Effect of Fungal Endophyte *Neotyphodium lollii* on the Freezing Tolerance of Perennial Ryegrass

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Introduction:

Perennial ryegrass (*Lolium perenne, L.)* is a common turfgrass widely grown for several key traits including quick germination, establishment and wear tolerance. Commercial varieties are commonly labeled as endophyte enhanced due to the mutualistic relationship with *Neotyphodium lolii* (Figure 1). These qualities make perennial ryegrass a common component in sports fields, golf courses and home lawns across a wide geographic range. Although perennial ryegrass is a cool-season grass it is one of the least winter hardy turfgrasses and does not perform consistently in northern climates. Current breeding efforts in increasing winter hardiness have yielded slow progress.

It is known that the fungal endophyte which commonly inhabits perennial ryegrass can confer tolerance to both abiotic and biotic stresses (Malinowski and Belesky, 2000). Research has also shown that the benefits endophytes offer can be related to stresses present at the location of coevolution (Kane, 2011). However, it is not known if endophytes can increase the winter hardiness of perennial ryegrass.

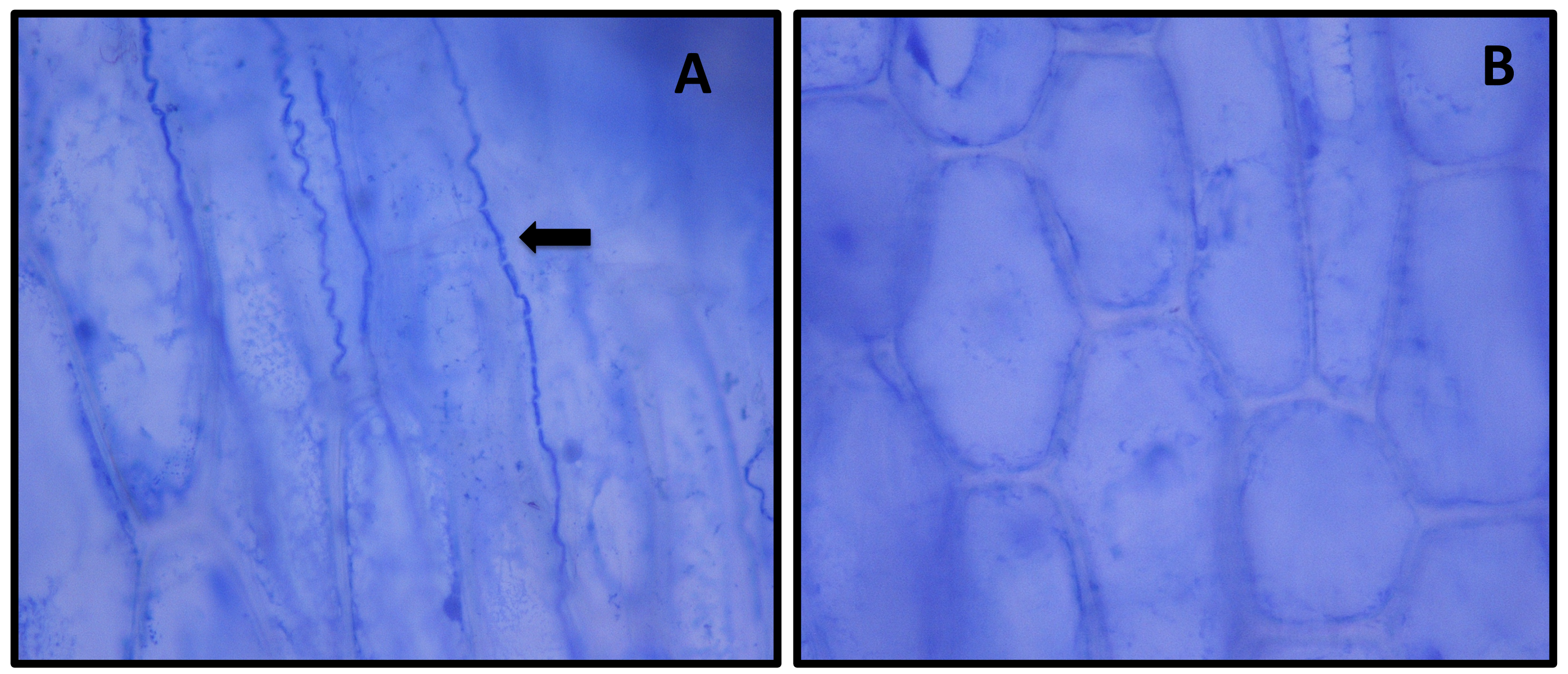

Figure 1. Compressed sheath tissue stained with aniline blue and enlarged to 40X. (A) Intercellular hyphal elements of *Neotyphodium lolii* in an E+ genotype (B) Sheath cells free of any hyphae in an E- genotype.

Objectives:

1. Determine the effect of endophyte on the freezing tolerance of perennial ryegrass.
2. Explore possible differential endophyte x host interactions using diverse germplasm.

Materials and Methods:

Plant Material

- Two accessions with greatly diverse backgrounds were chosen from the USDA Germplasm Resource Information Network.
- These accessions had been previously characterized for winter hardiness and freezing tolerance by Hulke et al., (2007; 2008).

Accession	Origin	Winter hardiness[1]	Freezing Tolerance (°C)[2]
PI 610806	Romania	4.9	-13.61
W6 11256	Turkey	1.5	-10.42

[1]Winter hardiness previously established by Hulke et al. (2007) using a 1-9 scale, 9 = best winter hardiness.

[2] LT50 established by Hulke et al. (2008).

Population Design

- Results from an immunoblot (ELISA) kit (Agronostics Ltd. Co.) identified populations consisting of only endophyte infected (E+) and uninfected (E-) plants from each accession (Figure 2).
- 10 genotypes per population were cloned across treatments and reps.
- Population size was designed based off of work by Bolaric et al., (2005) to maximize the effect of endophyte.

Figure 2. (A) Compressed sheath tissue near the crown was used to identify endophyte infected genotypes (B) This tissue was assayed using an ELISA specific to *Neotyphodium spp.* (C) Selected genotypes were propagated to produce the necessary quantity of clones (D) Clones were then grown to at least a five tiller growth stage.

Freezing Protocol

- In order to generate a lethal temperature 50 (LT50), an array of temperatures were used to allow a steady progression of dead to alive plant responses.
- Temperature treatments of -20, -18, -16, -14, -12 and -10 °C (Figure 3) were previously found to be effective.
- Prior to freezing, plants were acclimated for 14 days at 3 °C.
- For each freezing treatment, temperature within the freezing chamber was reduced to the target temperature at 1 °C h^{-1}.
- After the freezing treatment, plants were moved to a cold room for deacclimation for 3 days at 3 °C before returning to the greenhouse.

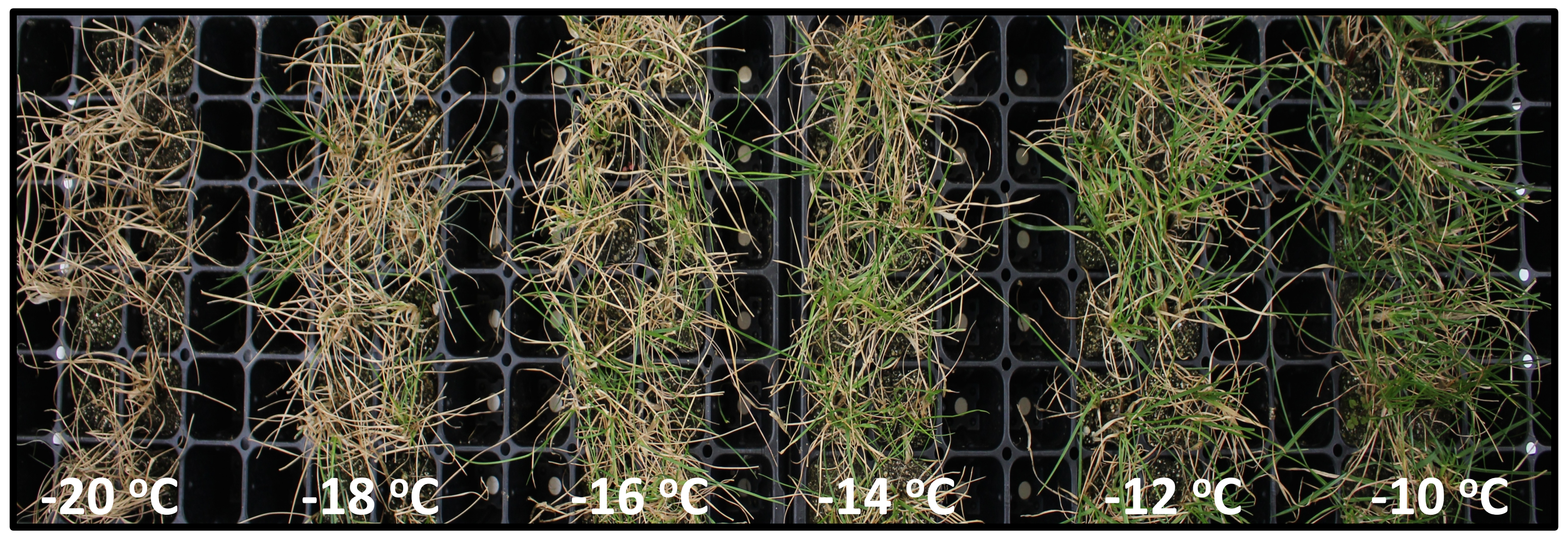

Figure 3. Progression of plant survival 21 days after freezing protocol was carried out.

Data Collection and Analysis

- A binomial scoring system was used to quantify the effects of freezing temperatures.
- These data generated proportions that were used to create LT50s for each population within each accession.
- Generalized linear models and graphs were generated in R to determine significant difference between populations.
- LT50 on a whole plant basis was calculated with 95% confidence intervals for each accession and endophyte status within accession.

Results:

- Analysis shows a significant difference between the two accessions, this is in agreement with the previous freezing tolerance data presented.
- Significant differences were found between the E+ and E- populations within PI 610806.
- No significant differences were found between E+ and E- populations within W6 11256.
- A graphical representation of plant survival shows the significant divergence at the LT50 in PI610806 E+ and E- populations and insignificant divergence in W6 11256 E+ and E- populations (Figure 4).

LT50, whole plant basis (°C)

Accession	95% lower bound	Mean	95% upper bound
E+ PI 610806	-16.58	-16.95	-17.32
E- PI 610806	-15.08	-15.47	-15.86
E+ W6 11256	-13.05	-13.39	-13.72
E- W6 11256	-12.95	-13.30	-13.65

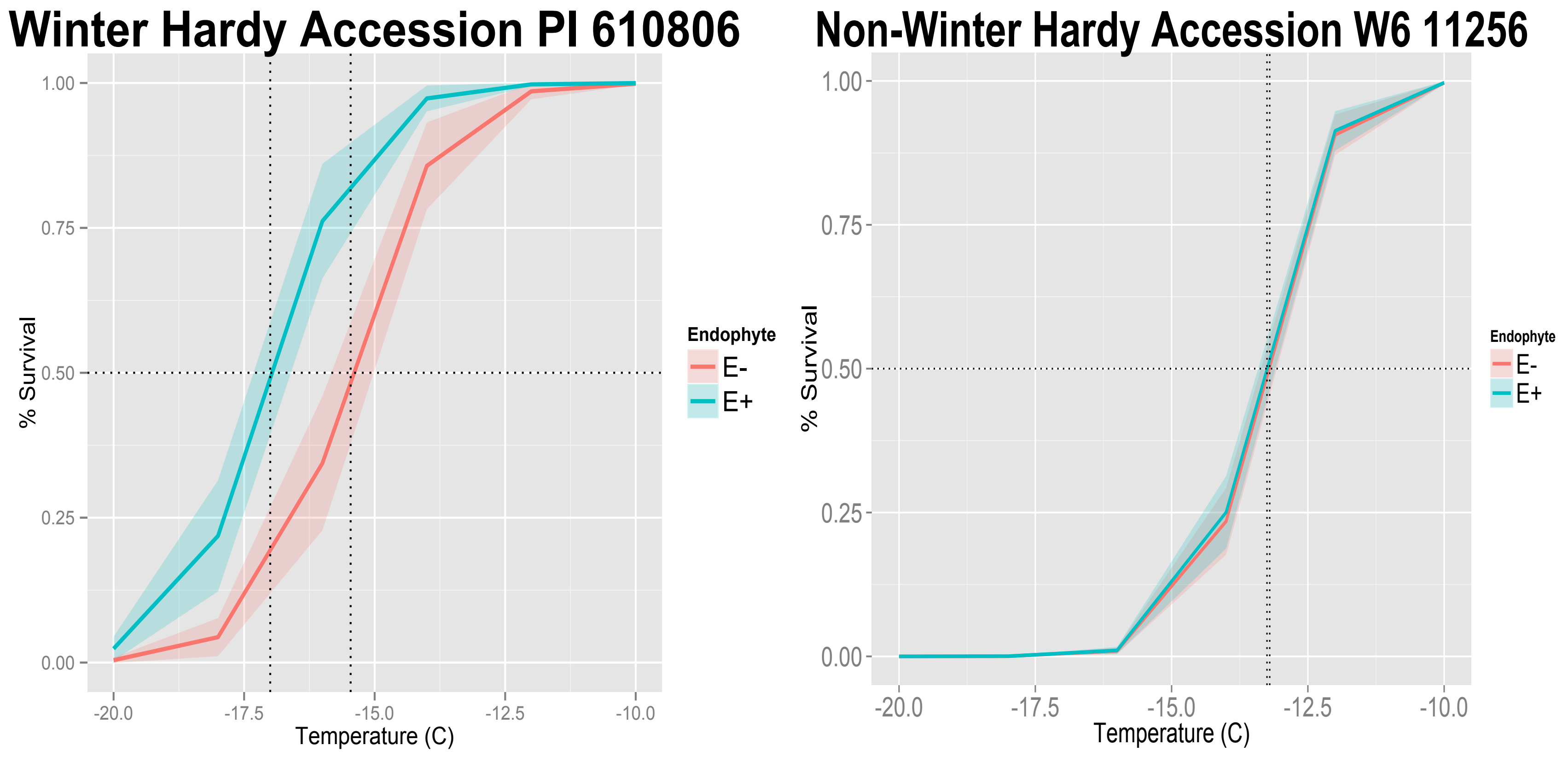

Figure 4. Logistic regressions for the two accessions, where the mean survival is represented by the solid color lines and the shaded regions represent the 95% CI for the prediction.

Conclusions:

- Endophyte infection had an effect on winter hardy accession PI 610806.
- This suggests that endophytes contribute to the plants survival by improving freezing tolerance.
- Results also indicate that effects are dependent on location of coevolution.
- This relationship can be exploited in a breeding program by introducing the endophyte from PI 610806 into germplasm with elite turf quality, but poor freezing tolerance.

Acknowledgements:

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References:

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