey) and OSU (black) over 7, 10, or 13 days. Letters indicate fishers least significant



The change in tiller number due to ice encasement was calculated for both MSU and OSU genotypes (Figure 5). OSU exhibited a more pronounced negative impact from ice encasement compared to the relatively more resilient MSU. Waterlogging for 3 d was detrimental for both winter wheat varieties.

The treatment's duration significantly affected the reproductive tiller numbers (Figure 5). There was a decrease from control day 0 levels of reproductive tillers due to 13 days of ice encasement. Whether waterlogging or no waterlogging was imparted did not have a significant effect on reproductive tiller number in this study.

Significant differences in root weight were observed between varieties, with MSU exhibiting a higher root dry weight (Figure 6A). This aligns with previous findings indicating MSU's greater tolerance to ice encasement compared to OSU. The heavier root weight suggests MSU may have an advantage compared to OSU to withstand various stresses. Regarding root length, little practical differences were found except for a minor reduction in root length due to waterlogging of plants (Figure 6 B,C).

In terms of yield (Table 1), although no significant differences were observed among the treatments, it is noteworthy that the control plants exhibited numerically higher yield compared to the waterlogged plants on most dates. Indicating that waterlogging of plants may have an influence on yield quantity.

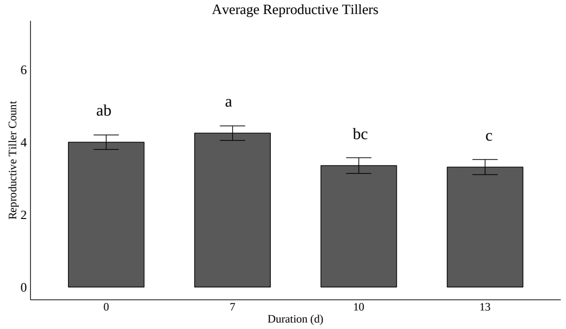


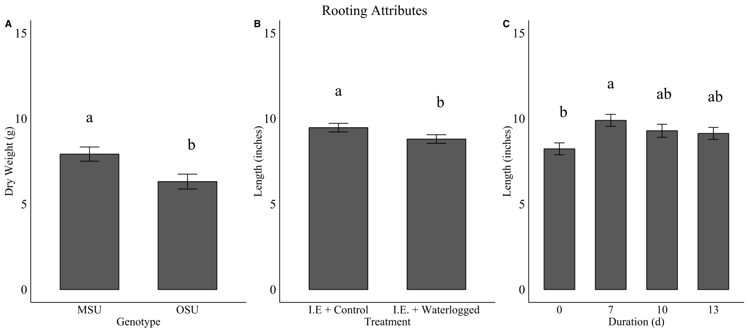
**Table 1.** Total yield (g) per variety and treatment. Letters indicate significant differences between duration of treatments (P<0.1).

| | Day 0 (b) | Day 7 (a) | Day 10 (a) | Day 13 (a) |
|---|---|---|---|---|
| **OSU Control** | 5.71 | 8.23 | 5.80 | 7.98 |
| **OSU Waterlogged** | 4.35 | 7.02 | 7.07 | 6.36 |
| **MSU Control** | 4.90 | 8.49 | 7.05 | 6.28 |
| **MSU Waterlogged** | 2.97 | 7.10 | 7.01 | 6.71 |

**Conclusions from Objective 1**

This study has revealed methodology, such as duration of treatment, that can be used in future research to better understand the influence of ice encasement and/or spring waterlogging on winter wheat health and growth. Interestingly, a short duration of waterlogging of just 3 days after ice encasement conditions caused measurable damage to internal plant structures and reduced plant growth and productivity. This indicates that management practices to alleviate excess water stresses in the spring such as improving drainage or other cultural practices to reduce standing water and improve waterlogged or saturated soils following winter are important. Organic matter can be incorporated to improve drainage on compacted sites. These practices should be done prior to the waterlogged condition since additional compaction can occur when working on overly wet soils. Tile drains and other grading improvements may be beneficial for winter wheat crops.

Figure 6. Average root dry weight (A) in MSU and OSU genotypes (B) average root length across treatments (C) average root length across duration of study of 0, 7, 10, or 13 days (C). Letters indicate fishers least significant difference between main effects (P<0.1). Error bars represent standard error.



## Methods and Results of Objective 2

To accomplish objective 2, we used one standard soft white winter wheat variety 'Jupiter'. Once vernalized and cold acclimated, the plants were placed at -1°C in a growth chamber. The Jupiter plants were then exposed to different soil moisture treatments which are described in Table 2 for durations of those watering winter watering treatments for 0, 10, or 20 days in growth chambers. Spring recovery treatments were performed in a greenhouse. The plants were sampled after treatments and lipid peroxidation was measured in crown tissues to understand how these treatments affect the plants' health by quantifying MDA. There were no major changes in MDA due to overwintering or watering treatments found in this study.

**Table 2.** Explanation of treatments for winter and spring conditions.

| Treatment | Soil or air moisture during dormancy (winter) | Soil or air moisture recovery (spring) |
|---|---|---|
| 1 | Control (soil field capacity) | Control (soil field capacity) |
| 2 | Dry (soil 8% VWC) | Control (soil field capacity) |
| 3 | Control (soil field capacity) | Dry (8% soil VWC) |
| 4 | Dry (soil 8% VWC) | Dry (8% soil VWC) |
| 5 | Control (soil field capacity) | Control (soil field capacity) with hot, humid air |
| 6 | Control (soil field capacity) | Dry (8% soil VWC) with hot humid air |

Following treatments and once the plants had reached full maturity, biomass was measured in the spikes (data not shown), tiller and leaves (Figure 7A), and roots (Figure 7B). For spike biomass, there was not much change due to treatment. The duration of winter reduced tiller and leaf biomass and root weight across all treatments. Without a harsh winter (day 0 plants), optimal soil moisture during cold acclimation and during spring (treatment 1) produced the highest level of above and below ground plant biomass. For day 0 plants, dry soil in the winter and spring and combinations therein were all detrimental to plant growth of above ground parts. For roots, dry conditions in both seasons and a hot and dry spring caused the greatest reduction in root biomass

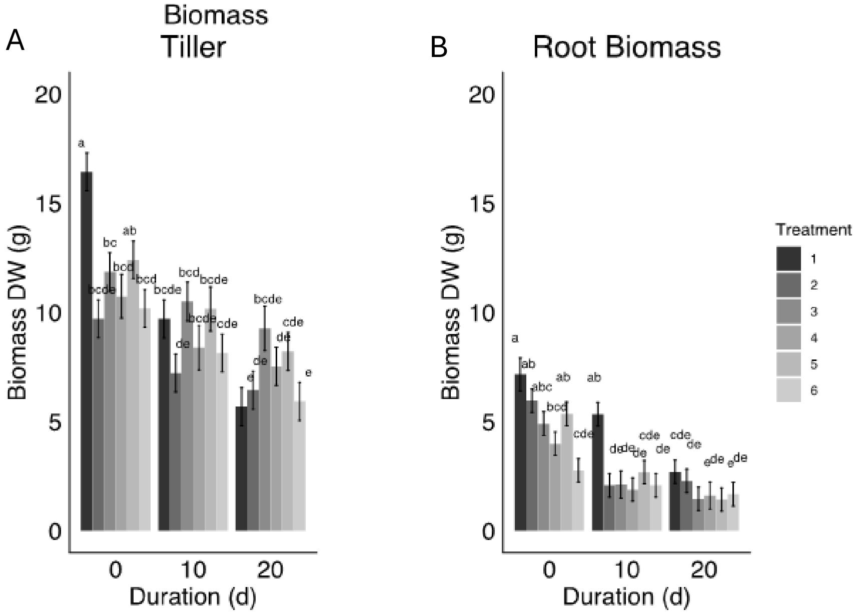
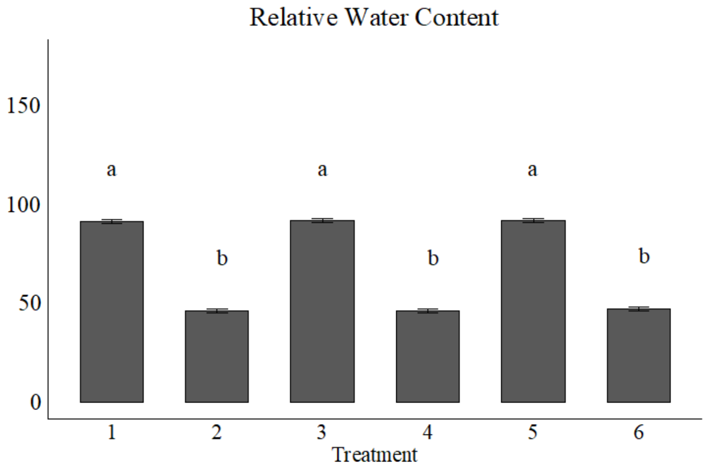


Figure 7. Biomass in (A) tillers and leaves (B) roots for respective treatments (1-6) and duration under treatment of day 0, 10, or 20. Letters indicate fishers least significance difference in the interaction between treatment and day (P<0.1). Error bars represent standard error.

(treatment 4 and 6). With winter treatment (days 10 and 20), dry soil in the spring (treatment 3) tended to benefit wheat plants, although there was a numerical difference but not statistical differences. Dry soil in the winter was the most detrimental to above and below ground growth (treatments 2 and 4). But we observed visually that the plants were greener with winter dry soil conditions compared to spring drought (Figure 8). Visual wilting occurred with both spring and winter dry conditions. This wilting was confirmed with relative water content of leaves (Figure 9). Spring drought (treatment 2) caused a much larger loss of water content compared to winter drought, since winter drought treated plants had a relative water content at control levels. Additional data should be gathered to quantify the loss of chlorophyll, plant cellular water, and stress occurring due to these simulated seasonal conditions.

Figure 8. Plants in the greenhouse following winter conditions treated with (left most group) control conditions, (left center) winter dry soil, (right center) spring dry soil, and (right most) winter and spring dry soil. Error bars represent standard error.

Figure 9. Relative water content of plants with a given soil moisture treatment after 20 days in winter conditions.



## Summary and Conclusions of Objective 2

It is possible that winter dry soil conditions may activate preservation defenses at the expense of growth, since growth and biomass were most reduced due to the winter dry soil conditions on several dates but the plants had a healthier visual appearance and relative water content of leaves. This winter drying could act as a stress preconditioning type of treatment that can improve a plant's survival of future stresses. Spring dry soil condition plants were visually less healthy than winter dry conditions, observed as greater yellowing and loss of leaf water content. Spring dry soil treated plants had a loss of biomass compared to control conditions also, but they were numerically higher levels of biomass compared to the winter dry treatment on some sampling dates. This is consistent with experiments in turfgrasses that showed drier conditions during cold acclimation was found to improve turfgrass winter survival in our previous studies. Additional investigation into seasonal soil moisture level conditions is needed, particularly as snow cover may become less prevalent and less persisting in Michigan resulting in less melt water available in spring and warmer spring conditions. These treatments and durations could be used to screen winter wheat plant breeding populations for seasonal resiliency.

## Future Work and Changes

Both studies are being repeated in summer 2024. Data analysis and interpretations are ongoing. Additional measurements will be taken such as chlorophyll content to better quantify physiological observations for objective 2.