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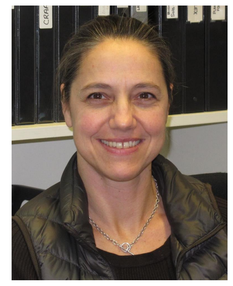

# Development of an indoor freezing tolerance test for switchgrass

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## Introduction

- ✓ The capacity to survive low sub-freezing temperature is the most important factor contributing to the winter survival of perennial grasses cultivated in northern regions.
- ✓ A screening method to determine the lethal temperature killing 50% of a population (LT50) of perennial forage crops was developed by our team (Bertrand et al. 2014; Castonguay et al. 2009).
- ✓ Improvement of freezing tolerance in alfalfa was associated with a decrease in starch and an increase in total soluble sugars concentrations in alfalfa crows during fall (Castonguay et al., 2011).

**The objective was to evaluate the LT50 and the non-structural carbohydrate concentrations of three contrasting switchgrass cultivars**

## Materials & methods

### Plant material

- Three switchgrass cultivars: Dacotah, Cave-in-Rock, and Kanlow
- Plant preparation (2015):
  - *July* - 640 plants per cv. were seeded in multi-cells, stratified at 4°C, and grown under 16h photoperiod and 25/22°C Day/Night temperature for five weeks;
  - *September* - plants were transferred for five weeks in a greenhouse (no fertilization, no heating) for fall acclimation;
  - *October* - plants were transplanted in pots (10 plants per pot)
    - 32 pots per cv. were used to assess (October 2015) LT50 and non-structural carbohydrate concentration (total soluble sugar and starch) of non-acclimated (NA) plants;
    - 32 pots per cv. were transferred in an unheated greenhouse for cold acclimation (CA) and overwintering for later assessment (February 2016) of the LT50 and the non-structural carbohydrate concentration of CA plants.

### Freezing tolerance and biochemical analysis

Evaluation of LT50

- Programation of a step-wise decrease of temperature (2°C/h) followed by a 1.5h-plateau at -2, -6, -8, -10, -12, -14, -16, -18, -20, -22, -24, -26°C.
- Retrieve 4 pots (40 plants) per temperature and transfer pots under regrowth conditions (16h photoperiod, 25/22°C)
- Count number of surviving plants after 4-wk

Non-structural carbohydrates

- Retrieve 4 pots (40 plants) of NA (October) and CA plants (February)
- Harvest crowns
- Freeze-dry, grind, extract sugar, quantify sugars by HPLC

## Results & discussion

### Freezing tolerance

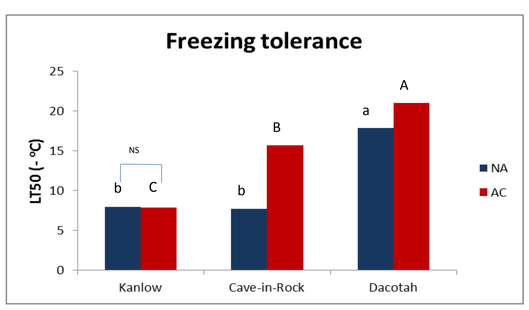


**Fig 1**. Freezing tolerance of non-acclimated (NA) and cold-acclimated (CA) plants of 3 cultivars of switchgrass expressed as $LT_{50}$.

- Lowland cv. Kanlow had a low freezing tolerance (LT50 of -8°C) under NA conditions and a similar freezing tolerance under CA conditions (LT50 of -8°C).
- Upland cv. Cave-in-Rock had a low freezing tolerance (LT50 of -8°C) under NA conditions and a significantly higher freezing tolerance under CA conditions (LT50 of -16°C).
- Upland cv. Dacotah, originating from the most northern region, had a high freezing tolerance (LT50 of -18°C) under NA conditions and a significantly freezing tolerance under CA conditions (-21°C).

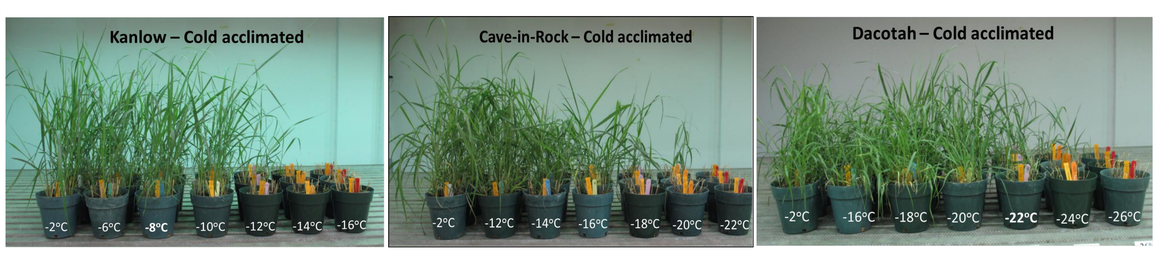


**Fig 2**. Visual assessment of the 4-wk regrowth of cold-acclimated switchgrass plants of cultivars Kanlow, Cave-in-Rock, and Dacotah

### Non-structural carbohydrates

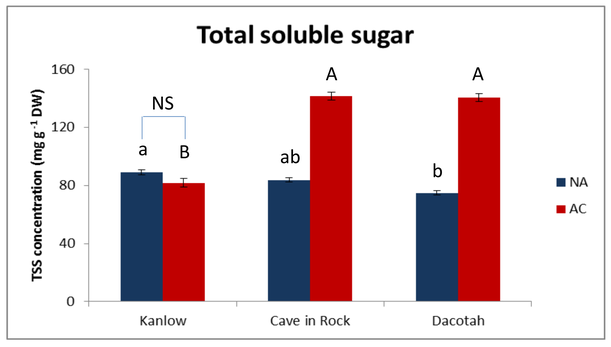


**Fig 4**. Concentration of total soluble sugars (TSS) in non-acclimated (NA) and cold acclimated (CA) crowns of three switchgrass cultivars.

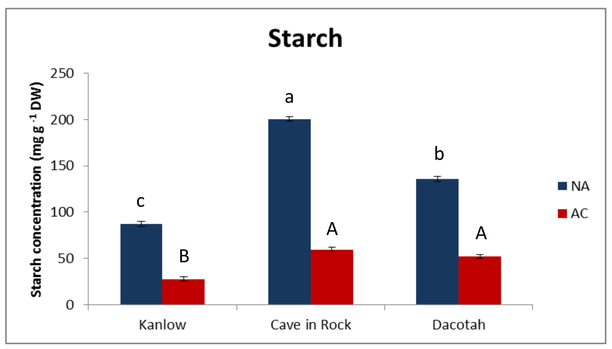


**Fig 5**. Starch concentration in non-acclimated (NA) and cold acclimated (CA) crowns of three switchgrass cultivars.

- ✓ TSS concentration was similar for the three cultivars under NA conditions.
- ✓ TSS concentration increased in response to CA only in the two upland cultivars (Cave-in-Rock and Dakotah).

- ✓ Starch concentration was lower in all CA compared to NA crowns in all three cultivars.
- ✓ Starch concentration in upland cultivars were higher than in the lowland cultivar in either NA and CA conditions.

## Conclusions

- **The indoor freezing tolerance test allowed us to precisely assess the LT50 of three switchgrass cultivars.**
- **Significant LT50 differences were observed between lowland and upland cultivars as well as between NA and CA plants.**
- **The concentration of cold-regulated carbohydrates was linked with the level of freezing tolerance reached by each cultivar: 1) larger accumulation of TSS in response to CA in upland cultivars with high levels of freezing tolerance, 2) lower starch concentration in the lowland cultivar that showed no hardening capacity.**

## References

Bertrand et al. 2014. Methods in Plant Biology 1166, pp. 35-41.
Castonguay et al. 2011. Crop Science 51: 2132-2144.
Castonguay et al. 2009. Crop Science 49: 809-818.

## Acknowledgements

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