Freezing Tolerance of Two Legume Cover Crops for Upper Midwest High Tunnel Conditions

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Introduction

Winter annual legume cover crops have the potential to provide both soil health benefits and nitrogen fertility to organic high tunnel systems in the Upper Midwest. Red clover (*Trifolium pratense*) and hairy vetch (*Vicia villosa*) are two popular legume species grown as winter annual cover crops that generally survive winters in the field. However, extreme daily temperature fluctuation in high tunnels during winter, when temperatures can range from 20°C during the day to -18°C at night, may affect freezing tolerance. Researchers have explored freezing tolerance for these plants under standard acclimation conditions meant to mimic the open field, but no research has studied freezing tolerance following conditions similar to those in high tunnels. Investigating differences in freezing tolerance between plants that experience high tunnel temperature fluctuations and plants that do not may shed light on challenges or opportunities for growing winter annual cover crops in high tunnels.


Red clover


Hairy vetch

Objectives

Quantify the effects of **standard** or **simulated high tunnel** acclimation conditions on legume response to freezing temperatures observed in the field, with specific objectives to:

1. Compare survival and calculate LT_{50} (temperature at which 50% of individual plants survive)
2. Quantify biomass accumulation after freezing events
3. Analyze nitrogen (N) content of vetch after freezing events

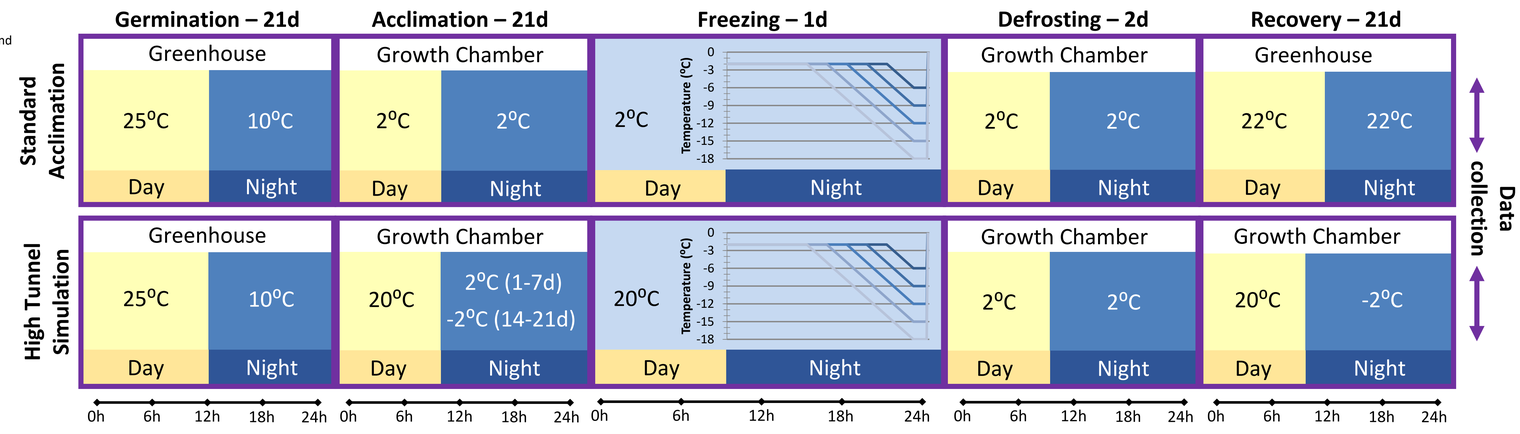
Methods

A factorial design was used with three factors: 1) Species (red clover, hairy vetch), 2) Acclimation conditions (standard, high tunnel simulation), and 3) Freezing treatment (-6 °C, -9 °C, -12 °C, -15 °C, or -18°C). This resulted in a total of 20 unique treatment combinations (2 species x 2 acclimation conditions x 5 freezing temperatures).

Thirty individual plant replicates were used for each treatment combination. Plants were germinated in the greenhouse and allowed to grow for 21 d, then moved to an acclimation treatment in a growth chamber for 21 d. Plants were then frozen overnight after soaking in water for 30 m and draining for 60 m. After freezing, plants defrosted for 2 d, and were then placed in secondary recovery for 21 d (Fig. 1). Each plant was fertilized weekly with 10 mL of organic fish/seaweed emulsion (Neptune's Harvest 2-3-1, Gloucester, MA), diluted to 100 ppm N. Plants were watered as needed.

After secondary recovery, plants were assessed for survival and living biomass was harvested. Biomass was dried at 60°C for 72 h, then weighed. Vetch biomass was ground, pooled to create large enough subsamples for analysis, then analyzed for percent N on a PYRO cube combustion analyzer (Elementar, Germany).

Figure 1. Light and temperature settings for all treatment combinations.



Results

Survival and LT_{50}

- Survival curve differences were driven by acclimation treatment.
- High survival at low freezing treatments limited accuracy of survival curves for high tunnel simulation.

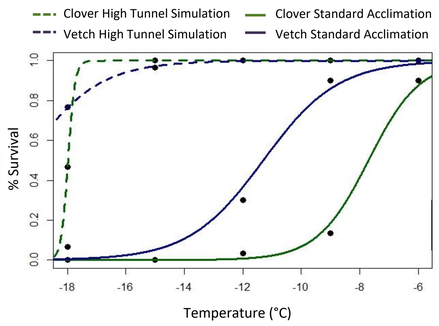


Figure 2. Logistic curves fitted to plant survival (0 = dead, 1 = alive) data for clover and vetch grown in high tunnel simulation and standard acclimation conditions. n = 30 plants for each data point.

- Plants grown in high tunnel simulation can survive lower freezing temperatures than plants grown in standard acclimation.
- LT_{50} for high tunnel simulations are less accurate because no freezing treatments experienced complete mortality.

Table 1. LT_{50} for clover and vetch grown in high tunnel simulation and standard acclimation conditions (± 1 SE). Values calculated using glm and predict function in R.

Species	High Tunnel Simulation	Standard Acclimation
Clover	-18.1 (± 1.7)	-7.6 (± 1.7)
Vetch	-19.2 (± 2.3)	-11.3 (± 1.6)

Biomass

- Clover accumulated less biomass following colder freezing treatments.
- Plants grown in high tunnel simulation accumulated more biomass following freezing than plants grown in standard acclimation.

- Vetch also accumulated less biomass following colder freezing treatments.
- Vetch from both acclimation treatments are sensitive to the difference between freezing temperatures of -6°C and -9°C.

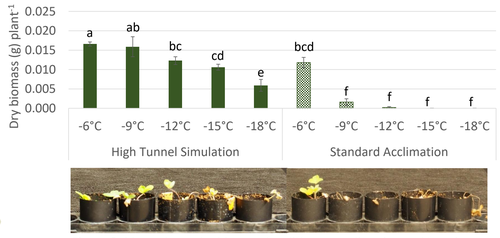


Figure 3. Mean clover dry biomass for different acclimation and freezing treatments, error bars represent 1 SE. Mean separation calculated using Dunn's test. For each acclimation/freezing treatment combination n = 30 plants. Pictures taken at time of biomass harvest, one representative plant for each acclimation/freezing treatment combination.

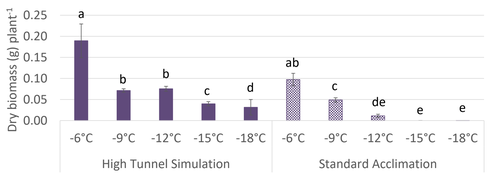


Figure 4. Mean vetch dry biomass for different acclimation and freezing treatments, error bars represent 1 SE. Mean separation calculated using Dunn's test. For each acclimation/freezing treatment combination n = 30 plants. Pictures taken at time of biomass harvest, one representative plant for each acclimation/freezing treatment combination.

Hairy Vetch Nitrogen Content

- Mean percent N in vetch was different over the range of freezing treatments, both within and between acclimation treatments.
- Opposing trends: high tunnel simulation plants have higher percent N following colder freezing treatments, whereas standard acclimation plants have lower percent N following colder freezing treatments.

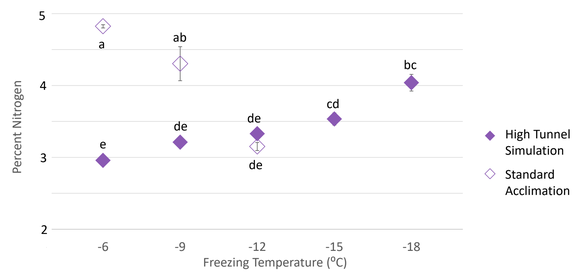


Figure 5. Mean percent nitrogen of vetch plants for all high tunnel simulation treatments and three standard acclimation treatments, error bars represent 1 SE. The two coldest treatments in standard acclimation did not regrow enough biomass for analysis. Mean separation was calculated using Tukey's HSD, n = 3 samples.

Conclusions

- The large temperature fluctuation in the high tunnel simulation did not reduce freezing tolerance for red clover or hairy vetch compared to standard acclimation.
- At all freezing temperatures, high tunnel simulation promoted more regrowth than standard acclimation in both species .
- Percent N in vetch varies with freezing temperature, directly for high tunnel simulation and inversely for standard acclimation. This suggests some fundamental difference in physiology between differently acclimated plants.

The results of this study suggest that winter annual cover crops may be able to withstand colder freezing events in a high tunnel than plants in the field. Future studies should compare survival of red clover and hairy vetch in the open field and in high tunnels to test this hypothesis.


Hairy vetch, standard acclimation, two weeks after -6 C freezing treatment.
Photo credit: T. Sooksa-nguan

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Hairy vetch flower.
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