

Chilling sensitivity of stored purple fountain grass propagules

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Abstract

The chilling sensitivity of purple fountain grass (*Pennisetum × advena* (Wipff and Veldkamp) (formerly known as *Pennisetum setaceum* ‘Rubrum’ (Forsk.) Chiov.)) was determined by storing propagules in plug trays at 0, 5, 10 or 15 °C in controlled environment chambers for 0, 2, 4 or 6 weeks in year 1 and at 7.5, 10, 12.5 or 15 °C for 0, 2, 4, 6 or 12 weeks in year 2 of the study. Chlorophyll fluorescence was measured as the ratio of variable to maximal chlorophyll fluorescence (Fv/Fm) during storage of the propagules. Following storage, propagules were transplanted and subsequent greenhouse performance was assessed based on survival percentage, visual ratings and tiller production. No propagules survived storage at 0 and 5 °C and only 40% propagules survived 2-week storage at 7.5 °C. All propagules survived 2-week storage at 10 °C, while 90, 70 and 60% survived 4-, 6- and 12-week storage at 10 °C, respectively. Although the visual appearance of plants stored for 2 weeks at 10 °C was initially impaired, the plants recovered based on visual ratings taken 4 weeks later. Additionally, tiller production of plants stored at 10 °C for 2 weeks in year 2 was lower than non-stored controls but was comparable after 6 weeks. All plants survived storage at ≥12.5 °C for up to 12 weeks and final visual ratings indicated that these plants were not adversely affected. Additionally, tiller production of plants stored at ≥12.5 °C was comparable to non-stored controls after 4 weeks in the greenhouse. Chlorophyll fluorescence declined when plugs were stored at ≤10 °C, and was associated with loss in plant quality and increase in mortality. However, the decline in Fv/Fm did not precede symptom expression, and hence its value as a tool for predicting chilling injury in stored purple fountain plugs appears to be limited.

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1. Introduction

Purple fountain grass (*Pennisetum × advena* Wipff and Veldkamp (formerly known as *Pennisetum setaceum* ‘Rubrum’ (Forsk.) Chiov.)) is a popular ornamental grass with significant commercial importance in the United States (Wang et al., 2000). *Pennisetum × advena* is thought to be a cross of *P. setaceum*, native to Northern Africa, and *Pennisetum macrostachys* (Brongn.) Trin., native to Malesia (Wipff and Veldkamp, 1999). Both parents are from tropical regions, and purple fountain grass is said to be cold-hardy only to USDA hardiness zone 9 (Gilman, 1999).

Being essentially pollen sterile with only 0.05% seed production upon self-pollination (Simpson and Bashaw, 1969), and not true-to-type, purple fountain grass must be propagated vegetatively by divisions and sometimes by cuttings. Rooted divisions are marketed 12 months of the year, though most are sold in late

winter and early spring months to be grown for spring sales. In colder climates, it is useful to produce propagules during the autumn months while light levels and temperatures are higher compared to winter, and then to store them through the winter until needed using minimal heat. However, appropriate storage temperatures have not been determined, and growers have reported chilling injury symptoms and plant death when kept in greenhouses set between 0 and 5 °C (personal communication with Dennis Crum, Four Star Greenhouses, Carleton, MI USA). Depending on the species, the onset of chilling injury symptoms is usually observed between 0 and 12 °C (Allen and Ort, 2001; Lange and Cameron, 1997; Paull, 1990), and symptom development is most often pronounced after plants are returned to warmer, non-chilling temperatures (Morris, 1982; Saltveit and Morris, 1990). The specific chilling sensitivity of purple fountain grass has not been previously characterized.

Chlorophyll fluorescence has been used in field and laboratory conditions to assess the state of Photosystem II (PSII; Maxwell and Johnson, 2000), and the ratio of variable to maximal chlorophyll fluorescence (Fv/Fm) has been used to predict

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chilling injury prior to visual symptom development in various fruits and vegetables (DeEll et al., 1999; Smillie et al., 1987). Changes in fluorescence parameters associated with chilling sensitivity have been studied in leafy green herbs and vegetables such as basil (*Ocimum basilicum* L.) and jute (*Corchoris olitorius* L.) (Meir et al., 1997; Tulio et al., 2002). For basil leaves, Fv/Fm declined after 2 d storage at 4 °C, while chilling injury symptoms were clearly observed only after 4 d at 4 °C (Meir et al., 1997). However, changes in Fv/Fv did not always correlate with subsequent chilling injury development at other temperatures and durations (Meir et al., 1997). Jute leaves developed chilling injury symptoms after 5 d at 0 °C, while Fv/Fm declined after 1 d (Tulio et al., 2002).

The objectives of this study were to (1) characterize the chilling sensitivity of purple fountain grass propagules as a function of temperature and duration on visual appearance and tiller production after storage and during greenhouse growing and (2) evaluate if chlorophyll fluorescence measured as Fv/Fm could be used as a tool to assess chilling injury during storage of purple fountain grass.

2. Materials and methods

2.1. Plant culture

In 2002 (year 1), purple fountain grass stockplants were grown in 13-cm square plastic containers (1.1 L) containing commercial soil-less medium composed of pine bark, peat, vermiculite, perlite with wetting agent and starter fertilizer (Sure-Mix, Michigan Grower Products, Galesburg, MI, USA). Stockplants were maintained in a glass greenhouse at 20 ± 2 °C day/night setpoint under 16-h photoperiod provided by sunlight and 400-W high pressure sodium (HPS) lamps. HPS lamps also provided supplemental lighting of $150 \mu\text{mol m}^{-2} \text{s}^{-1}$ during 06:00 to 17:00 HR when ambient light levels were below $200 \mu\text{mol m}^{-2} \text{s}^{-1}$ and ceased when ambient light exceeded $400 \mu\text{mol m}^{-2} \text{s}^{-1}$. Plants were irrigated when necessary with acidified well water (H_2SO_4 to a titratable alkalinity of $\sim 130 \text{ mg CaCO}_3 \text{ L}^{-1}$) containing water-soluble fertilizer (125N–12P–125K (mg L^{-1}) and 1.0Fe–0.5Mn–0.5Zn–0.5Cu–0.1B–0.1Mo (mg L^{-1}); MSU Special, Greencare Fertilizers, Chicago, IL, USA).

Stockplants were split into three-tiller divisions and placed in 72-cell (0.03 L cell volume) plug trays (Landmark Plastic Corporation, Akron, OH, USA) filled with a mixture of 50% commercial soil-less medium and 50% coarse perlite (Thermo-Rock, East Inc., New Eagle, PA, USA). The propagules were first grown in a propagation house for 7 d at 23 °C air temperature and 26 °C soil temperature, maintained by providing bottom-heat. The propagation house was maintained at 0.3 kPa vapor pressure deficit by steam injection. The propagules were then transferred to the same environmental conditions as stockplants and grown for 6 weeks until the roots could hold the medium. In 2003 (year 2), actively growing propagules were received in 72-cell (0.03 L cell volume) plug trays from a commercial grower (Henry Mast Greenhouses, Byron Center, MI, USA) on 13 March.

2.2. Storage experiment

In year 1, propagules were stored in plug trays in controlled environment chambers set at constant 0, 5, 10 or 15 °C for 2, 4 or 6 weeks. An additional set of propagules was stored at 0 °C for 1 week. In year 2, the propagules were stored at 7.5, 10, 12.5 or 15 °C for 2, 4, 6 or 12 weeks. Propagules in the chambers were lit with fluorescent lights with an intensity of $\sim 125 \mu\text{mol m}^{-2} \text{s}^{-1}$ for a 12- and 11-h photoperiod in years 1 and 2, respectively. A row of additional propagules lined the edges of the plug trays to ensure that the airflow in chambers did not damage the experimental propagules. Propagules were watered when necessary using acidified well water with nutrients described above. An additional group of propagules was used as non-stored controls for each temperature treatment. The non-stored controls were potted in 13 cm square plastic containers containing commercial soil-less medium described above and grown in the greenhouse on 19 June 2002 in year 1 and on 24 April 2003 in year 2 at the initiation of the experiments.

2.3. Post-storage performance evaluation

Propagules were removed from storage after 2, 4 or 6 weeks in years 1 and 2, 4, 6 or 12 weeks in year 2. In year 1, an additional set of propagules was sampled from 0 °C after 1 week. The sampled propagules were potted in 13 cm square plastic containers filled with previously described commercial soil-less medium and grown in a glass greenhouse under the same environmental conditions as stockplants for 6 weeks. In the greenhouse, PPF at plant height was measured at two locations using line quantum sensors containing 10 photodiodes (Apogee Instruments, Logan, UT, USA) connected to a CR10 datalogger (Campbell Scientific, Logan, UT, USA). Greenhouse air temperature was controlled using a climate control computer (Priva, Model CD750, De Lier, The Netherlands) and was monitored on each greenhouse bench at plant height using type E thermocouples (TT-E-40; Omega engineering, Stamford, CT, USA) placed in aspirated tubes connected to the CR10 datalogger. Light and temperature measurements were taken every 10 s by CR10 datalogger and hourly averages were recorded in a computer. For each group of propagules, the average daily light integral and average daily temperature were calculated for 6 weeks after the plants were transferred to the greenhouse. The average daily light integral was 19.6 ± 2.4 and $15.7 \pm 0.9 \text{ mol m}^{-2} \text{ d}^{-1}$ and the average daily temperature was 25.7 ± 0.6 and 23.0 ± 0.9 °C in years 1 and 2, respectively. Tillers were counted before the storage treatments and on weeks 2, 4 and 6 of growing in the greenhouse for surviving plants. Initial and final visual ratings were assigned after 1 and 5 weeks of growing in the greenhouse. Ten plants per treatment were rated on a 1–4 scale based on their visual appearance as follows: (1) no visible living tissue (dead), (2) unmarketable (more than 50% necrosis), (3) marketable with some injury (less than 50% necrosis) and (4) uninjured. Surviving plants were counted after 1 week in the greenhouse and percent survival calculated.

2.4. Chlorophyll fluorescence

In 2003, chlorophyll fluorescence was measured at the initiation of propagule storage and every week thereafter for 12 weeks. Before each measurement, plants were removed from each storage temperature and placed in a common area set at 22 °C for at least 1 h. Fluorescence was measured using a plant efficiency analyzer (Hansatech, King's Lynn, UK) on the second or third fully expanded leaf of each propagule (as per Bruce et al., 2001). In brief, each leaf was dark acclimated 5 cm from the leaf base using acclimation clips provided with the plant efficiency analyzer by closing the shutter of the clip for at least 15 min. Light with a peak wavelength of 650 nm was provided at 1200 $\mu\text{mol m}^{-2} \text{s}^{-1}$ to saturate PSII. Fluorescence was measured after fitting the sensor over the acclimation clip and opening the shutter clip exposing the leaf for 5 s. Chlorophyll fluorescence was expressed as Fv/Fm. Following chlorophyll fluorescence measurement, plants were returned to the storage treatments.

2.5. Data analysis

Due to the differences in size of starting material (7.4 and 15.5 tillers per plant in years 1 and 2, respectively), data from 2002 and 2003 were analyzed separately. Storage temperature and storage duration were organized in a factorial arrangement, with 10 experimental units nested in the storage durations. Repeated measurement analysis was performed on datasets for response variables including visual ratings, tiller number and Fv/Fm using SAS's PROC MIXED (SAS Institute, Cary, NC, USA). Least significant difference (LSD) procedure was used for paired comparisons with $P=0.05$ as a value for significance.

3. Results

3.1. Percent survival, visual ratings and tiller number

All non-stored controls survived and received average visual ratings of 3.95 and 4.0 in years 1 and 2, respectively (Figs. 1 and 2). However, propagule roots and shoots were completely necrotic after 1 week at 0 °C or 2 weeks at 5 °C, the shortest durations tested in the study (Fig. 1E and F). No plants from these treatments were living after 2 weeks in the greenhouse (Fig. 1A and B). About 75% of the propagules survived 2 weeks of storage at 7.5 °C in year 2, but most had a large percentage of necrotic tissue and regrowth in the greenhouse was reduced based on visual ratings (Fig. 2A and E). No purple fountain grass plants survived ≥ 4 weeks of storage at 7.5 °C (Fig. 1A). In both years, all plants survived storage at 10 °C for 2 weeks (Figs. 1C and 2B). In year 1, the average initial visual rating was 2.8 following 2-week storage at 10 °C, but plants recovered after 5 weeks in the greenhouse (Fig. 1G). In year 2, initial visual ratings did not decrease after storage at 10 °C for 2 weeks (Fig. 2F). In both years, 10 and 30% plants died after plugs were stored at 10 °C for 4 and 6 weeks, respectively and, in year 2, 12-week storage at 10 °C resulted in a 40% loss of plants. Plants stored at 10 °C for 4 weeks largely recovered after 5-week growth. However, following storage at 10 °C for ≥ 6 weeks, plants recovered poorly based on final visual ratings. All propagules survived storage at 12.5 °C for 12 weeks, the longest storage duration tested (Fig. 2C). Initial and final visual ratings of plants stored at 12.5 °C for ≤ 4 weeks and ≥ 2 weeks remained similar to the non-stored controls (Fig. 2G). In years 1 and 2, all propagules survived storage at 15 °C for 6 and 12 weeks, respectively (Figs. 1D and 2D), the longest storage durations tested. In year 1, average initial visual ratings

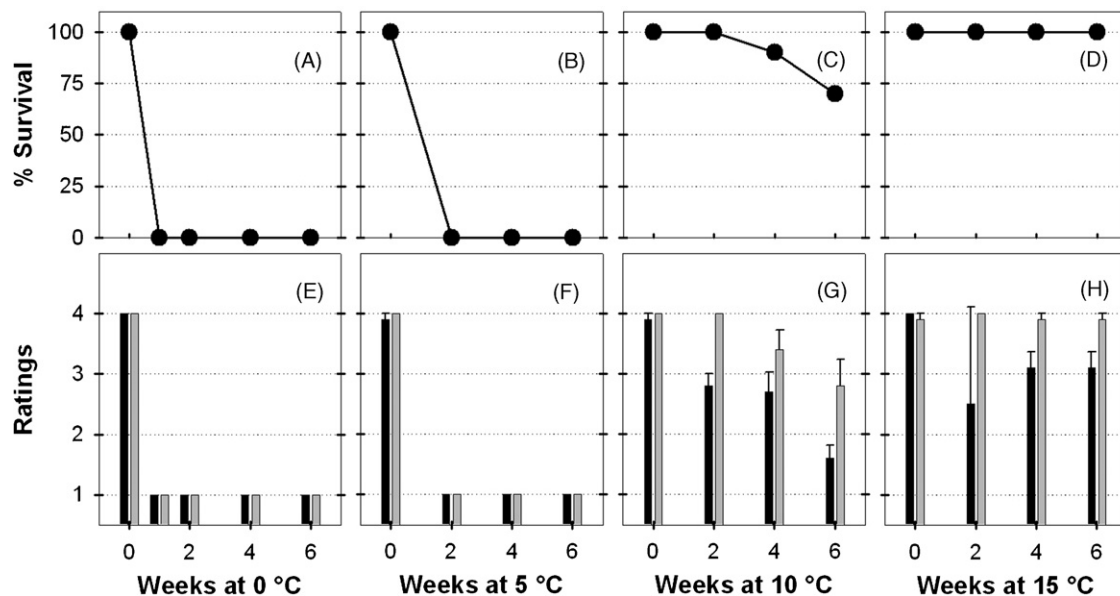


Fig. 1. Percent survival (A–D) and visual ratings (E–H) of purple fountain grass plants stored at 0, 5, 10 and 15 °C in year 1. Percent survival was based on the number of surviving plants 2 weeks after transplanting in the greenhouse. Black bars and gray bars represent visual ratings (1 = dead, 2 = greater than 50% necrosis, 3 = less than 50% necrosis and 4 = no injury) of plants grown in the greenhouse after storage for 1 and 5 weeks, respectively. Mean \pm S.E. of 10 plants.

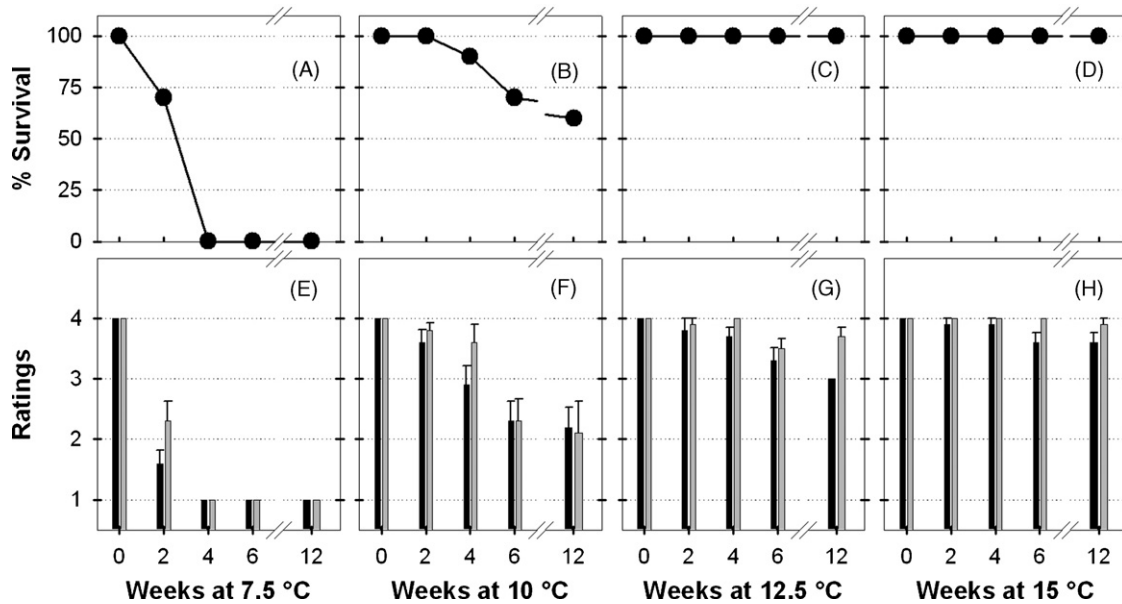


Fig. 2. Percent survival (A–D) and visual ratings (E–H) of purple fountain grass plants stored at 7.5, 10, 12.5 and 15 °C in year 2. Percent survival was based on the number of surviving plants 2 weeks after transplanting in the greenhouse. Black bars and gray bars represent visual ratings (1 = dead, 2 = greater than 50% necrosis, 3 = less than 50% necrosis and 4 = no injury) of plants grown in the greenhouse after storage for 1 and 5 weeks, respectively. Mean \pm S.E. of 10 plants.

declined after storage at 15 °C for 6 weeks, though plants were still marketable and recovered completely during the additional 4 weeks of greenhouse growth (Fig. 1H). In year 2, storage at 15 °C for ≤ 12 weeks did not affect the initial or final visual ratings (Fig. 2H).

Purple fountain grass plants generally formed fewer tillers after 2 weeks of growing following storage for any duration at ≤ 12.5 °C though tiller formation often recovered after longer regrowth durations (Figs. 3 and 4). Storage at 15 °C for 6 and 12 weeks in years 1 and 2, respectively did not adversely affect subsequent tiller production for 6 weeks in the greenhouse (Figs. 3B and 4D). Conversely, tiller formation of plants that survived 2-week storage at 7.5 °C was significantly reduced compared to non-stored controls (Fig. 4A). Following storage at 10 °C for ≥ 2 weeks, subsequent tiller production in the greenhouse was significantly reduced compared to non-stored controls for ≤ 4 and 2 weeks in years 1 and 2, respectively (Figs. 3A and 4B). The tiller numbers of

plants previously stored at 12.5 °C were not significantly different from controls when grown for ≥ 4 weeks in the greenhouse (Fig. 4C).

Generally, plants in year 2 formed more tillers and received higher initial and final visual ratings than plants in year 1 with the exception of final visual ratings after storage at 10 °C for 2 and 6 weeks. Propagules used as starting material in year 2 had almost twice the number of initial tillers compared to propagules used in year 1. Additionally, the average air temperature and daily light integrals in the greenhouse were higher in year 1 compared to year 2. It is possible that exposure to higher temperatures and light levels immediately following storage may have increased the severity of chilling injury symptoms in year 1.

3.2. Chlorophyll fluorescence

Prior to storage, the mean F_v/F_m of purple fountain grass propagules was 0.79. Mean F_v/F_m did not change even after

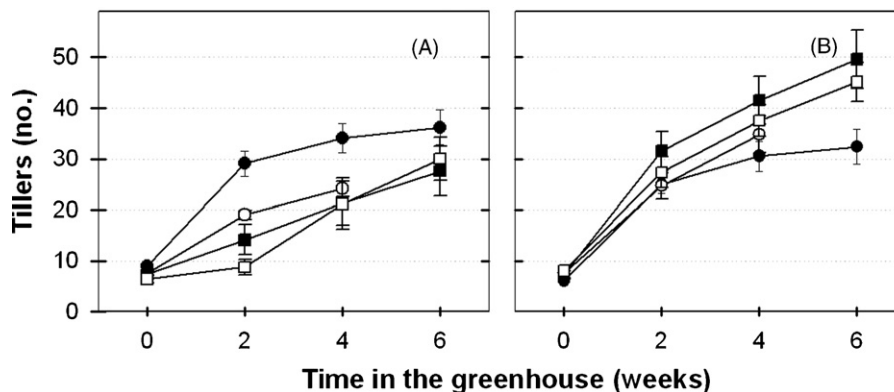


Fig. 3. Time course of tiller development during regrowth in the greenhouse of purple fountain grass plants previously stored at 10 °C (A) and 15 °C (B) for 0 (●), 2 (○), 4 (■) or 6 (□) weeks in year 1. Mean \pm S.E. of 10 plants.

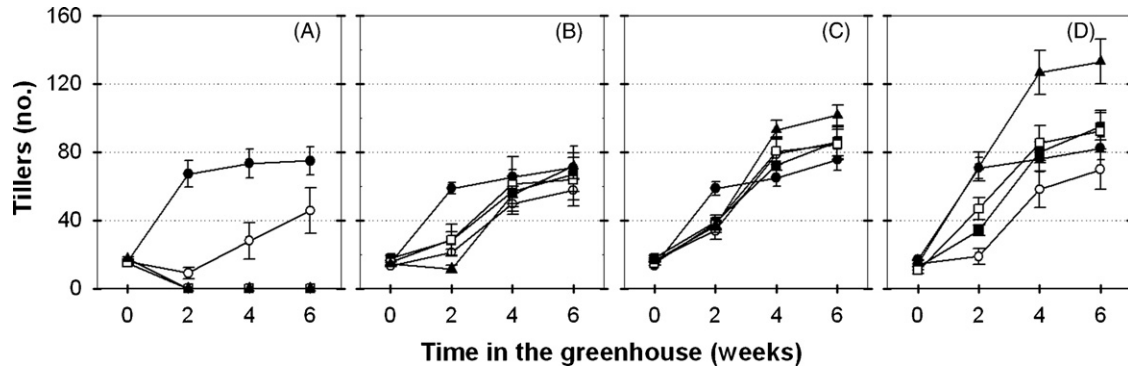


Fig. 4. Time course of tiller development during regrowth in the greenhouse of purple fountain grass plants previously stored at 7.5 °C (A), 10 °C (B), 12.5 °C (C) and 15 °C (D) for 0 (●), 2 (○), 4 (■), 6 (□) or 12 (▲) weeks in year 2. Mean \pm S.E. of 10 plants.

12-week storage at 12.5 or 15 °C (Fig. 5C and D), though some individual measurements were below 0.78 (Fig. 5G and H). During storage at 7.5 °C, Fv/Fm remained largely unchanged for 1 week but sharply declined to a mean value of 0.59 after 2 weeks (Fig. 5A) by which time, necrosis and plant death were already evident (Fig. 2A). Fv/Fm varied widely for individual plants when measured after 2 weeks at 7.5 °C (Fig. 5E). After 3 and 4 weeks at 7.5 °C, mean Fv/Fm was \sim 0.20 and 0.05, respectively, though there was a wide degree of variability. After 4 weeks at 7.5 °C, few plants had measurable Fv/Fm, and after 1 week in the greenhouse, all plants were dead (Fig. 2A).

Following storage at 10 °C for \leq 3 weeks, Fv/Fm for a few plants declined (Fig. 5F), though mean Fv/Fm remained essentially unchanged (Fig. 5B). When propagules were stored at 10 °C for \geq 4 weeks, mean Fv/Fm declined with considerable variability. The change in Fv/Fm was associated with a drop in survival (Fig. 2B), visual ratings (Fig. 2G) and subsequent tiller production of the survivors (Fig. 4B).

4. Discussion

Our results demonstrate that actively growing purple fountain grass is highly chilling sensitive at \leq 7.5 °C. Complete plant loss was observed after 4 weeks at 7.5 °C, 2 weeks at 5 °C and 1 week at 0 °C. Chilling injury was moderate at 10 °C; symptom development and plant mortality developed more slowly than at lower temperatures. All plants survived 2 weeks at 10 °C with little long-term loss of regrowth and over 50% plants survived 12-week storage at 10 °C. The severity of symptom expression depended on exposure temperature and duration, with lower temperatures and longer exposure times elevating the chilling injury. Additionally, our findings indicate that storage at sub-optimal temperatures had a long-term effect on subsequent greenhouse growth of purple fountain grass. The chilling sensitive temperature range of purple fountain grass, an interspecific hybrid with two parents of tropical origin, is consistent with that of other plants of tropical and subtropical origins (Allen and Ort, 2001; Paull, 1990).

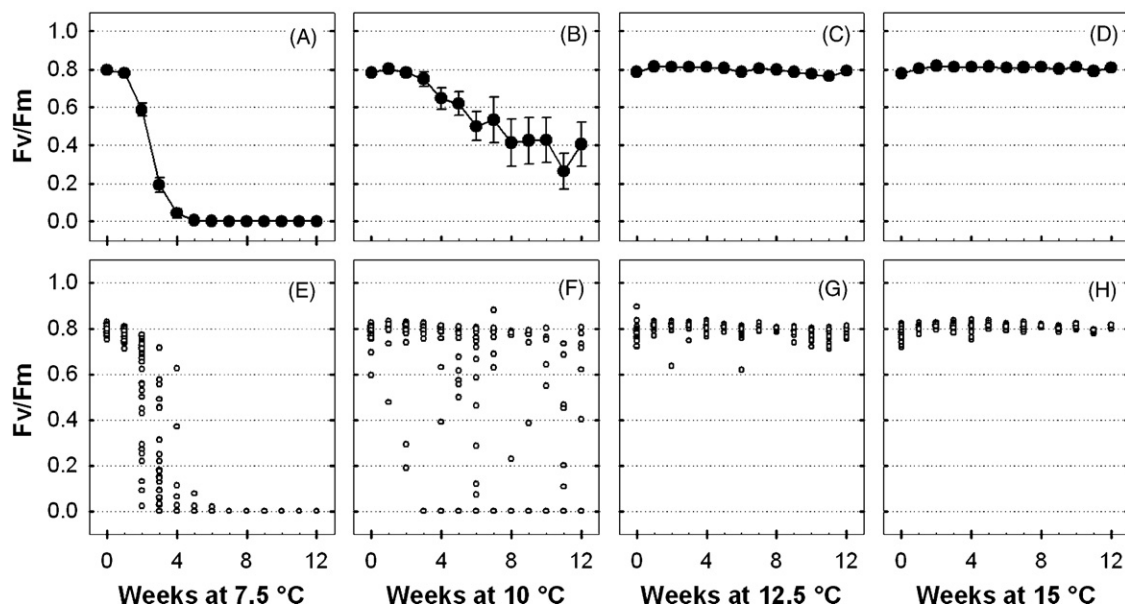


Fig. 5. Time course of changes in chlorophyll fluorescence expressed as Fv/Fm of purple fountain grass propagules during storage at 7.5 °C (A and E), 10 °C (B and F), 12.5 °C (C and G) and 15 °C (D and H). Mean Fv/Fm values \pm S.E. are given in (A)–(D) and Fv/Fm values for individual plants are given in (E)–(H).

Table 1

Number of observations (n) and percentage with visible ratings of 1 = dead, 2 = greater than 50% necrosis, 3 = less than 50% necrosis and 4 = no injury per range of Fv/Fm measurements for individual purple fountain grass plants stored at 7.5, 10, 12.5 and 15 °C

Fv/Fm	n	Visual rating (% in category)			
		1	2	3	4
0	33	97	3	0	0
0.001–0.7	16	31	50	13	6
0.701–0.76	20	20	20	15	45
0.761–0.8	59	0	2	22	76
0.801–0.82	56	0	5	36	59
0.821–0.84	16	0	0	6	94

Fv/Fm was measured immediately after plants were removed from storage. They were then transplanted and grown in the greenhouse for 1 week and visual ratings were made 1 week later.

Based on measurements made after all durations and temperatures, 97% of plants with Fv/Fm of zero died (Table 1). When Fv/Fm was greater than 0.76, all plants survived and regrowth ratings were generally good to excellent. When Fv/Fm was between 0.70 and 0.76, visual ratings after 1 week in the greenhouse ranged from dead to excellent. It was observed that during moderate chilling injury, individual leaves could express chilling injury symptoms on plants that otherwise grew and performed well. If Fv/Fm measurements were made on chilling injured leaves, they would predict poor regrowth for a plant that eventually performed well. This “false negative” would limit the use of chlorophyll fluorescence as a means of injury detection under these circumstances. In addition, the drop in chlorophyll fluorescence measured as mean Fv/Fm on an individual plant basis accompanied but did not precede macroscopic evidence of chilling injury for purple fountain grass plants stored at chilling temperatures. Thus, the use of Fv/Fm as a tool for predicting chilling injury in stored purple fountain plugs appears to be limited. A change in Fv/Fm preceded chilling injury symptom development in some but not all cases for basil leaves (Meir et al., 1997). Symptom development did not precede injury in jute leaves (Tulio et al., 2002). Madakadze et al. (2003) found that chlorophyll fluorescence strongly correlated with base temperature for seedling growth in cultivars of *Panicum virgatum*, *Andropogon gerrardii* Vitman, *Sorghastrum nutans* L. Nash and *Calamagrostis longifolia* (Hook) Scribn, though they did not show that the change in Fv/Fm preceded symptom development.

Our data indicate that purple fountain grass can be stored or transported for 2 weeks or less at 10 °C as long as the potential negative impact on visual appearance and tiller production for initial 2–5 weeks of subsequent growing is taken into account. Purple fountain grass plugs can be stored at 12.5 °C for up to 12 weeks in low light provided plants have 4 weeks or more to recover from minor leaf injury and by which time tiller production would be comparable to non-stored plants. It is probable

that exposing plants to acclimating temperatures (Lange and Cameron, 1997) or intermittent warming may increase chilling tolerance of this grass.

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