



High-throughput phenotyping of forage grasses under controlled conditions

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Abstract

Ice encasement can damage perennial forage grasses during winter and reduce spring growth. Ice cover restricts gas exchange, which can lead to oxygen depletion and the accumulation of harmful metabolites. Controlled screening can help compare plant responses, but the treatment must produce ice-related injury without mainly becoming a test of freezing tolerance. This study evaluated a controlled ice-encasement protocol, tested image-based biomass measurements, and compared controlled responses with field observations.

Module 1 developed and assessed a controlled ice-encasement protocol for meadow fescue, tall fescue, and timothy. Plants were established in a greenhouse, cold acclimated, placed in freezers set to approximately -5°C , and covered with a shallow ice layer. Treatment durations were 5, 9, and 15 days for the two fescue species and 10, 22, and 30 days for timothy. Plants were photographed repeatedly during greenhouse recovery, and final plant cover was measured by image analysis. Module 2 evaluated whether image analysis could reliably reflect plant biomass. Image-derived measurements were compared with harvested green and dry biomass in a subset of 40 timothy pots. Module 3 used a semi-controlled field ice-encasement experiment at Lännäs, Sweden, as a field reference for the controlled experiment.

In Module 1, final plant cover differed among cultivars, but longer ice treatments did not always lead to lower cover. Mean cover in meadow fescue fell from 2.5% after 5 days to 0.6% after 15 days. Tall fescue had almost no cover after 5 days but higher cover after 9 and 15 days. Timothy had its highest mean cover, 18.2%, after 22 days. These differences were not tested for statistical significance. Treatment durations and recovery times differed among species, so the results could not show which species was most tolerant of ice encasement.

In Module 2, image-derived measurements were positively correlated with harvested green biomass ($r = 0.61$, $p < 0.001$) and dry biomass ($r = 0.50$, $p = 0.001$). The correlation was higher for green biomass than for dry biomass. These results showed that image analysis could detect some of the differences in plant biomass.

In the field experiment, Grindstad, a timothy cultivar, and Karolina, a tall fescue cultivar, were consistently among the greenest ice-treated subplots. Meadow fescue generally showed little green growth. Karolina performed relatively well in both the controlled and field experiments. However, the cultivar rankings for timothy and meadow fescue differed between the two experiments.

The controlled protocol was feasible, but several factors, including temperature differences among freezer locations and differences in recovery time, made the results difficult to interpret. The shallow laboratory ice layer also differed from the field treatment. Image analysis can support repeated assessment of plant recovery and growth without harvesting the plants. More uniform temperatures, equal recovery times, and further field testing are needed before the protocol can be used for reliable ice-encasement screening.

Keywords: ice encasement; forage grasses; high-throughput phenotyping; RGB imaging; biomass; winter survival; field experiment

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Abbreviations

HTP	High-throughput phenotyping
LD50	Exposure duration causing 50% mortality
MF	Meadow fescue
RGB	Red, green and blue
SDG	Sustainable Development Goal
SLU	Swedish University of Agricultural Sciences
SMHI	Swedish Meteorological and Hydrological Institute
TF	Tall fescue
TT	Timothy
UAV	Unmanned aerial vehicle

1. Introduction

Perennial forage grasses are important to livestock production in northern and temperate Europe. They provide feed for dairy and meat production, protect soil, support carbon storage, and help maintain agricultural landscapes. In the European Union, permanent grassland covered 50.7 million ha in 2023, corresponding to 31.6% of utilised agricultural area (European Commission, 2026). In northern Sweden, perennial forage crops are especially important as they cover about 80% of the agricultural landscape and form the main feed base for livestock systems (Swedish University of Agricultural Sciences [SLU], 2026). Stable forage production is therefore important for farm profitability and livestock production in the region.

Improving the resilience of forage grass systems also supports several sustainable development goals (SDG). More stable forage production contributes to sustainable food production and climate-resilient agriculture under SDG 2. It also supports adaptation to climate change under SDG 13 and more sustainable use of terrestrial ecosystems under SDG 15 (United Nations, 2015). Grasslands can deliver these benefits only if they remain productive and persistent under changing weather patterns. This is a major challenge for Sweden and other northern European regions, where winter conditions are becoming less stable.

Climate change does not remove winter stress. Instead, it can change the type of winter stress that plants experience. Warmer winters can reduce some direct freezing events, but they may also increase rain-on-snow events, freeze-thaw cycles, waterlogging, de-acclimation, and the formation of surface ice (Dalmannsdóttir et al., 2017; Ergon, 2017; Thorsen et al., 2010; Thorsen & Höglind, 2010). These events can be harmful for perennial forage grasses. Snow usually acts as an insulating layer. When snow melts and refreezes, or when water accumulates frozen soil, solid ice layers may form over the plants. Such ice layers can reduce gas exchange and create stress conditions that differ from direct freezing injury.

Winter damage often appears in patches rather than across an entire field. This makes injury difficult to predict and difficult to evaluate from a single field of observation. Photographs taken in grassland near Lännäs showed clear variation in spring conditions over short distances (Figure 1). These observations were outside the experimental rings and were used only as a general field context. Winter injury in northern Sweden may reflect variation in snow cover, temperature, ice encasement, soil moisture, and pathogens (SLU, 2026). This variability is one reason to combine controlled experiments with a separate field reference.



Figure 1. Patchy spring condition observed in grassland near Lännäs, Sweden. These observations were made outside the experimental rings and were used only as general field context.

Ice encasement is not the same as freezing tolerance. Freezing tolerance describes the ability of acclimated plant tissues to survive extracellular ice formation, cellular dehydration, and membrane stress at sub-zero temperatures. Ice encasement can occur at temperatures close to 0 to -2 degrees C, but the plants are covered by ice. The ice restricts gas exchange. Oxygen can become limited, while carbon dioxide and anaerobic metabolites can accumulate. Carbohydrate reserves may also decline during the stress period. When the ice melts, sudden re-aeration may cause additional oxidative damage (Gendjar et al., 2024; Gudleifsson, 2010; Höglind et al., 2010; Merewitz, 2021). This distinction is important for experimental design. If the treatment temperature is too low, the measured response may reflect freezing tolerance rather than true ice-induced injury. If the treatment is too short, damage may be too weak to separate genotypes. Therefore, a controlled ice-encasement protocol must be validated for treatment temperature and duration to ensure that the measured response reflects ice-induced injury and anaerobic stress, rather than freezing tolerance alone.

Previous studies have shown that forage grass species and cultivars differ in ice encasement tolerance. Timothy (*Phleum pratense* L.) is often more tolerant of ice encasement than perennial ryegrass (*Lolium perenne* L.), and northern-adapted cultivars can show better survival under winter stress than cultivars from milder regions (Höglind et al., 2010). In Icelandic studies, grasses were generally more ice tolerant than legumes and winter cereals, and timothy ranked among the more tolerant species (Gudleifsson, 2010). Recent studies on turfgrasses have also shown that ice encasement affects recovery, gas composition, sugars, amino acids, membrane health, and cuticular wax traits (Chalise & Merewitz, 2025; Gendjar et al., 2024; Merewitz, 2021). Although turfgrass and forage systems differ in

management, these studies provide useful physiological insight into how perennial grasses respond to ice cover.

For breeding, the main challenge is throughput. Traditional winter survival evaluations often depend on field scoring, visual ratings, or destructive measurements after stress. These methods are useful, but they are slow and can be subjective. They are also difficult to scale when many genotypes need to be screened. High-throughput phenotyping (HTP) can help reduce this bottleneck. RGB imaging can measure plant cover, projected area, colour, and regrowth over time without destroying the plants (Jiang & Li, 2020; Yang et al., 2020). In forage and grass systems, image-based methods have been used to estimate biomass, growth, drought response, and breeding-relevant traits in both controlled and field environments (Biswas et al., 2020; Castro et al., 2020; De Swaef et al., 2021; Jiang & Yang, 2022).

Image-based phenotyping must be checked against direct plant measurements before it can be used as a biomass proxy. Under standardised, well-irrigated greenhouse conditions, repeated RGB images can record changes in visible plant areas without destroying the plants. Harvested green and dry biomass provide a direct reference for testing whether an image-derived measurement reflects real differences in plant amount. This comparison is important because a two-dimensional image may not fully capture vertical growth, leaf overlap, or tissue density.

This study consisted of three experimental modules. Module 1 developed and evaluated a controlled ice-encasement protocol for meadow fescue, tall fescue, and timothy, with the aim of determining whether the protocol could provide an interpretable and useful method for studying forage grass responses to ice encasement under controlled conditions. Module 2 used repeated image-based phenotyping under well-irrigated greenhouse conditions and tested whether an image-derived measure of plant amount reflected harvested green and dry biomass. Module 3 used a semi-controlled field ice-encasement experiment at Lännäs, Sweden, as a field reference to examine whether responses observed under controlled conditions were also reflected under field conditions.

2. Critical literature review

2.1 Ice encasement should be treated as a distinct winter stress

The most important conceptual point in the ice-encasement literature is that ice damage is not simply another form of freezing injury. Gudleifsson (2010) provided one of the clearest demonstrations of this distinction in herbage crops. In field observations and artificial ice-encasement tests at -2 degrees C, he showed that plant groups differed strongly in their ability to survive ice cover. Grasses were generally more tolerant than clovers, brassicas, alpine plants, and winter cereals. Within the grasses, timothy and Bering hairgrass were among the most tolerant species. The same study also showed that ice encasement changes plant metabolism. During ice cover, timothy consumed carbohydrates and accumulated carbon dioxide, ethanol, lactate, and other metabolites. This supports the view that injury under ice is linked to restricted gas exchange and anaerobic metabolism, not only to low temperature.

Höglind et al. (2010) reached a similar conclusion from a different experimental angle. They compared timothy and perennial ryegrass cultivars sampled from three Norwegian sites and tested for frost tolerance, ice-encasement tolerance, and water-soluble carbohydrates. Timothy tolerated much longer ice exposure than perennial ryegrass, with maximum LD50 values of about 63 days for timothy and 20 days for perennial ryegrass. They also found that cultivar origin mattered. Northern-origin cultivars showed higher winter adaptation than southern-origin cultivars. This agrees with the broader Nordic understanding that winter survival is shaped by local adaptation.

However, the two studies also show that ice tolerance cannot be explained by one simple mechanism. Gudleifsson (2010) emphasised metabolite accumulation under anoxia. Höglind et al. (2010) found that the higher ice tolerance of timothy was associated with a slower change in water-soluble carbohydrate content during ice encasement, but the amount of carbohydrate at the start of treatment did not clearly predict survival. This is important. It suggests that carbohydrate reserve alone is not a sufficient screening trait. A genotype may have adequate reserves before stress but still differ in respiration rate, anaerobic metabolism, membrane stability, or recovery after re-aeration. For breeding, this means that a useful screening method should measure plant performance after the stress, not only pre-stress physiological status.

These studies provide the basis for the present experiment. They show that ice encasement is a damaging stress in northern forage systems; that species and cultivars differ, and that timothy is often more tolerant than other grasses. They also show why treatment temperatures and duration must be chosen carefully. If

the temperature is too low, the protocol may become a freezing tolerance test. If the treatment is too short, it may not cause enough injury to separate genotypes.

2.2 Field tests are realistic, but they are not enough

The second theme in the literature is the tension between field realism and experimental control. Gudleifsson (2010) argued that field tests of ice tolerance are difficult to reproduce because stress intensity depends on winter weather and local environmental conditions. The experimental area at Lännäs was flat, but the amount and duration of ice could still vary with snow cover, leakage from the rings, soil conditions, wind exposure, and temperature fluctuations. Field damage is therefore biologically meaningful but statistically difficult to reproduce across winters.

Gudleifsson and Bjarnadottir (2014) developed this point into a methodological framework. They described five main stages for laboratory testing of ice-encasement tolerance: plant growth, cold acclimation, ice-encasement stress, recovery, and damage assessment. They also stated that laboratory tests are the most common and effective approach when large plant material must be screened, because temperature, plant age, acclimation, stress duration, and recovery conditions can be controlled. At the same time, they clearly recognised that laboratory tests do not fully reproduce the field. Plants may be tested with or without soil; air spaces in soil can reduce anaerobic stress; removing soil can increase uniformity but also increase labour; and the timing of scoring affects the result.

This methodological discussion creates a direct gap for applied forage breeding. The literature contains strong laboratory methods, but many are not designed for high-throughput breeding workflows. Some protocols use tiller bundles, washed roots, destructive sampling, or repeated manual scoring. These approaches are valuable for physiological research but less practical when many forage genotypes must be screened quickly. The field, in contrast, captures natural variation but is too variable to provide a stable first-stage screen. The present study addresses this gap by developing a controlled protocol that is simple enough for breeding work while using a semi-controlled field experiment at Lännäs as a reference point.

2.3 Evidence from timothy breeding shows both promise and complexity

Pashapu (2025) extended the discussion from species-level tolerance to genetic adaptation within timothy. The thesis showed that timothy cultivars with different adaptation backgrounds differ in both freezing tolerance and ice-encasement tolerance. In the reported ranking, the northern-adapted cultivars Engmo and

Snorri had higher winter hardiness than the southern-adapted Grindstad, while Noreng was intermediate. This supports the pattern observed by Höglind et al. (2010): northern adaptation is generally associated with better winter survival.

At the same time, Pashapu (2025) also complicates a simple adaptation story. Field survivors from natural winter conditions had lower freezing and ice-encasement tolerance than plants raised from the original seed lots. This is counterintuitive, because one might expect natural field selection to enrich more tolerant individuals. The thesis also reported that ice-encasement stress responses overlap with hypoxic and freezing stress responses at the molecular level. This supports the idea that ice encasement is a combined stress. It includes low temperature, restricted oxygen, metabolite toxicity, re-aeration injury, and possibly disease pressure. Importantly, the thesis noted that laboratory ice encasement can be more severe than natural field ice conditions and may not always detect the same adaptive responses as field selection.

A controlled protocol should therefore not be treated as a substitute for field testing. It can provide a repeatable first-stage screen, but its biological meaning still needs to be checked against field observations. The present study follows this approach by combining controlled ice encasement, repeated recovery imaging, and a semi-controlled field experiment at Lännäs, Sweden.

2.4 Modern ice-encasement studies add recovery dynamics, but not yet forage-breeding throughput

Recent turfgrass studies show how ice-encasement research is moving beyond simple survival scoring. Chalise and Merewitz (2025) studied annual bluegrass under 0, 40, and 60 days of ice encasement. They measured green cover during recovery, cuticular wax traits, malondialdehyde, electrolyte leakage, and recovery rate. The study found that longer ice encasement reduced recovery and decreased cuticular wax coverage, especially C26-hexacosanol. Wax coverage was negatively related to membrane damage and positively related to recovery. This provides useful evidence that post-stress recovery is not only a visual outcome; it is connected to measurable physiological damage.

Gendjar et al. (2024) also used controlled ice encasement in turfgrass and measured plant recovery, gas evolution, sugars, and amino acids. Together with the review by Merewitz (2021), these studies strengthen the view that ice encasement should be understood as a dynamic stress process. Injury develops during ice cover, but recovery after melting is equally important. A plant that looks green immediately after ice removal may still decline during re-aeration. Therefore, repeated post-treatment imaging is more informative than a single final score.

The limitation is that these modern studies are mainly in turfgrass systems. Turfgrass studies are useful because they often have strong controlled

environment designs and detailed physiology. However, turfgrass management differs from forage breeding. Turfgrass systems use different species, cutting height, recovery goals, and economic criteria. The treatment durations can also be much longer than those needed for a practical early-stage forage screen. Thus, the literature has modern recovery-based methods, but these methods have not yet been translated into a low-cost, high-throughput protocol for forage grass germplasm under greenhouse and freezer conditions.

2.5 High-throughput phenotyping can help, but it must be biologically anchored

The broader HTP literature shows that image-based phenotyping can reduce labour and increase temporal resolution. RGB imaging can measure canopy cover, projected area, colour, senescence, and regrowth (Jiang & Li, 2020; Yang et al., 2020). In forage systems, UAV and controlled-environment imaging have been used to estimate biomass, drought response, and growth dynamics (Castro et al., 2020; De Swaef et al., 2021; Jiang & Yang, 2022). These studies support the use of imaging in forage research, but image-derived measurements still need direct biological calibration. Comparing an image measure with harvested green and dry biomass provides such a test. Imaging alone also does not solve the ice-encasement problem.

For ice encasement, imaging is useful only when the treatment causes the intended type and level of stress. A weak treatment will produce little separation in the images. A treatment that is too cold may produce mainly freezing injury. Repeated GoPro/RGB imaging can follow recovery over time, but the measurements are meaningful only if the ice treatment itself is well controlled.

2.6 Research gap and the current study

The reviewed literature provides a strong foundation but leaves several gaps. First, most classical ice-encasement studies establish that species and cultivars differ, but their methods are often too labour-intensive for routine breeding. Second, field studies capture realistic winter injury but are strongly affected by weather, snow cover, ice properties, temperature fluctuations, and disease. Third, modern turfgrass studies show the value of repeated recovery assessment and physiological measurements, but they do not directly address forage grass breeding material. Fourth, recent timothy work shows that laboratory and field responses may not always match, so controlled tests need field reference points.

These gaps define two methodological needs in the present study. First, a practical protocol is needed that can produce measurable ice-induced injury under controlled conditions without mainly testing freezing tolerance. It must be simple enough for multiple forage genotypes and sensitive enough to detect differences in recovery. Its results also need a field reference because natural ice stress includes environmental variation that cannot be reproduced fully in a freezer. Second, the image-analysis method needs validation against direct measurements. Under well-irrigated greenhouse conditions, harvested green and dry biomass can be used to test whether image-derived plant amount reflects real biomass differences.

The current study responds to these needs through three modules. Module 1 develops and evaluates the controlled ice-encasement protocol. Module 2 tests the correspondence between an image-derived measurement and harvested green and dry biomass under well-irrigated greenhouse conditions. Module 3 provides a semi-controlled field reference at Lännäs. Each module therefore evaluates a different part of the phenotyping approach.

3. Materials and methods

3.1 Study overview

The study was organised into three experimental modules. Module 1 developed and tested a controlled environment protocol for ice-encasement injury in forage grasses. Module 2 evaluated whether image-derived measurements reflected harvested green and dry biomass under well-irrigated greenhouse conditions. Module 3 documented a semi-controlled field ice-encasement experiment at Lännäs and used it as a field reference for the controlled experiment.

The controlled ice-encasement experiment was carried out at Svalöv using greenhouse-grown plants, cold acclimation, freezer treatment, ice application, and recovery imaging. Figure 2 places this experiment alongside the other two modules. In the well-irrigated greenhouse experiment, harvested green and dry biomass were used as reference measurements to test the reliability of the image-derived measure. In the Lännäs field experiment, metal rings were used to create an ice layer over selected plots during winter (Figure 25).



Figure 2. Representative settings for the three experimental modules. From left to right: trays in the freezer during the controlled ice-encasement experiment (Module 1), greenhouse-grown pots used to validate the image-derived measure against harvested biomass (Module 2), and the semi-controlled field experiment at Lännäs (Module 3).

3.2 Module 1: Controlled ice-encasement protocol and germplasm testing

3.2.1 Plant material

Three forage grass groups were included in the controlled ice-encasement experiment: meadow fescue (*Festuca pratensis* Huds.), tall fescue (*Festuca arundinacea* Schreb.), and timothy (*Phleum pratense* L.). Each species group included a check of genotype and additional cultivars or breeding-relevant entries.

For meadow fescue, the entries were Tored, SW Revansch, Lipoche, Valtteri, Cosmopolitan, Castelle, and Santtu. Tored was used both as a non-ice control and as an ice-treated check. For tall fescue, the entries were Swaj, Karolina, Barelite,

Tamiko, Eleanora, Iliade, and RGT Noug. Swaj was used both as a non-ice control and as an ice-treated check. For timothy, the entries were Grindstad, Snorri, Rakel, Switch, Rhonia, Maoi, and Nuutti. Grindstad was used both as a non-ice control and as an ice-treated check.

Row / Col.	Shelf 1						Shelf 2			Shelf 3		
	1	2	3	4	5	6	7	8	9	10	11	12
1	1	7	6	6	1	4	5	5	1	5	3	8
2	2	8	7	8	5	6	3	3	8	7	6	5
3	3	1	4	4	2	1	7	7	2	1	8	4
4	4	8	3	3	8	6	2	2	3	2	5	2
5	5	2	1	7	7	4	1	8	4	7	1	3
6	6	5	2	5	3	8	4	6	6	4	7	6

 Blue = 5 days treatment	 Green = 10 days treatment	 White = 15 days treatment
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MF	TF	TT
1 Tored	1 Swaj	1 Grindstad
2 Tored	2 Swaj	2 Grindstad
3 SW Revansch	3 Karolina	3 Snorri
4 Lipoche	4 Barelite	4 Rakel
5 Valtteri	5 Tamiko	5 Switch
6 Cosmopoli	6 Eleanora	6 Rhonia
7 Castelle	7 Iliade	7 Maoi
8 Santtu	8 RGT Noug	8 Nuutti

Figure 3. Randomisation plan used for the controlled ice-encasement experiment, showing pot positions, genotype codes, and treatment-duration groups for the three forage grass species.

3.2.2 Experimental layout and randomisation

Pot positions were assigned according to the randomisation plan shown in Figure 3. The experiment was prepared in three batches. Each batch contained all three species, with 72 pots per species (216 pots per batch and 648 pots in total). Within each species, genotypes were distributed across rows and columns using the same basic randomisation structure; the 72-pot arrangement for one species is shown in Figure 4. The check genotype for each species was included both as a non-ice control and as an ice-treated check. Each treatment duration included three replicates, with 24 pots assigned to each duration per species.



Figure 4. Example of the 72-pot arrangement used for one species within a batch, with pot positions labelled according to the randomisation plan.

3.2.3 Tray preparation and sowing

White plastic trays were used to hold the plant beakers. Two freezers were used, and each freezer could hold up to 72 beakers on the two upper shelves used for the experimental trays. To fit the freezers and allow pots to be removed at different treatment durations, trays were divided into sections before the experiment.

Each beaker was filled with peat-based soil to approximately two thirds of its height. As shown in Figure 5, the soil was gently compressed using an empty tray so that soil volume and density were as uniform as possible among beakers. The soil level was checked visually and adjusted when needed.



Figure 5. Preparation of beakers for the controlled ice-encasement experiment by filling and gently compressing peat-based soil to obtain a uniform soil volume.

The prepared trays were placed in a water-filled box so that the soil could become saturated by capillary uptake, as shown in Figure 6. After saturation, the trays

were removed from the box and allowed to drain fully. This step was used to standardise soil moisture before sowing and later freezing.

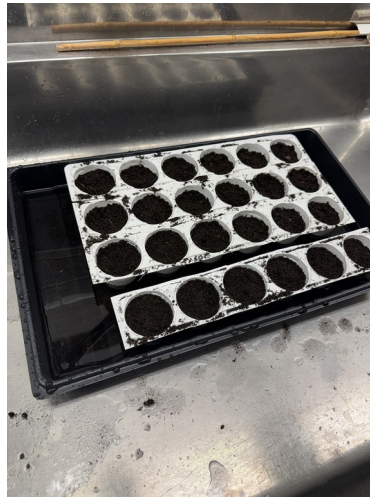


Figure 6. Saturation of the prepared beakers by capillary uptake from water in the outer tray before drainage.

For batches established by direct sowing, fifteen seeds were sown in each beaker and covered with a thin layer of soil (Figure 7). Plants were grown under standard greenhouse conditions until establishment. After emergence and early growth, seedlings were thinned to ten uniform plants per beaker.

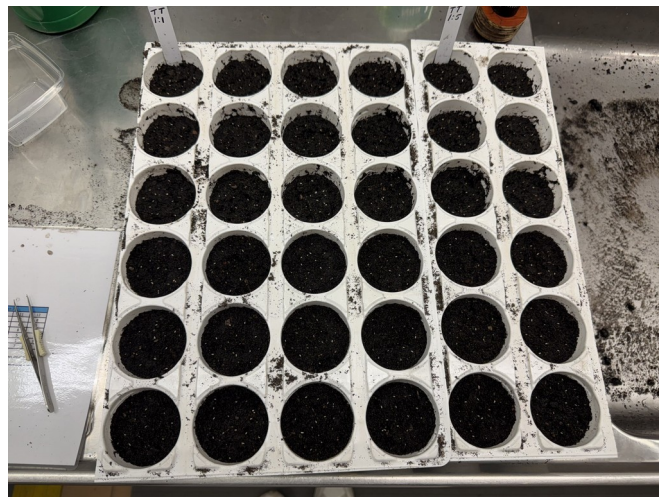


Figure 7. Sowing of 15 seeds per beaker before covering with a thin layer of soil.

The second batch was established using a transplanting step. Seedlings were first grown in larger containers, as shown in Figure 8, and were then transferred to the pot positions specified in the randomisation plan so that each genotype matched its assigned position. Ten seedlings were transplanted into each pot.



Figure 8. Seedlings for the second experimental batch before transplantation into the genotype-assigned pots; ten seedlings were transplanted into each pot.

3.2.4 Cold acclimation

Before cold acclimation, plants were allowed to establish under greenhouse conditions in the tray arrangement shown in Figure 9. Once establishment was complete, the plants were moved to vernalisation or cold-acclimation conditions according to the standard forage grass protocol used at the facility. In the main experiment, plants were established approximately five weeks before vernalisation. Vernalisation lasted approximately eight weeks before the start of freezer treatment. This step was included because winter stress responses in perennial forage grasses depend strongly on prior cold acclimation.



Figure 9. Established plants arranged in trays in the greenhouse before transfer to cold-acclimation conditions.

3.2.5 Preparation before ice treatment

After cold acclimation, trays were prepared for freezer treatment. Before treatment, the plants were clipped to a uniform height so that all pots entered the freezers with a comparable canopy height. As shown in Figure 10, the trays were then submerged in water to make soil moisture as even as possible. They were lifted from the water and allowed to drain, after which water and soil were removed from the outside of the beakers.



Figure 10. Saturation and drainage of established plants before freezer treatment to standardise soil moisture.

The holes in the trays were sealed with duct tape to reduce leakage during ice formation, as shown in Figure 11. Tape was first applied across one direction so that all holes in each row were covered. A second layer was then applied in the opposite direction to improve sealing. Based on preliminary tests, the two freezers were set to -5°C . At least five days were needed for the soil in all pots to freeze completely. Water was added only after the soil was fully frozen.



Figure 11. Tray holes sealed with two layers of duct tape to limit leakage during ice formation.

Temperature was recorded continuously throughout freezer treatment using four data loggers. Two loggers were placed in each freezer, with one logger on each of the two upper shelves that held the experimental trays, as illustrated for one freezer in Figure 12. This arrangement measured temperature at the levels where the samples were positioned.



Figure 12. Placement of two temperature data loggers on the upper two sample shelves in one freezer; the same arrangement was used in both freezers.

3.2.6 Ice application

A cold ice-water mixture was prepared in a 10 L bucket by adding three scoops of ice and then filling the bucket with cold water to the 5 L mark (Figure 13, left). Each tray was briefly removed from the freezer, and cold water was added to the marked level in the beakers assigned to the ice treatment (Figure 13, right). The

water formed a shallow ice layer approximately 1 cm above the soil surface. The tray was then returned to the freezer before the next tray was treated.



Figure 13. Preparation and application of the ice treatment. From left to right: the cold ice-water mixture and the addition of the mixture to the frozen beakers.

The temperatures recorded on the sample shelves were kept close to -5 degrees C during the treatment period. This temperature was selected to maintain ice cover while reducing the risk that the treatment mainly measured direct freezing tolerance. The intended stress was an ice-induced injury caused by restricted gas exchange and anaerobic conditions.

3.2.7 Treatment duration

The protocol development phase considered treatment duration as a key variable because early tests showed limited visible injury after short exposure. Meadow fescue and tall fescue were exposed to ice encasement for 5, 9, and 15 days, whereas timothy was exposed for 10, 22, and 30 days. These species-specific durations were used to test whether short, intermediate, and longer exposures produced measurable differences in post-treatment plant cover among genotypes. A non-ice control of the check genotype was included at each duration.

Because freezer capacity was limited, the species groups were treated in sequence rather than at the same time. Meadow fescue and tall fescue from batch 1 were treated first. Timothy from batches 1 and 2 entered the freezers after those treatments had ended. As a result, the species groups had different numbers of greenhouse recovery days by the common assessment date.

3.2.8 Post-treatment transition and recovery

At the end of each treatment duration, trays were removed from the freezer in the condition shown in Figure 14 and transferred to a vernalisation chamber for two days. This step allowed a gradual transition from the frozen state before

greenhouse recovery. After the transition period, trays were moved to the greenhouse for recovery.



Figure 14. Tray condition after removal from the freezer and before the two-day transition in the vernalisation chamber.

Plants were allowed to recover under greenhouse conditions. Two days after transferring to the greenhouse, trays were photographed from above using the setup shown in Figure 15. Additional photographs were taken during recovery to follow green tissue recovery and regrowth over time. This repeated imaging approach was chosen because ice injury may appear gradually after thawing and re-aeration.



Figure 15. Top-view recovery imaging setup, with the GoPro camera positioned above a tray in the greenhouse.

3.2.9 Recovery imaging

Recovery images were captured using a GoPro camera positioned above the trays. Images were taken from a top-view angle at approximately three-day intervals so that green cover and canopy recovery could be followed over time. The same imaging approach was used throughout the recovery period to minimise variation caused by camera position. The Module 1 results reported in this thesis used the common calendar-date assessment on 6 May 2026.

3.3 Module 2: Validation of image-derived measurements against harvested biomass

3.3.1 Plant material and experimental scale

The source greenhouse experiment included three forage grass groups: timothy, perennial ryegrass, and tall fescue. Each species included 100 genotypes with four replicates per genotype. As illustrated in Figure 16, this gave 400 pots per species and 1200 pots in total. Module 2 used this material to test the reliability of the image-derived measure, with harvested biomass used as the reference.



Figure 16. Greenhouse scale of the source experiment for Module 2, with 400 pots per species arranged on benches.

The plant material included advanced breeding material, parental material, and progeny from selected crosses. This broader material provides a range of plant sizes for image-based phenotyping. The validation analysis reported in this thesis used a selected subset of timothy pots.

3.3.2 Pot preparation and growth conditions

Plants were grown in 1.5 L pots with a diameter of approximately 14 cm and a surface area of approximately 154 cm². The pots were filled with fertilised soil

and arranged on greenhouse benches, as shown in Figure 17. Seeds were broad sown in each pot according to the greenhouse order sheet, and all pots were managed in the same way until a well-established plant stand had developed.



Figure 17. Pots filled with fertilised soil and arranged on a greenhouse bench before seedling establishment.

The experiment was maintained under well-irrigated conditions throughout the growth and imaging period. This avoided an imposed drought treatment while the image-derived measure was evaluated against harvested biomass.

3.3.3 Randomisation

Pots were arranged randomly using the labelled seed packets and order sheet shown in Figure 18. Each pot had a plot number, entry number, genotype name, row position, and column position. This randomised layout was used to reduce the influence of greenhouse position on growth and image-derived traits.



Figure 18. Materials used to implement the randomised greenhouse layout. From left to right: labelled seed packets organised by pot position and the order sheet used during sowing.

3.3.4 Clipping between imaging rounds

Plants were clipped to a common height after each imaging round, as illustrated in Figure 19. This provided a common starting height for the next regrowth period. Clipping was carried out separately for each species according to the greenhouse schedule, but the same procedure was used across species.

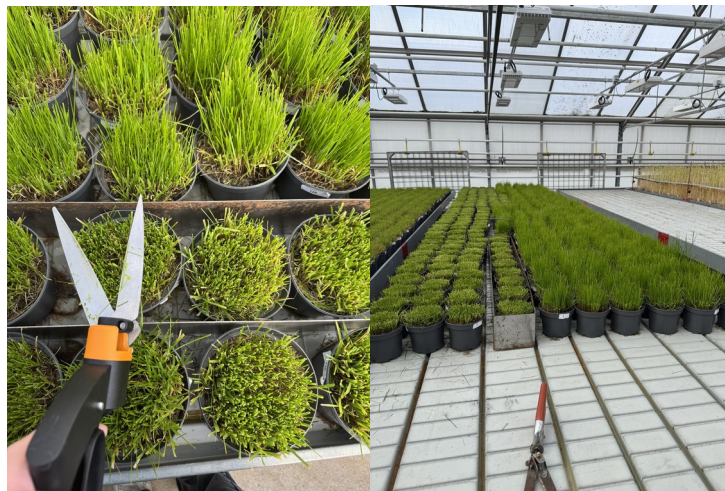


Figure 19. Uniform clipping after an imaging round. From left to right: clipping individual pots to a common height and the greenhouse rows after clipping.

3.3.5 Repeated imaging during regrowth

Pots were photographed repeatedly during regrowth using the GoPro setup shown in Figure 20. One pot was photographed at a time to reduce distortion and improve consistency among images. At each round, images were taken before clipping. The plants were then clipped to a common height and allowed to regrow

before the next round. The image series therefore recorded the regrowth that had accumulated between clippings.



Figure 20. Repeated GoPro imaging of individual pots during regrowth.

Although clipping created a common starting height, genotypes regrew at different rates. By the next imaging round, canopy height was uneven among pots (Figure 21).



Figure 21. Uneven canopy height before a later imaging round. Plants had been clipped to a common height after the previous image, but regrowth differed among pots before the next image was taken.

3.3.6 Manual biomass sampling for validation

A destructive biomass subset was collected to validate the image-derived measurement. Ten genotypes with clear visual differences in plant amount were selected within the sampled species. For each selected genotype, four pots were harvested, giving 40 pots per species.

Plants were cut at pot level as part of the biomass sampling workflow summarised in Figure 22. All aboveground biomass from each pot was weighed immediately to obtain green mass. The samples were then dried and weighed again to obtain dry matter mass. These measurements provided a direct biomass reference for evaluating whether the image-derived measurement represented real differences in plant amount. Biomass measurements were conducted for timothy and tall fescue; the correlation analysis reported in this thesis used the timothy subset ($n = 40$ pots).



Figure 22. Sampling biomass workflow. From left to right: selected pots, clipping of aboveground biomass at pot level, and bagged samples placed in the drying cabinet before dry-mass determination.

3.4 Module 3: Semi-controlled field ice-encasement experiment at Lännäs

3.4.1 Field site and plant material

The semi-controlled field experiment was established at the Lännäs site shown in Figure 23 to study ice encasement injury under outdoor conditions. The experiment included ten forage grass varieties: two timothy varieties, four meadow fescue varieties, and four tall fescue varieties. The timothy varieties were Snorri and Grindstad. The meadow fescue varieties were Tored, SW Revansch, Valtteri, and Cosmopolitan. The tall fescue varieties were Swaj, Karolina, Barelite, and Tamiko.



Figure 23. Experimental field site at Lännäs, showing established plots and treatment markers.

3.4.2 Field design

The field design comprised three blocks arranged according to the randomised plan in Figure 24. Each variety had one ice treatment and one control treatment in each block, giving 60 plots in total. Varieties and treatments were randomised within blocks according to this plan.

Column	Block	3	3	3	3	3	3	3	3	3	3
6	Treatment	Ice1	Control	Ice1	Control	Control	Control	Control	Ice1	Control	Control
5	Treatment	Control	Ice1	Control	Ice1	Ice1	Ice1	Ice1	Control	Ice1	Ice1
	Main plot	21	22	23	24	25	26	27	28	29	30

Column	Block	2	2	2	2	2	2	2	2	2	2
4	Treatment	Ice1	Control	Ice1	Ice1	Ice1	Ice1	Control	Ice1	Control	Control
3	Treatment	Control	Ice1	Control	Control	Control	Control	Ice1	Control	Ice1	Ice1
	Main plot	11	12	13	14	15	16	17	18	19	20

Column	Block	1	1	1	1	1	1	1	1	1	1
2	Treatment	Ice1	Control	Ice1	Control	Control	Ice1	Control	Control	Ice1	Ice1
1	Treatment	Control	Ice1	Control	Ice1	Ice1	Control	Ice1	Ice1	Control	Control
	Main plot	1	2	3	4	5	6	7	8	9	10
	Row	1	2	3	4	5	6	7	8	9	10

Figure 24. Randomised three-block field plan showing the positions of Ice and Control plots for the ten varieties.

3.4.3 Establishment and pre-winter preparation

The field was sown on 2 September 2025, and the plants were allowed to establish under natural field conditions. On 10 November 2025, the metal rings shown in Figure 25 were placed in the field to create a controlled ice layer over selected treatment plots during winter. No fertiliser was applied during the experiment.



Figure 25. Metal rings used for semi-controlled field ice-encasement treatment. From left to right: a ring installed around an experimental subplot, a side view of the installed ring, and rings stored before placement in the field.

3.4.4 Snow removal and ice formation

In January 2026, snow was present at the site. Flags were placed to mark the ice-treatment plots, as shown in Figure 26, before snow was removed from the corresponding metal rings. Control plots were left under natural conditions and were not manipulated.



Figure 26. Flags marking the field subplots assigned to the ice treatment.

Water was added to the ice-treatment rings in three steps. On 22 January 2026, 4 L of water was added to each marked treatment ring. On 23 January 2026, an additional 8 L of water was added because some rings leaked. On 28 January 2026, water was added again to make the ice layer more uniform. The final target ice layer was approximately 8 cm, fully covering the green plant parts inside the rings. When new snow fell during the treatment period, snow was removed from the ice-treatment rings to maintain the treatment.

3.4.5 Ice duration and spring observation

The ice layer remained for about two months. Figure 27 shows the Lännäs plots during snowmelt on 19 March 2026, and most of the ice had melted by 27 March. By April 2026, the ice had disappeared. Field photographs were taken during the ice period and after melting to document the ice layer inside the rings and the spring condition of the plots. The final paired Ice and Control photographs used for the spring-recovery assessment were taken on 13 May 2026.

Regional temperature context was obtained from the Swedish Meteorological and Hydrological Institute. The historical Lännäs station was no longer active in 2026, so daily mean, minimum, and maximum air temperature were obtained for Västmarkum A (station 138070; 63.1243° N, 18.2518° E), the nearest active station with the required daily series, approximately 30 km from the field site. Quality-controlled data were used through April 2026, and provisional recent data were used for 1–13 May (SMHI, 2026).



Figure 27. Ice-treatment plots at Lännäs during snowmelt on 19 March 2026.

3.4.6 Visual assessment of spring condition

The final Ice, Control, and Overview photographs from all 30 main plots were examined visually. Photographs were compared side by side, as illustrated by the examples in Figure 28, with attention to the amount and distribution of visible green shoots and the extent of browning inside each metal ring. The Ice photographs were ranked by visual greenness. The eight greenest subplots and the eight subplots with the most extensive browning were selected for illustration. Each Ice photograph was also compared with the corresponding Control photograph from the same genotype and block. No automated image analysis, numerical plant-cover calculation, or inferential test was used. The visual categories are descriptive, and near-complete browning does not demonstrate plant death.



Figure 28. Examples of the final spring photographs used for the visual assessment of green shoots and browning within the metal rings.

3.4.7 Context observations outside the experimental rings

Additional photographs of the surrounding grassland, including the paired views in Figure 29, showed patchy variation in spring conditions outside the experimental rings. Because these photographs came from a separate area with an unknown local stress history, they were used only as general context and not to infer the cause of responses in the flat experimental field.



Figure 29. Patchy spring condition in grassland near Lännäs, outside the experimental rings.

4. Results

4.1 Module 1: Plant cover at the final assessment after controlled ice encasement

The results reported in this section used timothy (TT) data from batches 1 and 2 and tall fescue (TF) and meadow fescue (MF) data from batch 1. As shown in Figures 30–32, plant cover at the final image assessment on 6 May 2026 varied among cultivars and treatment-duration groups in all three species. Meadow fescue and tall fescue were evaluated after 5, 9, and 15 days of ice encasement, whereas timothy was evaluated after 10, 22, and 30 days. Each figure presents cultivar means from three replicates, the overall mean across ice-treated cultivars, and the mean of the non-ice control.

The duration profiles differed among species. Mean cover in meadow fescue decreased across the three duration groups. Tall fescue had a negligible cover in the 5-day group but higher values in the 9- and 15-day groups. Timothy reached its highest overall mean in the 22-day group rather than the 10-day group. Thus, the final-cover values did not show a common monotonic response to treatment duration. Because the duration series differed between timothy and the two fescue species, numerical comparisons among species were not made.

4.1.1 Meadow fescue

In the 5-day panel of Figure 30, mean plant cover among ice-treated meadow fescue cultivars ranged from 0.7% to 4.9%. SW Revansch had the highest mean cover (4.9%). Tored ranked second (3.0%), while Lipoche and Valtteri both had a 2.9% cover. Castelle, Cosmopolitan, and Santtu had lower mean values of 1.5%, 1.3%, and 0.7%, respectively.

The range was narrower in the 9-day group, extending from 0.8% for Tored to 3.1% for SW Revansch. SW Revansch remained the highest-ranked cultivar, and Valtteri ranked second with 2.5% cover. Lipoche had 1.5% cover, followed by Santtu at 1.2%. Cosmopolitan and Castelle both had 0.9% cover. The marked reduction in Tored, from 3.0% at 5 days to 0.8% at 9 days, shifted this cultivar from the upper to the lower end of the descriptive ranking.

The mean cover was lower again in the 15-day group, and no cultivar exceeded 1.1%. Valtteri had the highest value (1.1%), followed by Lipoche (0.9%). SW Revansch and Cosmopolitan both had 0.8% cover, whereas Tored and Castelle had the lowest values of 0.2% and 0.1%, respectively. Every meadow fescue cultivar had a lower final-cover value in the 15-day group than in the 5-day group. Valtteri, Lipoche, and SW Revansch nevertheless remained among the higher-ranked cultivars across the duration series.

The mean across ice-treated cultivars declined from 2.5% at 5 days to 1.6% at 9 days and 0.6% at 15 days. The corresponding control means were 2.4%, 1.7%, and 0.4%. The absolute difference between the ice-treated mean and the control mean was therefore no greater than 0.2 percentage points at any duration, and the direction of the difference changed across groups.

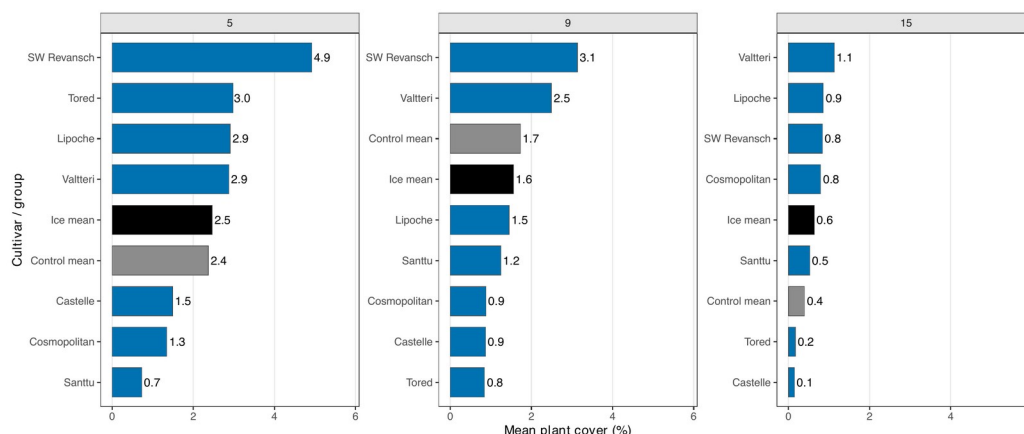


Figure 30. Mean plant cover of meadow fescue cultivars at the final image assessment on 6 May 2026 after 5, 9, and 15 days of controlled ice encasement. Coloured bars show ice-treated cultivar means ($n = 3$ per cultivar and duration), black bars show the mean across ice-treated cultivars, and grey bars show the non-ice control mean.

4.1.2 Tall fescue

Figure 31 shows that final plant cover in the 5-day tall fescue group was below the plotted resolution: all cultivar means, the ice-treated mean, and the control mean rounded to 0.0%. The 9-day group showed a wider range, from 0.0% for Swaj to 2.8% for Tamiko. Karolina and Barelite also had comparatively high values of 2.6% and 2.3%, respectively. Eleanora had 1.2% cover, while RGT Noug and Iliade had 0.3% and 0.1%.

The ranking changed substantially in the 15-day group. Eleanora had the highest mean cover (4.0%), compared with 1.2% in the 9-day group. Karolina remained near the top of the ranking with a 1.9% cover. Iliade and RGT Noug had 0.8% and 0.6%, respectively. In contrast, Barelite decreased from 2.3% at 9 days to 0.2% at 15 days, and Tamiko decreased from the highest value at 9 days (2.8%) to a value that rounded to 0.0% at 15 days. Swaj rounded to 0.0% at both durations.

The mean across ice-treated tall fescue cultivars was 0.0%, 1.3%, and 1.1% in the 5-, 9-, and 15-day groups, respectively. The corresponding control means were 0.0%, 1.7%, and 0.7%. Thus, the ice-treated mean was 0.4 percentage points below the control at 9 days, but 0.4 percentage points above the control at 15 days. The reversal in direction and the marked changes in cultivar ranking did not

indicate a consistent descriptive separation between the ice-treated group and the control.

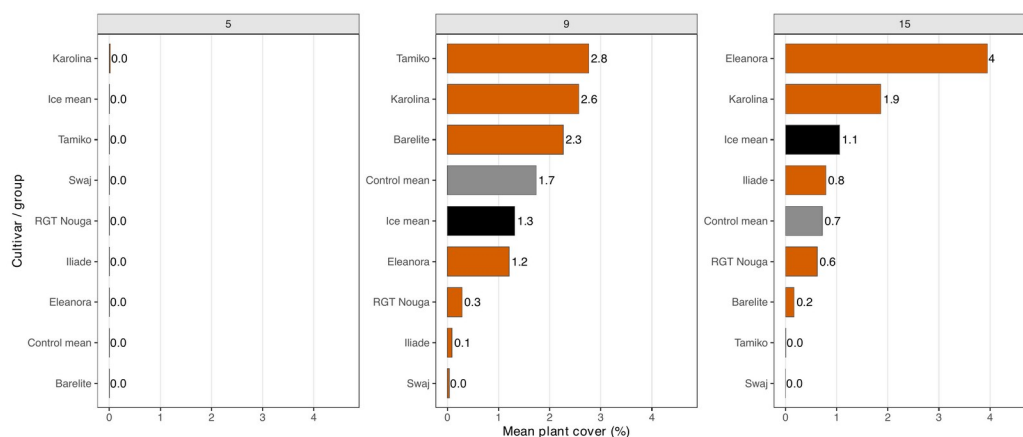


Figure 31. Mean plant cover of tall fescue cultivars at the final image assessment on 6 May 2026 after 5, 9, and 15 days of controlled ice encasement. Coloured bars show ice-treated cultivar means ($n = 3$ per cultivar and duration), black bars show the mean across ice-treated cultivars, and grey bars show the non-ice control mean.

4.1.3 Timothy

In the 10-day panel of Figure 32, mean plant cover among ice-treated cultivars ranged from 0.2% to 7.4%. Switch had the highest mean cover (7.4%), followed by Maoi (6.1%) and Snorri (6.0%). Grindstad had a 3.2% cover. Rhonia, Nuutti, and Rakel had lower values of 2.3%, 1.0%, and 0.2%, respectively. The mean across ice-treated cultivars was 3.7%, compared with 2.6% for the control.

All cultivar means were higher in the 22-day group than in the 10-day group. At 22 days, cultivar means ranged from 14.1% for Maoi to 24.4% for Snorri. Switch ranked second with 22.5%, and Grindstad ranked third with 19.8%. Rakel, Nuutti, and Rhonia had mean cover values of 16.8%, 15.4%, and 14.2%, respectively. The ice-treated mean was 18.2%, which was 0.8 percentage points above the control mean of 17.4%.

The mean cover in the 30-day group ranged from 4.1% for Maoi to 18.8% for Nuutti. Nuutti, Snorri (18.7%), and Switch (17.8%) formed a closely spaced upper group. Rakel and Rhonia had 12.1% and 11.8% cover, whereas Grindstad had 10.0%. The ice-treated mean was 13.3%, compared with 11.9% for the control. The ice-treated mean was therefore lower at 30 days than at 22 days but remained at 1.4 percentage points above the 30-day control mean.

Snorri and Switch ranked among the three cultivars with the highest mean cover at all three treatment durations. Other cultivars changed positions more strongly. Nuutti moved from the second-lowest value at 10 days to the highest value in 30 days. Maoi showed the opposite pattern: it ranked second at 10 days

but had the lowest mean cover at both 22 and 30 days. Grindstad ranked third at 22 d but was near the lower end of the 30-day ranking.

Across the three timothy duration groups, the ice-treated mean increased from 3.7% at 10 days to 18.2% at 22 days and then decreased to 13.3% at 30 days. The corresponding control means followed by the same general profile (2.6%, 17.4%, and 11.9%). The final-cover summaries therefore did not show a progressive decline with increasing ice duration.

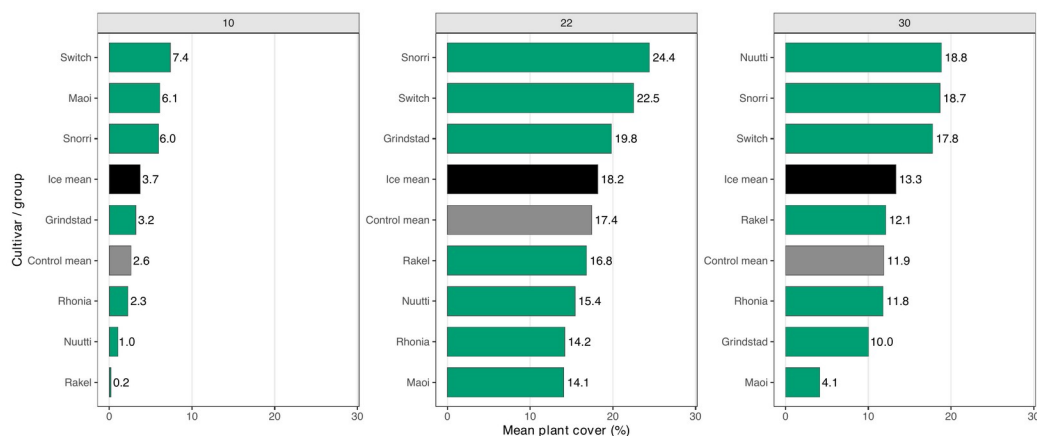


Figure 32. Mean plant cover of timothy cultivars at the final image assessment on 6 May 2026 after 10, 22, and 30 days of controlled ice encasement. Coloured bars show ice-treated cultivar means ($n = 3$ per cultivar and duration), black bars show the mean across ice-treated cultivars, and grey bars show the non-ice control mean.

4.1.4 Freezer conditions

Both freezers were operated at the same nominal set point, but the shelf-level logger records were not identical between freezers or between the two monitored shelves. Frost also accumulated in different locations within the two units (Figure 33). Because only one point was measured on each shelf, the temperatures experienced at individual pot positions could not be determined.



(a) Freezer 1



(b) Freezer 2

Figure 33. Spatially uneven frost accumulation in the two freezers used for Module 1. The experimental pots occupied the two upper shelves. The different frost patterns indicate differences in airflow or heat exchange, but the photographs do not quantify temperature at individual pot positions.

4.2 Module 2: Validation of white-pixel count against measured biomass

Harvested green and dry mass were used as reference measurements to test the reliability of the image-derived white-pixel count in 40 timothy pots. White pixels are non-black pixels representing visible plant material in each image.

As shown in Figure 34, the white-pixel count was positively correlated with both reference measurements. The correlation was $r = 0.61$ ($p < 0.001$) for green mass and $r = 0.50$ ($p = 0.001$) for dry mass. These results show that white-pixel count tracked biomass differences to a moderate extent, with a somewhat stronger relationship for green mass.

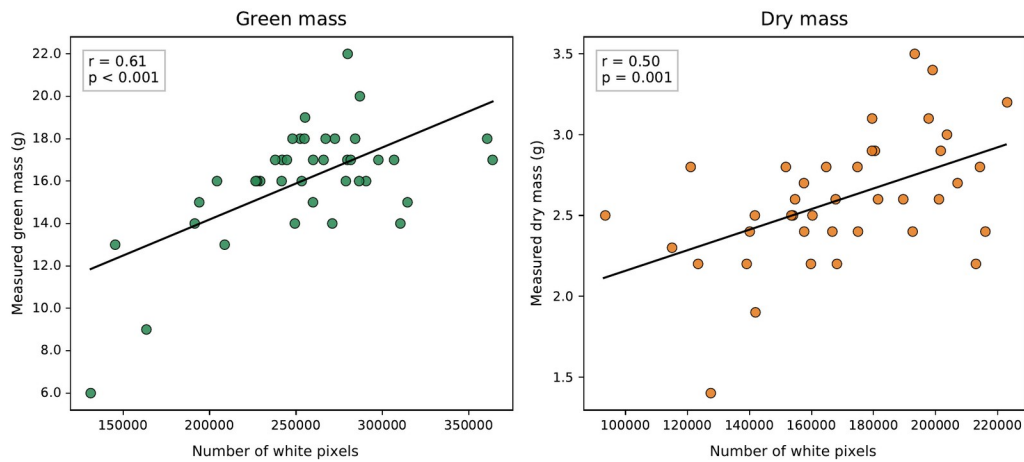


Figure 34. Relationships between the number of white pixels in the images and measured green mass (left) or dry mass (right) in the timothy validation subset ($n = 40$ pots). White pixels are the non-black pixels and represent visible plant material. Each point represents one pot, and the lines show fitted linear relationships. Pearson correlation coefficients and p values are shown within each panel.

4.3 Module 3: Visual spring condition after field ice encasement at Lännäs

Visual inspection of the final field photographs showed clear contrasts among the 30 Ice subplots. The greenest group was dominated by Grindstad, a timothy genotype, and Karolina, a tall fescue genotype. All three field replicates of both genotypes were included among the eight greenest Ice subplots. The remaining two positions were occupied by one Tored meadow fescue subplot and one Swaj tall fescue subplot. At the other end of the visual ranking, several subplots were almost entirely brown and had few or no clearly visible green shoots. These photographs show strong variation among genotypes, but they do not by themselves demonstrate plant death or statistically significant differences.

4.3.1 Visual ranking of individual Ice subplots

Karolina in Block 3 and Grindstad in Block 2 showed the densest and most evenly distributed green shoots. Grindstad in Block 1 and Karolina in Block 1 were also clearly green. The third field replicates of these genotypes, Grindstad in Block 3 and Karolina in Block 2, were less green but remained in the upper visual group. One Tored subplot in Block 2 and one Swaj subplot in Block 3 completed the group of eight greenest Ice subplots.

The subplots with the most extensive browning included Tored, Snorri, Barelite, Valtteri, and Cosmopolitan. Most of the soil surface was exposed in

these photographs, and the plant material inside the rings was predominantly brown. Two Snorri subplots, two Barelite subplots, and two Valtteri subplots were included in this lower visual group. Table 1 shows the eight greenest Ice subplots and the eight subplots with the most extensive browning.

Table 1. Visual ranking of the eight greenest Ice subplots and the eight Ice subplots with the most extensive browning at Lännäs, Sweden, on 13 May 2026. Within each group, rank 1 indicates the greenest subplot or the subplot with the most extensive browning, respectively.

Visual group	Photograph 1	Photograph 2	Photograph 3	Photograph 4
Most green Ranks 1–4				
	Rank 1 Karolina (TF) Block 3, Plot 24	Rank 2 Grindstad (TT) Block 2, Plot 17	Rank 3 Grindstad (TT) Block 1, Plot 2	Rank 4 Karolina (TF) Block 1, Plot 8
				
	Rank 5 Grindstad (TT) Block 3, Plot 22	Rank 6 Karolina (TF) Block 2, Plot 20	Rank 7 Toreð (MF) Block 2, Plot 18	Rank 8 Swaj (TF) Block 3, Plot 30
Near- complete browning Ranks 1–4				
	Rank 1 Toreð (MF) Block 1, Plot 3	Rank 2 Valtteri (MF) Block 3, Plot 23	Rank 3 Snorri (TT) Block 1, Plot 1	Rank 4 Barelite (TF) Block 1, Plot 9
				
	Rank 5 Snorri (TT) Block 3, Plot 29	Rank 6 Cosmopolitan (MF) Block 3, Plot 27	Rank 7 Valtteri (MF) Block 2, Plot 15	Rank 8 Barelite (TF) Block 2, Plot 13

Note. Ranks are based only on visual comparison of the final Ice photographs. The photographs are unannotated copies of the original images and were reduced only for display. Near-complete browning indicates very little visible green tissue and does not confirm plant death.

4.3.2 Visual comparison of Ice and Control subplots

Many Ice subplots were browner or had fewer green shoots than the matching Control subplots, although the size of the difference varied among genotypes and blocks. The Control photograph showed how much green growth the same genotype had in the same block without the added ice treatment. This made it easier to judge whether poor spring conditions were associated with the added ice or with generally weak spring growth.

Timothy (TT). Grindstad showed substantial green growth in all three Ice photographs and was also green in the corresponding Controls. In contrast, the Snorri Ice subplots were largely brown, while visible green shoots remained in the Control photographs. The visual difference between Ice and Control was therefore clearer for Snorri than for Grindstad.

Tall Fescue (TF). Karolina was visibly green in both treatments and remained the visually greenest tall fescue genotype in the Ice photographs. One Karolina Ice subplot was less dense than the other two, showing that the field response was not identical across blocks. Swaj retained scattered green shoots in several photographs. Tamiko and Barelite were generally sparse and brown in the Ice treatment, although their Controls often retained more visible green tissue.

Meadow Fescue (MF). Visual spring condition was generally weak, and several Ice and Control subplots had little green tissue. Tored varied strongly among blocks: one Ice subplot was included in the upper visual group, whereas another was among the most extensively browned. Valtteri was weak in both treatments, and two of its Ice subplots were included in the lower visual group. Cosmopolitan and SW Revansch showed scattered green shoots in some plots, but this pattern was not consistent across blocks.

4.3.3 Descriptive correspondence with the controlled-environment screening

Timothy (TT). The field pattern did not match the controlled environment ranking in Module 1. Snorri performed comparatively well in the controlled experiment but was largely brown in the field Ice photographs. Grindstad showed the opposite pattern and was consistently among the greenest field subplots.

Tall Fescue (TF). Karolina showed the clearest agreement between the two environments. It was among the stronger tall fescue genotypes in the controlled experiment and was also clearly green in the field Ice photographs. The other tall fescue genotypes did not show the same consistent pattern.

Meadow Fescue (MF). The field photographs did not show a clear match with the controlled ranking. SW Revansch and Valtteri had comparatively strong results in parts of the controlled experiment, but their field Ice photographs were mostly sparse or brown. Tored also varied strongly among field blocks.

Overall, the visual field assessment showed only partial agreement with the controlled environment screening. Karolina provided the clearest example of similar performance in both settings, whereas the timothy and meadow fescue rankings differed. The field assessment was visual, and the two experiments differed in environment and stress duration. The comparison is therefore descriptive and does not constitute a formal validation of field performance.

5. Discussion

5.1 Main findings

This study addressed three distinct methodological questions. Module 1 asked whether a controlled ice-encasement protocol could produce interpretable differences in meadow fescue, tall fescue, and timothy. Module 2 asked whether a simple image-derived measure of plant amount agreed with harvested green and dry biomass under well-irrigated greenhouse conditions. Module 3 provided a field reference for the controlled experiment. The three modules therefore evaluated the stress protocol, the image measurement, and the transfer of controlled responses to the field, respectively.

The controlled workflow was practical and followed the main biological stages of winter stress, but the final-cover results did not show a consistent response to treatment duration. In Module 2, harvested biomass was used as the reference, and the image-derived measure showed moderate positive relationships with both green and dry mass. The field comparison showed partial correspondence. Karolina, a tall fescue genotype, was the clearest example of relatively strong performance in both settings, while the rankings of timothy and meadow fescue differed between the freezer and the field. The protocol is worth developing further, but the current data are not sufficient for a final ranking of ice-encasement tolerance.

5.2 Module 1: What the controlled protocol achieved and what needs refinement

The overall sequence of the protocol was appropriate for studying winter injury. Greenhouse establishments provided actively growing plants. Cold acclimation then prepared them for winter conditions before the soil was frozen, and water was added. After ice treatment, a short transition in the vernalisation chamber reduced the abrupt change from the freezer to the greenhouse. The recovery period was an essential part of the test. Damage caused during ice cover or re-aeration may appear only after thawing, while plants that initially look damaged may regrow from surviving crowns. This agrees with the staged approach described by Gudleifsson and Bjarnadottir (2014) and with recent work showing that recovery after ice removal contains important information about injury (Gendjar et al., 2024; Merewitz, 2021).

The choice of approximately -5°C was intended to keep the ice stable while limiting the contribution of direct freezing injury. This distinction is important. Ice encasement restricts gas exchange, promotes oxygen depletion, and allows carbon dioxide and anaerobic metabolites to accumulate. These processes differ

from injury caused mainly by extracellular freezing and cellular dehydration (Gudleifsson, 2010; Höglind et al., 2010). The selected temperature was therefore a practical compromise, not proof that freezing injury was absent. At -5°C , freezing stress can still contribute to the response. In addition, the present design included a non-ice control only for the check genotype at each duration. It could not separate ice effects from low-temperature effects for every genotype.

A useful screening protocol should produce enough injury to separate genotypes without causing complete and uniform death. The duration results did not yet meet this criterion consistently. Mean meadow fescue cover declined from 5 to 15 days, but the ice-treated mean remained very close to the corresponding check control at every duration. Tall fescue showed almost no cover in the 5-day group and higher cover after 9 and 15 days, which is not a plausible dose response to increasing ice exposure. Timothy also lacked a progressive decline: the overall mean was highest after 22 days, and the ice-treated mean was slightly above the check of control at all three durations. The changes among duration groups therefore reflected more than treatment severity alone.

The longer durations used for timothy were justified because this species is known to tolerate prolonged ice cover. Höglind et al. (2010) reported maximum LD50 values of about 63 days for timothy under their conditions, compared with about 20 days for perennial ryegrass. The maximum exposure of 30 days in the present study may therefore have been too short to create a stable separation among timothy genotypes. Extending the timothy series beyond 30 days is reasonable if recovery remains high. However, simply lowering the freezer temperature would be a poor substitute for longer exposure because it would increase the risk of turning the protocol into a freezing test. Duration should be extended only after the actual temperature distribution has been brought under better control.

The timing of assessment also affected interpretation. Recovery images were taken approximately every three days, but the analysis used plant cover on one calendar date, 6 May 2026. Pots removed after shorter treatments had been spent longer in greenhouse recovery than pots removed after longer treatments. Meadow fescue and tall fescue were also treated before the timothy batches, so timothy had the shortest recovery period by 6 May. The final value combined treatment of injury, time since thaw, and subsequent growth. Duration-specific check controls partly reflected the different recovery times, but only for one genotype per species. A stronger analysis would compare genotypes at the same number of days after ice removal, for example 7, 14, and 21 days. Recovery rate, time to renewed growth, and area under the recovery curve could then use the full repeated-imaging series.

Temperature variation inside the freezers was another important limitation. Both units had the same nominal set point, but logger records differed between

freezers and between the two used shelves. The photographs also show that frost accumulated along different walls and surfaces in the two freezers (Figure 33). These visible patterns are consistent with uneven airflow or heat transfer, although the photographs are not temperature measurements. One logger on each shelf described the general shelf environment but could not resolve conditions at all pot positions. Plants close to a cold wall or a large frost deposit may therefore have experienced a different thermal history from plants near the centre of the same shelf.

The ice layer also differed between the controlled and field experiments. In the freezer, water formed a shallow layer of about 1 cm above the soil surface. In the field, the target layer was about 8 cm and fully covered the green plant parts inside each ring. The controlled treatment may therefore have stressed the crown and soil surface while leaving part of the shoot exposed. The field treatment more closely represented whole-canopy encasement and probably restricted gas exchange more strongly. Ice depth, air spaces in the soil, and contact between ice and plant tissue can all change the severity of anoxia (Gudleifsson & Bjarnadottir, 2014). This difference is one reason why the two environments should not be expected to produce identical rankings.

Final plant cover also reflects inherent growth and regrowth, not only stress tolerance. Because most genotypes lacked their own non-ice control, a genotype with high cover after treatment may have been vigorous before treatment rather than unusually tolerant of ice. Future runs should include paired ice and frozen non-ice controls for every genotype. Recovery could then be expressed relative to the corresponding control or to pre-treatment cover. With three replicates and no uncertainty estimates in the current figures, the observed rankings should remain descriptive.

The next protocol iteration should first map temperature at several pot-level positions in each loaded freezer. Freezer, shelf, and position should be treated as experimental blocks, and genotypes should be balanced across them and repeated in independent runs. Once temperature variation is better controlled, a small pilot should identify a suitable ice-encasement duration for each species. The selected duration should cause moderate injury and differences in recovery without causing uniform death. Timothy could be tested beyond 30 d if shorter treatments continue to show little separation. Standardised ice depth, paired ice and frozen non-ice controls, and assessment at fixed times after thaw would make the results easier to interpret. Measurements of oxygen or carbon dioxide under the ice could also confirm that the treatment causes restricted gas exchange rather than mainly freezing injury.

5.3 Module 2: Do image-derived measurements reflect biomass?

The Module 2 results support the use of image-derived plant amount as a proxy for biomass in the timothy validation subset. White-pixel count was positively correlated with green mass ($r = 0.61$) and dry mass ($r = 0.50$). Both relationships were statistically supported. In practical terms, the image measurement captured about 37% of the variation in green mass and 25% of the variation in dry mass, based on r squared. The images therefore contained useful biomass information, but they did not replace direct weighing.

The stronger relationship with green mass is biologically reasonable. A top-view RGB image records visible leaf area and canopy spread. These features are closely related to the amount of fresh, hydrated tissue present at harvest. Dry mass also depends on tissue water content, leaf thickness, stem proportion, and tissue density. Plants with similar projected area can therefore differ in dry weight. Overlapping leaves and upright growth can also hide biomass from a two-dimensional image.

The result agrees with earlier phenotyping studies. RGB imaging has been used to measure cover, projected area, colour, senescence, regrowth, and forage biomass (Castro et al., 2020; De Swaef et al., 2021; Jiang & Li, 2020; Jiang & Yang, 2022; Yang et al., 2020). Module 2 tested this relationship directly. Harvested green and dry mass served as the reference values for the image-derived white-pixel count. The positive correlations show that white-pixel count captured part of the measured biomass variation.

Several limits should be kept in mind. The reported analysis used 40 timothy pots selected to show clear visual differences. This selection increased the range of plant sizes and may have strengthened the observed relationship. It does not show that the same correlations apply to the full population, to other species, or to plants with a narrower range of biomass. White-pixel count is also a simple segmentation measure. Leaf angle, overlap, shadows, pot background, and lighting can add error. Validation in the full genotype set and in tall fescue would show how stable the relationship is across material. Calibration with more advanced area or volume traits may improve prediction, especially for dry mass.

Within these limits, Module 2 answered its intended question. The positive correlations show that the image-derived measurement reflected real biomass differences in this timothy subset. Direct biomass measurements remain important for calibration, but repeated imaging can add temporal resolution without destroying the plants.

5.4 Weather context for the Lännäs field experiment

The 2026 temperature record supports the observed timing of ice formation and melting. The former Lännäs SMHI station was no longer active, so regional weather context was taken from Västmarkum A, the nearest active station with the required daily temperature series, about 30 km from the field site. The mean temperature at this station was -8.2°C in January and -11.8°C in February 2026. The daily means on the three water-addition dates were -4.3 , -6.8 , and -16.7°C on 22, 23, and 28 January, respectively, and the daily maximum remained below 0°C on each date. Conditions were therefore suitable for rapid freezing of the added water (Swedish Meteorological and Hydrological Institute [SMHI], 2026).

From 22 January to 27 March, the regional daily mean averaged -6.1°C , and 43 of 65 d had a mean below 0°C . This helps explain why the artificial ice persisted for about two months. March then shifted towards melting conditions. Its monthly mean was $+1.4^{\circ}\text{C}$, and 25 d had a minimum below 0°C and a maximum above 0°C . On 19 March, when the plots were photographed during snowmelt, the daily minimum and maximum were -0.8 and $+8.1^{\circ}\text{C}$. On 27 March, when most ice had melted, they were -3.4 and $+7.7^{\circ}\text{C}$. Mean temperature was $+3.5^{\circ}\text{C}$ in April and $+5.8^{\circ}\text{C}$ from 1 to 13 May, supporting continued thaw and spring growth before the final field photographs.

These station data provide regional context rather than the temperature inside the metal rings. The station is about 30 km away, and snow removal, ring depth, ice thickness, wind exposure, and local soil conditions could all change the plot-level thermal regime. The weather record is therefore useful for explaining when freezing and melting were likely, but it cannot identify the stress experienced by each subplot.

5.5 Correspondence between the controlled and field experiments

Module 3 was a field reference, not a test designed to prove that Module 1 was correct. The field experiment combined a thicker ice layer, natural temperature fluctuations, snow, soil moisture, possible disease pressure, and freeze-thaw events. The final assessment was visual and did not include an automated cover estimate or an inferential test. Visible greenness on 13 May could reflect winter survival, spring phenology, and current growth, while browning did not by itself prove plant death. Some green shoots may also have come from seeds that had blown into a subplot and germinated, so not every green shoot necessarily belonged to the sown genotype. Exact agreement with the freezer rankings was therefore unlikely.

The clearest correspondence occurred in tall fescue. Karolina was among the stronger genotypes after 9 and 15 d in Module 1, and all three Karolina field

replicates were among the eight greenest ice-treated subplots. This repeated field pattern makes Karolina the strongest cross-environment candidate in the current data. The other tall fescue genotypes were less consistent. Barelite and Tamiko ranked relatively high after 9 d in the freezer but were sparse or brown in several field photographs, while Swaj was weak in the controlled results but retained green shoots in one of the upper-ranked field subplots.

Timothy showed a clear mismatch. Snorri and Switch were among the strongest controlled-environment genotypes across the tested durations, but Switch was not included in the field comparison, and Snorri was largely brown in two field ice subplots. Grindstad showed the opposite pattern. Its controlled result was intermediate and declined relative to other timothy genotypes at 30 d, yet all three Grindstad field replicates were among the greenest group. Meadow fescue also lacked a stable match. SW Revansch and Valtteri ranked relatively high in parts of Module 1 but were mostly sparse or brown in the field. Tore varied from one of the greenest field subplots to one of the most browned.

It is therefore not possible to name one most ice-tolerant genotype across all three species. The species received different treatment durations, recovery time was not standardised by days after thaw, most genotypes lacked paired controls, and the field ranking was descriptive. Among the genotypes tested in both environments, Karolina showed the most consistent evidence of good recovery. This should be treated as a candidate result to test again, not as a final tolerance classification.

The disagreement with other genotypes does not mean that the controlled experiment failed. It shows that the freezer treatment captured only part of the field stress. A repeatable controlled screen can still be useful if it measures a defined response and gives stable rankings across independent runs. Field experiments remain necessary to determine whether that response contributes to performance under variable winters. Repeating the Lännäs comparison across years and using numerical cover or biomass measurements would provide a stronger test of correspondence.

5.6 Overall assessment

Module 1 established a workable sequence for growing, acclimating, freezing, encasing, thawing, and repeatedly imaging forage grasses. At this stage, its main value is protocol development. The inconsistent duration profiles, unequal recovery time, temperature variation within the freezers, shallow laboratory ice, and limited control structure prevent the current final-cover values from being used as a final tolerance screen. The next experiments should improve temperature mapping, include paired controls, compare plants at the same time after thawing, and select a suitable duration for each species.

Using harvested green and dry biomass as the reference, Module 2 showed that the image-derived measurement reflected biomass differences to a moderate extent. Module 3 showed why the controlled protocol still needs a field reference. Karolina performed relatively well in both environments, but the timothy and meadow fescue rankings did not transfer consistently. Overall, the results support further development of the controlled ice-encasement protocol and continued use of image analysis for repeated measurements. Controlled and field experiments should be interpreted together because they describe different parts of winter response and cannot replace one another.

6. Conclusion

The controlled ice-encasement workflow provided a practical method for plant establishment, cold acclimation, soil freezing, ice application, thawing, and greenhouse recovery. The current results should be viewed as an evaluation of the protocol rather than a final ranking of ice-encasement tolerance. Temperature varied between freezer locations; recovery time was not fully standardised, and the shallow laboratory ice layer differed from the field treatment. Future experiments should measure temperature at several pot positions and adjust freezer to maintain a thermal environment that is as stable and uniform as possible. Each genotype should have a matched frozen, non-ice control. Plants should also be assessed after the same number of recovery days, and a suitable treatment duration should be identified separately for each species.

Module 2 showed that image analysis could detect meaningful differences in plant biomass. Image-derived measurements were positively related to both harvested green and dry biomass, with a stronger relationship for green biomass. Repeated image analysis can therefore be used to follow plant growth and regrowth without destroying the plants. Harvested biomass measurements are still needed for calibration and when precise biomass values are required.

The field reference showed only partial correspondence with the controlled experiment. Karolina, a tall fescue genotype, had the clearest evidence of relatively good performance in both environments, but the rankings of timothy and meadow fescue did not transfer consistently. This does not mean that the controlled protocol failed. The controlled and field experiments represent different stress conditions and answer different questions. The controlled protocol can provide a repeatable way to study ice-encasement responses, while field experiments remain necessary to determine whether these responses are also important under variable winter conditions.

References

- Biswas, D. K., Coulman, B., Biliget, B., & Fu, Y.-B. (2020). Advancing bromegrass breeding through imaging phenotyping and genomic selection: A review. *Frontiers in Plant Science*, 10, Article 1673. <https://doi.org/10.3389/fpls.2019.01673>
- Castro, W., Marcato Junior, J., Polidoro, C., Osco, L. P., Gonçalves, W., Rodrigues, L., Santos, M., Jank, L., Barrios, S., Valle, C., Simeão, R., Carromeu, C., Silveira, E., Jorge, L. A. de C., & Matsubara, E. T. (2020). Deep learning applied to phenotyping of biomass in forages with UAV-based RGB imagery. *Sensors*, 20(17), Article 4802. <https://doi.org/10.3390/s20174802>
- Chalise, D. P., & Merewitz, E. (2025). Leaf and crown cuticular wax responses in annual bluegrass (*Poa annua* L.) plants exposed to ice encasement. *Journal of Agronomy and Crop Science*, 211(1), Article e70009. <https://doi.org/10.1111/jac.70009>
- Dalmanndóttir, S., Jørgensen, M., Rapacz, M., Østrem, L., Larsen, A., Rødven, R., & Rognli, O. A. (2017). Cold acclimation in warmer extended autumns impairs freezing tolerance of perennial ryegrass (*Lolium perenne*) and timothy (*Phleum pratense*). *Physiologia Plantarum*, 160(3), 266-281. <https://doi.org/10.1111/ppl.12548>
- De Swaef, T., Maes, W. H., Aper, J., Baert, J., Cougnon, M., Reheul, D., Steppe, K., Roldán-Ruiz, I., & Lootens, P. (2021). Applying RGB- and thermal-based vegetation indices from UAVs for high-throughput field phenotyping of drought tolerance in forage grasses. *Remote Sensing*, 13(1), Article 147. <https://doi.org/10.3390/rs13010147>
- Ergon, Å. (2017). Optimal regulation of the balance between productivity and overwintering of perennial grasses in a warmer climate. *Agronomy*, 7(1), Article 19. <https://doi.org/10.3390/agronomy7010019>
- European Commission. (2026). *Grassland and livestock dynamics: How grazing management sustains and shapes European grasslands* (Analytical Brief No. 13). Directorate-General for Agriculture and Rural Development. https://agriculture.ec.europa.eu/document/download/b397715c-d526-4dd7-af26-adbdfe43af6d_en?filename=analytical-brief-13-grassland_en.pdf

- Gendjar, M., Chalise, D. P., Beaudry, R., & Merewitz, E. (2024). Influence of controlled condition ice encasement of creeping bentgrass and annual bluegrass on plant recovery, gas evolution, and metabolites. *Journal of the American Society for Horticultural Science*, 149(4), 230-242. <https://doi.org/10.21273/JASHS05394-24>
- Gudleifsson, B. E. (2010). Ice tolerance and metabolite accumulation of herbage crops in Iceland and impact of climate change. *Icelandic Agricultural Sciences*, 23, 111-122.
- Gudleifsson, B. E., & Bjarnadottir, B. (2014). Estimating ice encasement tolerance of herbage plants. In D. K. Hinch & E. Zuther (Eds.), *Plant cold acclimation: Methods and protocols* (Methods in Molecular Biology, Vol. 1166, pp. 225-240). Springer. https://doi.org/10.1007/978-1-4939-0844-8_17
- Höglind, M., Bakken, A. K., Jørgensen, M., & Østrem, L. (2010). Tolerance to frost and ice encasement in cultivars of timothy and perennial ryegrass during winter. *Grass and Forage Science*, 65(4), 431-445. <https://doi.org/10.1111/j.1365-2494.2010.00762.x>
- Jiang, Y., & Li, C. (2020). Convolutional neural networks for image-based high-throughput plant phenotyping: A review. *Plant Phenomics*, 2020, Article 4152816. <https://doi.org/10.34133/2020/4152816>
- Jiang, Y., & Yang, Y. (2022). High-throughput phenotyping for plant growth and biomass yield of switchgrass under a controlled environment. *Grass Research*, 2, Article 4. <https://doi.org/10.48130/GR-2022-0004>
- Merewitz, E. (2021). Current outlook on ice encasement stress and management strategies in turfgrasses. *HortTechnology*, 31(5), 561-565. <https://doi.org/10.21273/HORTTECH04907-21>

- Pashapu, A. R. (2025). *Investigations of frost and ice cover tolerance and genetic changes over time in cultivars of timothy (Phleum pratense L.) with different climate adaptation (NMBU Thesis 2025:28)* [Doctoral dissertation, Norwegian University of Life Sciences]. NMBU Open Research Repository. <https://hdl.handle.net/11250/3187789>
- Swedish Meteorological and Hydrological Institute. (2026). *Air temperature observations at Västmarkum A (station 138070): Daily mean, minimum, and maximum values* [Data set]. SMHI Open Data. Retrieved August 17, 2026, from <https://www.smhi.se/data/hitta-data-for-en-plats/ladda-ner-vaderobservationer/airtemperatureMean24h/138070>
- Swedish University of Agricultural Sciences. (2026). *Vall-Reparera: Precision agriculture to repair winter-damaged swards in Northern Sweden*. <https://www.slu.se/en/research/research-catalogue/projekt/v/vall-reparera/>
- Thorsen, S. M., Roer, A.-G., & van Oijen, M. (2010). Modelling the dynamics of snow cover, soil frost and surface ice in Norwegian grasslands. *Polar Research*, 29(1), 110-126. <https://doi.org/10.1111/j.1751-8369.2010.00157.x>
- Thorsen, S. M., & Höglind, M. (2010). Assessing winter survival of forage grasses in Norway under future climate scenarios by simulating potential frost tolerance in combination with simple agroclimatic indices. *Agricultural and Forest Meteorology*, 150(9), 1272-1282. <https://doi.org/10.1016/j.agrformet.2010.05.010>
- United Nations. (2015). *Transforming our world: The 2030 Agenda for Sustainable Development*. <https://sdgs.un.org/2030agenda>
- Yang, W., Feng, H., Zhang, X., Zhang, J., Doonan, J. H., Batchelor, W. D., Xiong, L., & Yan, J. (2020). Crop phenomics and high-throughput phenotyping: Past decades, current challenges, and future perspectives. *Molecular Plant*, 13(2), 187-214. <https://doi.org/10.1016/j.molp.2020.01.008>

Popular science summary

Grass grown for livestock must survive winter to provide feed in spring. Ice can cover plants for weeks, limiting gas exchange with the air. Oxygen may run low, and harmful substances may build up in plant cells. Finding grasses that recover well after ice cover could help farmers maintain a reliable supply of feed.

This thesis tested a method for studying grasses under ice, compared photographs with harvested plant weight, and examined recovery in a field experiment in northern Sweden.

In the freezer experiment, meadow fescue, tall fescue and timothy were grown in pots and gradually prepared for cold conditions. The pots were then placed in freezers set to about -5°C , and water was added to form a shallow ice layer. After different periods under ice, the plants returned to a greenhouse. Photographs recorded how much green plant cover was visible during recovery.

The procedure was practical to carry out, but longer ice treatments did not always lead to less green cover. Temperatures differed between freezer locations, and some plants had more time to recover before the final assessment. The ice layer was also much shallower than in the field. These differences made it difficult to separate damage caused by ice from the effects of temperature and later growth. The results therefore do not establish which grass species or variety is most tolerant of ice cover.

The second experiment tested whether photographs could indicate how much plant material was present. Image measurements were compared with the fresh and dry weight of plants harvested from 40 selected timothy pots. Pots with more visible plant material generally had greater weight. The relationship was moderate and was stronger for fresh weight than for dry weight. Photographs can therefore help follow growth without cutting the plants, but weighing is still needed for accurate biomass measurements. These findings apply to the timothy plants tested; other plant material needs further assessment.

The field experiment at Lännäs provided a comparison under outdoor winter conditions. Karolina, a tall fescue variety, showed relatively good recovery in both the freezer and the field. The timothy and meadow fescue varieties did not show a consistent match between the two settings. Field observations were based on visible green shoots and browning, so they could not confirm plant death or prove differences in tolerance.

Together, the experiments provide a starting point for improving winter tests in forage breeding. Future tests need more uniform temperatures, the same recovery time for all plants, and matching plants kept frozen without added ice for each variety. Photographs can support repeated measurements, while field trials are needed to check whether the results hold under changing winter conditions.

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