Southern Forest Nursery Management Cooperative

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RAPID DETERMINATION OF FREEZE DAMAGE TO LOBLOLLY PINE SEEDLINGS

by
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INTRODUCTION

Uncontrollable weather conditions, such as a freeze event, can result in significant seedling mortality regardless of quality of seedlings, site preparation or planting (Cameron and Lowerts 2007). Sudden freeze events can be damaging to forest seedling nursery crops, especially in the southeastern U.S. when sudden freeze events occur immediately preceded by above-normal warm temperatures (Zheng et al. 2012, Krasowski et al. 1993, South et al. 1993). While cold acclimation occurs with reduced photoperiod (sunlight) and lower temperatures, southern pines can de-acclimate within hours of unseasonable warm weather (Krasowski et al. 1993). This is of particular concern with increasing warm winters and periodic hard freezes in the southeastern U.S. A challenge in determining the sudden impact of a freeze event on forest seedlings is that it may take more than 2 weeks afterward for visible signs of damage to appear (Cameron and Lowerts 2007). Visible damage may include tip dieback or winter desiccation and stem and root discoloration (Semerci et al. 2020, South 2006). Significant reductions of survival can occur by outplanting seedlings that are unknowingly damaged by freeze events (South 2006). Since sudden freeze events preceded by above normal warm temperatures can occur during lifting season, decisions of whether seedlings were damaged in the nursery become more time sensitive.

To reduce the time-period in determining if seedlings have suffered irreversible freeze damage, following a freeze event, a rapid determination for nursery seedling health is needed. One such method may be the use of chlorophyll fluorescence that has been used to evaluate a plant's ability to tolerate low temperatures but can also determine the extent of damage caused by those stresses (Murchie and Lawson 2013, Maxwell and Johnson 2000, Groom and Baker 1992). Measurements of chlorophyll fluorescence can provide information on the state of the photosynthetic efficiency in photosystem II (PSII) which can frequently be the first sign of stress in the plant (Maxwell and Johnson 2000). More specifically, examining the ratio of; 1) variable fluorescence to 2) maximum fluorescence (F_vF_m) will provide a measure of the maximum efficiency of PSII, thus giving information of seedling damage to PSII by cold temperatures (Maxwell and Johnson 2000).

The objective of this study was to determine if chlorophyll fluorescence could be used as a tool to measure and evaluate the extent of freeze damage directly after a controlled freeze event. Our goal was to predict eventual seedling damage (mortality, growth reduction, etc) by correlating the reduction in PSII (F_vF_m) caused by a freeze event to the resulting severity of freeze injury or mortality in loblolly pine seedlings and to investigate the recovery time and loss of growth caused by the resulting freeze injury.

MATERIALS AND METHODS

Included in this study were nine genetic families representing three provenances (Coastal, Piedmont and Northern) of one-year old containerized loblolly pine seedlings obtained from the Tree Improvement Program at North Carolina State University. All paternal parents were a pollen mix comprised of pollen from 20 trees common to that region (Table 1). Two seedlings from each family were randomly assigned to 4 replications of 2 treatments which consisted of a freeze treatment and control treatment (18 seedlings total per replication and treatment). Seedlings were left in the greenhouse for a month with the temperature set to 35˚F to allow seedlings to acclimate naturally. Two weeks prior (1/14/21) to the experimental freeze, seedling were moved outside with containers covered in sand to reduce the chance of root damage during cold nights. During this 2-week period, low nighttime temperatures averaged 39.2˚F (Figure 1).

Seedlings in the freeze treatment were subjected to an experimental freeze on February 2, 2021. Seedlings were placed in a chest freezer, controlled by a 2-stage digital temperature thermometer (Inkbird ITC-308) set at 50˚F for 1 hour. Temperature was decreased at a rate of 9˚F per hour until a minimum temperature of 5˚F was reached. Seedlings then remained at 5˚F for 1 hour then gradually brought back to 50˚F again at a rate of 9˚F per hour. After the experimental freeze was complete, seedlings were once again placed in the greenhouse for post-freeze event measurements until outplanting.

Initial heights and root collar diameter (RCD) measurements were taken prior to the experimental freeze on both treatments. Pre- and post-freeze measurements of light adapted chlorophyll fluorescence were taken on one detached needle from each seedling using a Licor 6800 portable gas exchange system (Licor Inc, Lincoln, NE). Post-freeze measurements were taken 1, 3, 6, 8, 10, and 13 days after the freeze treatment. A visual assessment of foliage damage was taken 14 days post freeze. For the visual assessment, an average percent of damage foliage was obtained by 3 separate evaluations by SFNMC staff. After 41 days post-freeze, seedling mortality was recorded, and all seedlings (control and treated) were outplanted and watered every other day to observe growth throughout the growing season. Measurements were randomized by replication and treatment each measurement period.

RESULTS

Prior to the experimental freeze, there were no differences in provenance interactions or treatment effects on initial RCD, height or F_vF_m (Table 2). There was a difference in initial RCD with the Piedmont provenance having a smaller RCD (4.04 mm) compared to the Northern and Coastal provenances (average 4.31 mm) (Table 2). There were no effects of provenance on initial F_vF_m with an average F_vF_m of 0.602 across all provenances and treatments (Table 2).

To determine the immediate damage to PSII caused by the control freeze event, we measured F_vF_m prior to the experimental freeze and the day after in both the control and freeze treatments. There were no differences between the provenances caused by ambient conditions in the control treatment with an average reduction in F_vF_m of approximately 5% (Figure 2). However, the artificial freeze reduced F_vF_m in the freeze treatment from 41 to 72% with the Northern provenance suffering significantly less damage compared to the Coastal and Piedmont provenances (Figure 2).

There was no provenance difference in F_vF_m in the control treatment with F_vF_m ranging from 0.507 to 0.647 over the 13-day measurement period after the experimental freeze (data not shown). On average, across the 13-days post-freeze, F_vF_m in the Northern provenance declined to 0.350 but still was significantly higher than both the Coastal and Piedmont provenances which had an average F_vF_m of 0.203 (Figure 3a). F_vF_m in all provenances, in the freeze treatment, tended to decrease until 6 days after the freeze then slowly increase. This is also shown in figure 3b, as the percent of control F_vF_m (or percent of non-damaged F_vF_m). F_vF_m in the Northern provenance was on average 62% of the undamaged control which was significantly higher than the Coastal and Piedmont provenances which was on average 34% of the control (Figure 3b).

The initial reductions in F_vF_m were used to predict foliar damage and mortality of loblolly pine seedling subjected to the experimental freeze event. Percent reductions in F_vF_m caused by the experimental freeze was calculated from F_vF_m measurements taken one day prior to the experimental freeze and one day post-freeze. Reductions in F_vF_m ranged from 35-79%. Percent foliar damage was assessed 14-day post-freeze event by averaging a percent foliar injury score by three SFNMC staff. Across all genetic families, there was a linear relationship between percent reduction in F_vF_m and percent foliar damage with percent reduction in F_vF_m explaining 93% of the variation in foliar damage (Figure 4). Even more interesting was a strong linear correlation (R^2=0.83) between initial percent reduction in F_vF_m with mortality 41 days post freeze (Figure 5).

DISCUSSION

Unseasonably warm winters followed by sudden freeze events have become more frequent in the southeastern U.S. This combination can be extremely problematic to the forest nursery industry where the majority of seedlings produced are loblolly pine, which can be more susceptible to freeze damage (Merry et al. 2017). Freeze damaged seedlings, that have a decreased chance of survival once outplanted, can cost the forest nursery industry financially by loss of seedling crop, or by nursery-planter relationship by unknowingly selling freeze damaged seedlings (South 2006). For this reason, our objective was to determine if chlorophyll fluorescence could be used to rapidly assess damage in loblolly pine seedlings caused by a freeze event and whether the immediate assessment could be used to predict long-term damage.

Undamaged seedling F_vF_m in this study was approximately 0.60, which is similar to other studies on cold acclimated loblolly pine during winter months (Zheng et al. 2012, Hymus et al. 1999). Chlorophyll fluorescence was successful in capturing the immediate decline in F_vF_m caused by freezing stress injury to the photosynthetic machinery in loblolly pine seedlings illustrated by the approximate 72% decline in F_vF_m from pre to post freeze (Figure 2). Zheng et al. 2012 reported a 50% reduction in F_vF_m in mature loblolly pine after exposure to 5˚F experimental freeze in a region in China that experiences these low temperatures frequently.

Trees have the ability to recover from damage caused by freezing stress. In our study, we used F_vF_m to evaluate seedling recovery for freeze damage during the two weeks after the experimental freeze. After 13 days, all provenances of loblolly pine tested increased F_vF_m with the Northern, Coastal and Piedmont provenances operating at 71, 45, and 51%, respectively, of F_vF_m in the control treatment. While the seedlings were showing signs of recovery, 2 weeks post freeze, F_vF_m was reduced on average of 44% compared to the control (non-frozen seedlings). Merry et al. 2017, reported that recovery of mature eastern white pine took close to 3 months to recover to pre-freeze conditions. The time needed for full recovery of loblolly pine exposed to a sudden freeze event still needs to be examined.

Forest seedlings will inevitably be damaged by sudden freezing events. However, being able to predict long-term damage or survivability based on the degree of damage can be useful for nursery managers to make informed decisions about seedling quality. Visual foliar damage that would be useful for nursery growers, managers and reforestation companies did not fully manifest until 2 weeks post-freeze in this study. However, we found a strong linear relationship between initial reduction in F_vF_m as measured by the fluorometer and subsequent visible foliar damage 2 weeks after the experimental freeze. As F_vF_m decreased due to freeze injury (or % reduction in F_vF_m increased) the percent of foliar damage that would eventually appear increased. A similar relationship between reductions in F_vF_m and foliar damage was reported for Douglas-fir seedlings (Fisker et al. 1995). Not only was the initial reduction in F_vF_m caused by freeze injury related to foliar damage, but we were also able to predict the percent mortality as well.

Genetics can determine the susceptibility of loblolly pine to damage caused by freeze events. Understanding the impact of loblolly pine family genetics on their sensitivity to freeze damage might one day help nurseries identify vulnerable genotypes and prioritize protection within the nursery. In this study, we had three provenances of loblolly pine with 3 genetic families in each provenance. The Northern provenance significantly responded better to the experimental freeze than both the Coastal and Piedmont provenances. Over two weeks post-freeze, seedlings from the Northern provenance maintained F_vF_m over 51% of undamaged control seedlings while seedlings in the Coastal and Piedmont provenances were mainly less than 50%. This is not surprising as Northern provenances are less susceptible to freeze events than Coastal or Piedmont seed sources. Greater resilience to both foliage damage and mortality (41 days post freeze) was also demonstrated in seedlings from the Northern provenance compared to the Coastal and Piedmont provenances. The greatest mortality was in the seedlings from the Coastal provenance where minimum winter temperature averaged 15.6 ˚F compared with 6.6 ˚F in the Northern provenance, which suffered the least mortality.

One of the original goals of this study was to monitor growth after outplanting during the growing season to understand the impact of freeze damage on seedling development. Unfortunately, all loblolly pine seedlings in the freeze treatment died within a month after outplanting, even with 100% survival in the control seedlings. While some seedlings, especially in the Northern provenance, were showing signs of post-freeze recovery while still in the greenhouse, they eventually died due to the added stress of outplanting. Four days after outplanting, temperatures fell to 27 ˚F and was followed by several warm sunny days. While this may have contributed to the mortality of seedlings after outplanting, we believe the severity of the freeze treatment created too much damage on the photosynthetic machinery and seedlings were not adequately recovered to withstand the additional stress caused by outplanting. The experimental freeze in this study was 5˚F, which is below the minimum winter temperatures of all seedlings in the study and certainly not common in the southeastern U.S. We chose 5˚F because we wanted to put maximum stress on the seedling to increase the possibility of ascertaining damage caused by the freeze with chlorophyll fluorescence.

MANAGEMENT IMPLICATIONS

Seedling freeze damage caused by freezing temperatures changes the photochemistry in PSII of loblolly pine seedlings, which is detectable with chlorophyll fluorescence (Rizza et al. 2001, Lichtenthaler and Rinderle 1988). Damage caused by the experimental freeze in this study was detectable using chlorophyll fluorescence and the degree of damage along with predictability of future damage was also predicted. Therefore, it may be possible to use chlorophyll fluorescence as a rapid diagnostic tool in the nursery to determine the future health (survival and growth) of outplanted seedlings. However, some tweaking (more research) is needed using realistic freezing temperatures experienced in the southeast along with the extent of time needed to sufficiently recover to survive outplanting. This study shows promising results that suggest that chlorophyll fluorescence may be used as a rapid, low-cost tool to quickly assess freeze damage in loblolly pine seedling so that nurseries have the tools needed to make informed decisions on seedling quality.

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Table 1. Maternal and paternal parent description, number of genetic families represented in each maternal and paternal mix and the range in minimum winter temperature (MWT) for one-year old containerized loblolly pine seedlings used in this experiment.

Maternal Parent	Paternal Parent*	# of Genetic Families	MWT (˚F)
Coastal provenance	Coastal pollen mix	3	15.44-21.42
Northern provenance	Northern pollen mix	3	5.08-8.04
Piedmont provenance	Piedmont pollen mix	3	9.25-10.59

*The pollen mix for each provenance comprised of pollen from 20 trees common to that region.

Table 2. Mean (±SE) initial root collar diameter (RCD), height (HGT) and the ratio of variable to maximum fluorescence (F_vF_m) by treatment and provenance in one-year old containerized loblolly pine seedlings. Different letters indicate significant difference in means (α = 0.05).

	Initial RCD (mm)	Initial HGT (cm)	Initial F_vF_m
Provenance			
Coastal	4.26 ± 0.09 a	20.0 ± 0.53	0.599 ± 0.015
Northern	4.36 ± 0.05 a	21.0 ± 0.28	0.600 ± 0.009
Piedmont	4.04 ± 0.06 b	21.3 ± 0.62	0.616 ± 0.005
Treatment			
Control	4.17 ± 0.08	21.1 ± 0.46	0.593 ± 0.009
Freeze	4.27 ± 0.06	20.5 ± 0.38	0.611 ± 0.008
p>f			
Treatment	0.219	0.383	0.391
Provenance	**0.019**	0.246	0.553
Treatment*Provenance	0.661	0.801	0.985

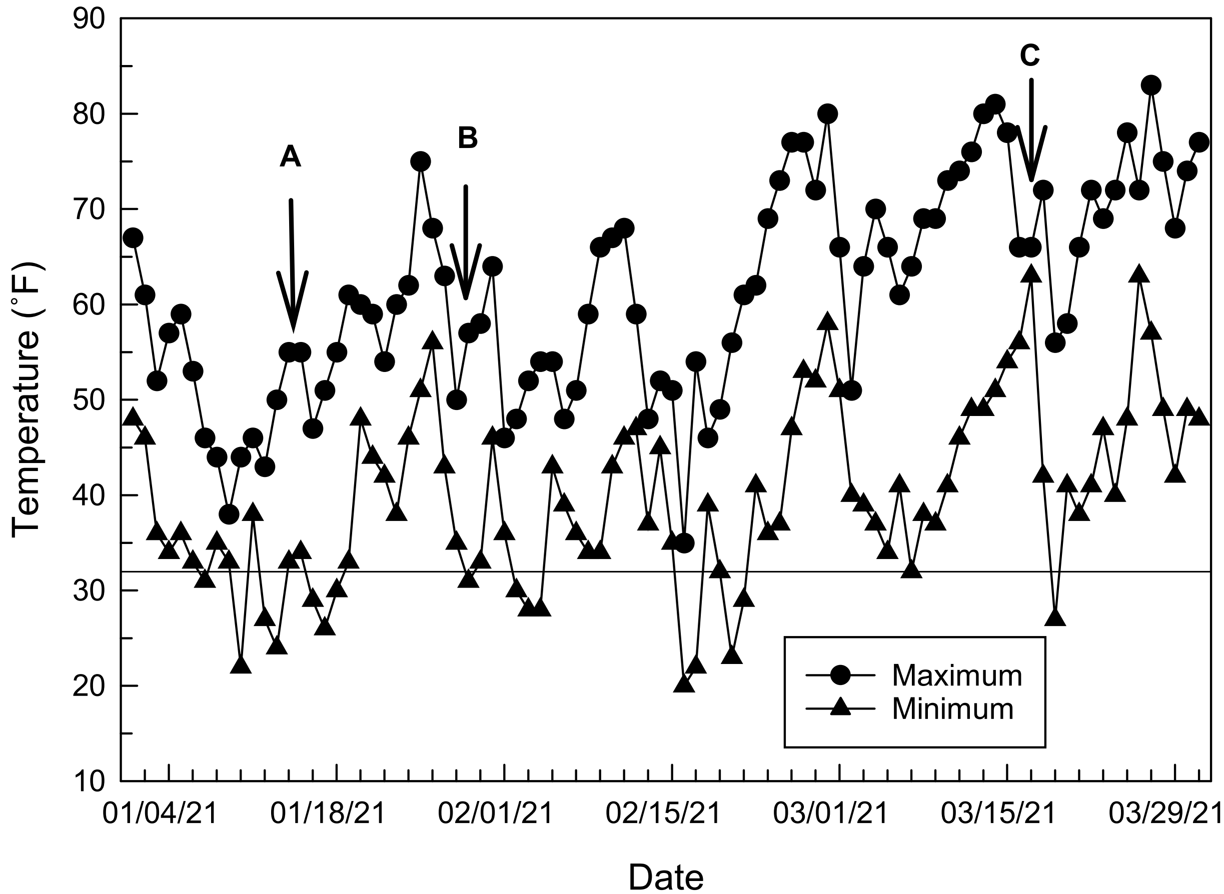

Figure 1. Daily minimum and maximum outside temperatures in Auburn, AL from January 1, 2021 through March 31, 2021. (A) indicates the date that seedlings were moved outside 2 weeks prior the freeze treatment, (B) indicates the date when seedlings were moved back into the greenhouse and freezing treatment initiated and (C) indicates the date when seedlings were outplanted. Temperature inside the greenhouse limited to a low of 35˚F.

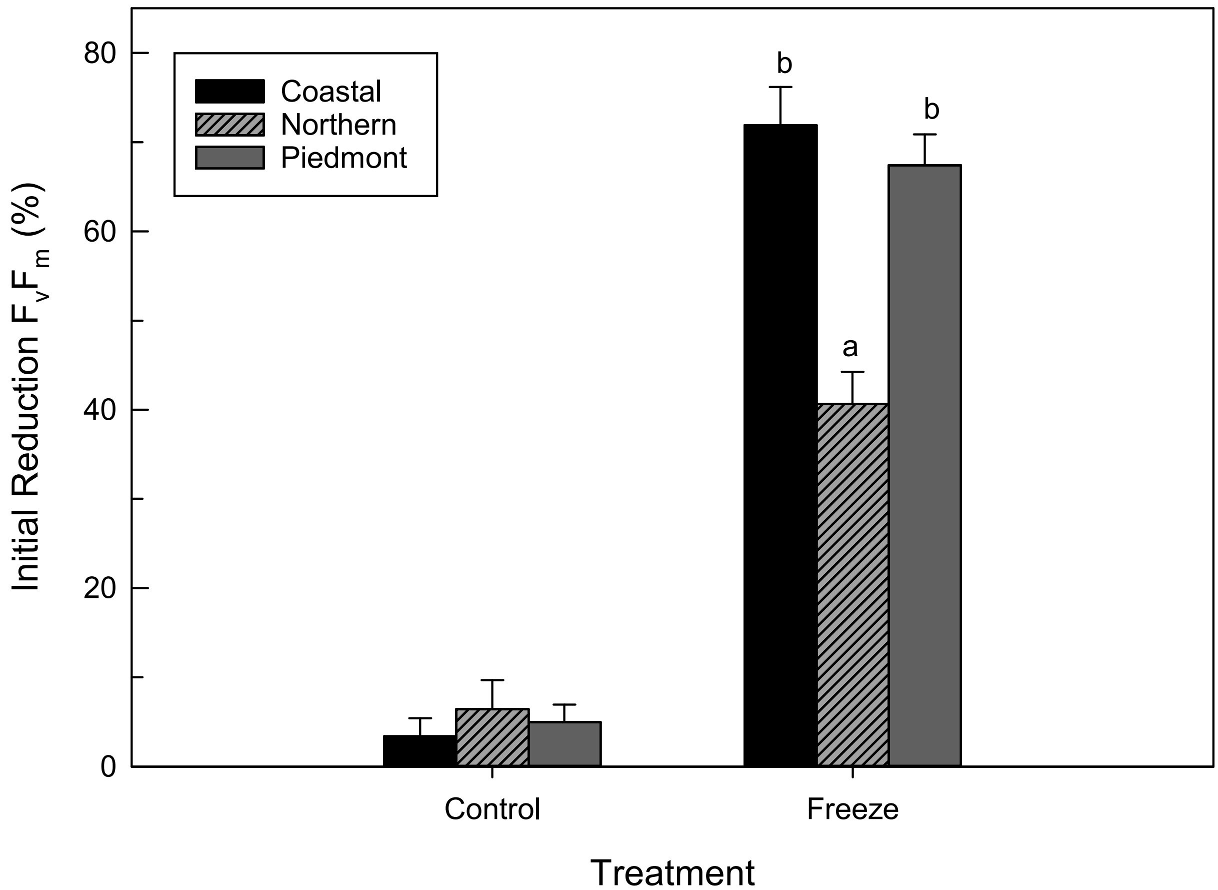

Figure 2. Mean (±SE) initial percent reductions in F_vF_m in one-year old containerized loblolly pine caused by ambient conditions (control treatment) and experimental freeze of 5 ˚F (freeze treatment). Different letters indicate a significant difference between provenances (α = 0.05).

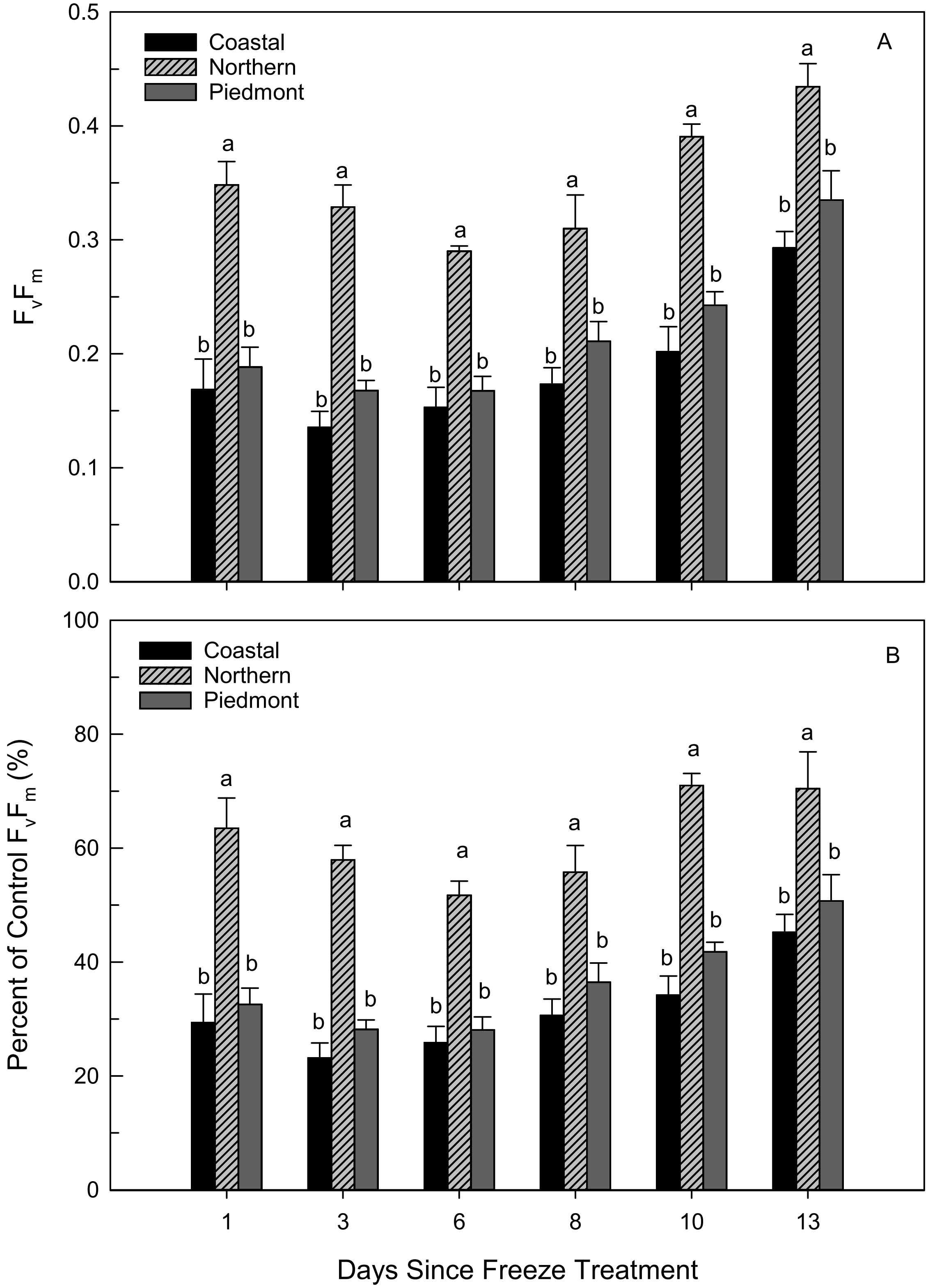

Figure 3. Mean (±SE) (A) F_vF_m and (B) percent of undamaged control F_vF_m in the freeze treatment during the 2 weeks post experimental freeze on one-year old containerized loblolly pine. Different letters indicate a significant difference between provenances ($\alpha = 0.05$).

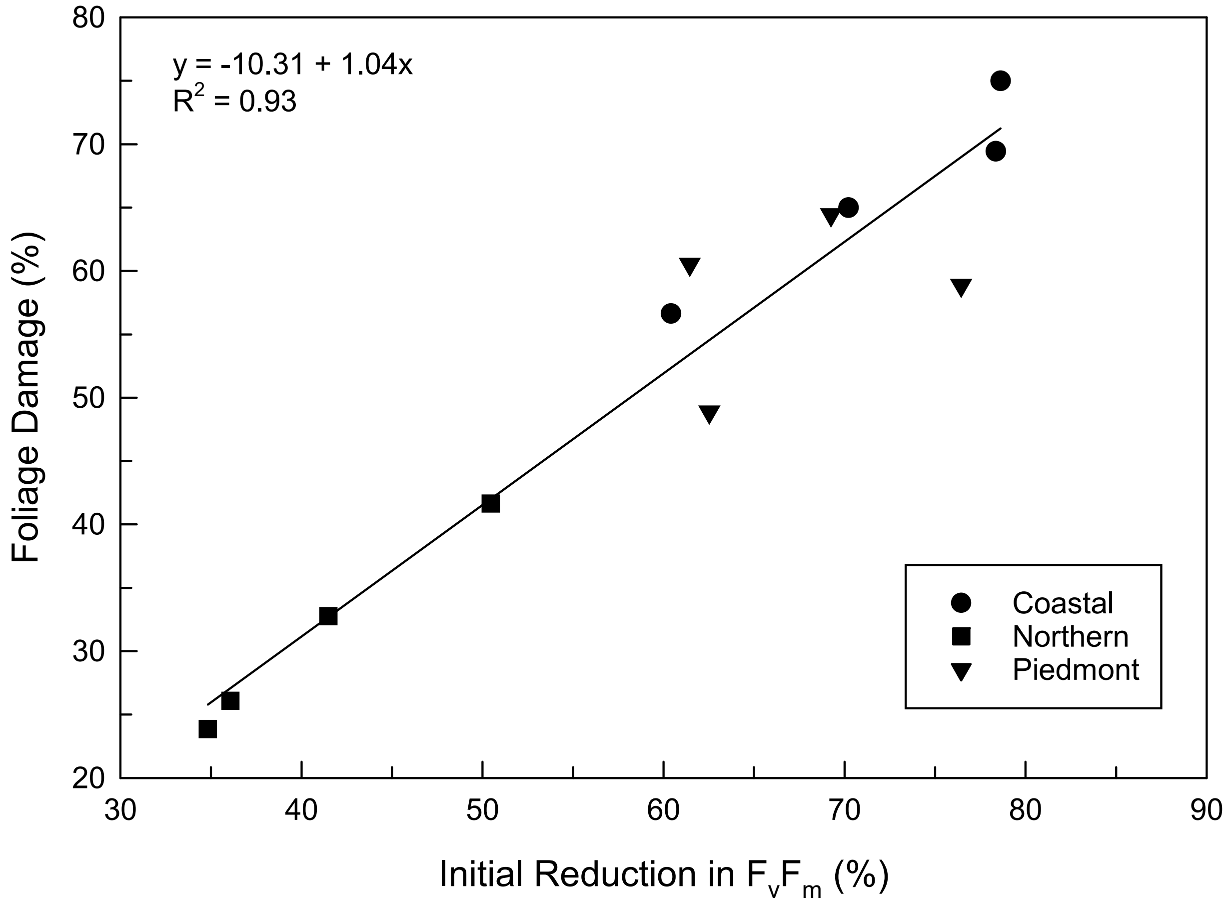

Figure 4. Relationship between initial percent reduction in F_vF_m and percent foliar damage 2 weeks post experimental freeze in one-year old containerized loblolly pine seedlings.

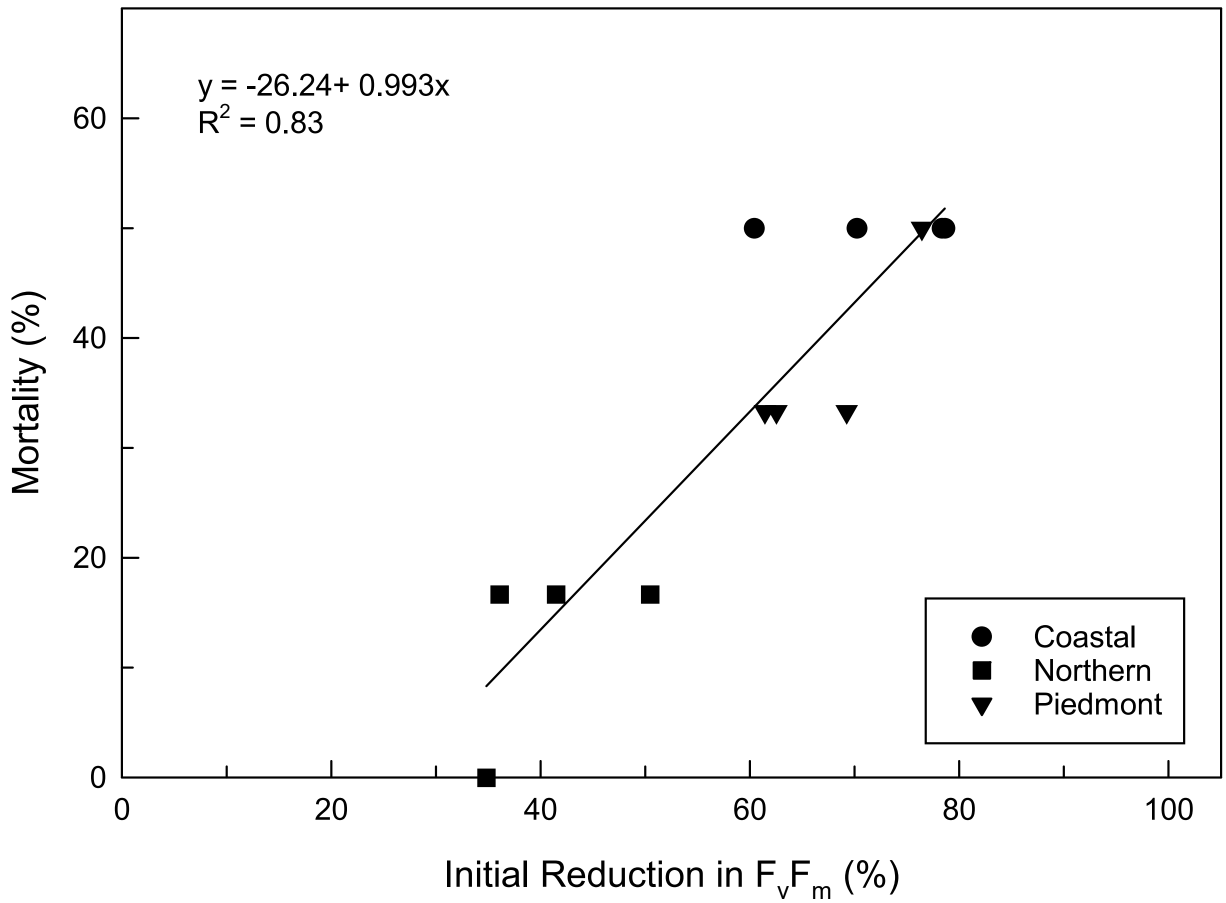

Figure 5. Relationship between initial percent reduction in F_vF_m and percent mortality 41 days post experimental freeze in one-year old containerized loblolly pine seedlings.