Temperature Effects on Interspecific Interference among Two Native Wetland Grasses and Tall Fescue

Jeffrey J. Steiner,* Tim G. Brewer, and Stephen M. Griffith

ABSTRACT

Successful reintroduction of native species into landscapes requires an understanding of how introduced species invaded and became established. This study was conducted to determine the effect of temperature on interspecific seedling interference of two wetland grasses [sloughgrass (Beckmannia syzigachne Steud.) and tufted hairgrass (Deschampsia cespitosa L.)] native to the Pacific Northwest and tall fescue (Festuca arundinacea Schreb. cv. Titan), a non-native grass species. The relationships of species interference to the thermal response of Photosystem II (PS II) fluorescence reappearance ratio (FRR) and glutathione reductase (GR) thermal stability were also investigated using the Mantel product moment correlation ($r_m$). Three combinations of two-species replacement series experiments (sloughgrass–hairgrass mixture, sloughgrass–tall fescue mixture, and hairgrass–tall fescue mixture) were conducted in growth chambers, planted in five proportions (0.0:1.0, 0.25:0.75, 0.5:0.5, 0.75:0.25, and 1.0:0.0), and grown at four temperatures (5, 10, 20, and 30°C). The FRR and GR were measured at eight temperatures ranging from 5 to 40°C. Tall fescue aggressiveness, relative to sloughgrass and hairgrass, increased with increasing temperature. Sloughgrass and hairgrass ranked second and third, respectively, and were only more aggressive than tall fescue at 5°C. Peak FRR occurred at 15, 20, and 22.5°C for sloughgrass, hairgrass, and tall fescue, respectively. Seedling dry mass of species was correlated with the stability of GR and the average efficiency of the PS II apparatus over the range of growing temperatures ($r_m = −1.00$ and 0.96, respectively). Tall fescue had greater PS II efficiency and GR stability under elevated temperatures than sloughgrass and hairgrass, which may explain why tall fescue has been able to dominate some wetland landscapes of the temperate Pacific Northwest.

A grassland dominated by tufted hairgrass was one of three major prairie communities in the Willamette River basin, and sloughgrass was an important codominant in depressed wetland areas (Moir and Mika, unpublished data, 1972). Understanding the adaptive characteristics that influence individual performance and relative aggressiveness of plants will provide insights to help determine the best approaches for rehabilitating disturbed sites and maintaining stands of native species.

Typically, the term competition is reserved for those interactions among plants that result in a reduction in the number of plants surviving in a mixture (Silvertown and Doust, 1993). Thus, the term interference is used to describe those interactions among species grown in mixtures where one species contributes more than the other to the total yield of the two competitors, and it implies the two species are utilizing a common limited resource and have different competitive abilities (Harper, 1977). A substitutive, or replacement series, experimental design is a method to assess the relative aggressiveness of different species. It separates the effects of plant density and population proportion by holding total mixture density constant while species proportions are varied (deWit, 1960). Inter- and intraspecific plant interference effects can be separated because each species is also grown alone. The concept of density-independent yield (law of constant final yield) is assumed for this design, and an important assumption is that yields in population mixtures can be estimated from monoculture yields. Interspecific interference is estimated in a replacement series by comparing deviations from yields of two competitors grown alone. Because emergence time is an important factor for determining dominance and greatly influences interference (Ross and Harper, 1972), stands established using same-age and same-size seedlings can be used to determine the effects of other competitive factors (Harper, 1977).

Other studies investigating the relative effects of environmental factors on interspecific interference included oat (Avena sativa L.) and barley (Hordeum vulgare L.) grown in mixtures under differing conditions of soil pH (deWit, 1960) and the effect of soil depth on a mixture of four oat species (A. sativa, A. strigosa Schreb., A. fatua L., and A. ludoviciana Dur.) (Trenbath and Harper, 1973). The impacts of temperature, shading, and soil moisture amount have been investigated for competition of the broadleaf species summer cypress [Kochia scoparia (L.) Schrad.] with barley and wheat (Triticum aestivum L.) (Messersmith et al., 2000). The effect of two different temperature regimes on the relat-

---

**Abbreviations:** FRR, fluorescence reappearance ratio; GR, glutathione reductase; PS II, Photosystem II; RCC, relative crowding coefficient.


The thermal response of Photosystem II (PS II) variable fluorescence reappearance following illumination has been used to identify optimal growing temperatures for agriculturally important species (Burke, 1990; Ferguson and Burke, 1991; Burke and Oliver, 1993; Burke, 1995). Enzyme response to varying temperatures measured by the apparent Michaelis–Menten constant may be a useful indicator of plant environmental adaptation (Haulsladen and Alشير, 1994). Glutathione reductase (GR) activity has been used to determine the thermal tolerance limits for several crops (Mahan et al., 1990; Anderson et al., 1992; Burke and Oliver, 1993; Burke and Upchurch, 1995). Glutathione reductase is involved in prevention of enzyme and membrane oxidation, regulation of gene expression associated with abiotic stress response, and detoxification during conditions of photodestructive stress (Halliwel and Foyer, 1978; Creissen et al., 1991). Variable fluorescence recovery and GR activity responses have not been used to distinguish relative competitive ability among different species based on optimal physiologic temperatures.

The objectives of this research were to determine (i) the effect of temperature on relative interspecific seedling interference of native Pacific Northwest sloughgrass and hairgrass and introduced tall fescue using a replacement series experimental design and (ii) whether relative interference can be predicted by either the thermal response of PS II variable fluorescence reappearance following illumination of leaves or by the thermal stability of GR.

**MATERIALS AND METHODS**

**Substitutive Competition Assay**

Sloughgrass seeds were collected from wild populations near Corvallis, OR (44.3°N, 123.15°W). Tufted hairgrass seeds were obtained from the USDA-ARS Plant Materials Center, Corvallis, OR. 'Titan' tall fescue seeds were obtained from a commercial seed company.

All seeds were germinated on blotter paper (7 d in 20 mg L−1 KNO3 at 5°C), and 60 same-age and same-size seedlings were transplanted into 2.7-L plastic pots with 15-cm top diameter that were filled with a potting mixture adjusted to a pH of 6.4. The three combinations of two-species mixtures were planted in the proportions: 0:1, 25:75, 50:50, 75:25, and 1:0 (3390 plants m−2). The 0:1 and 1:0 proportions represent the monoculture treatments for the first and second species of each two-species combination, respectively. The planting density used was determined from preliminary experiments where a constant final yield was obtained at 5°C within 7 wk. The plants were provided nonlimiting amounts of water (wastered to soil water-holding capacity every 2 d) and fertilizer [watered to soil water-holding capacity weekly with Peters Professional nutrient solution (Scotts, Marysville, OH) using 473 mg N L−1]. Injured and dead plants were replaced soon after initial planting with surplus same-age and same-size seedlings to maintain the initial population density. There were five replicate pots of each monoculture and mixed treatment for each species.

Temperature was controlled using an eight-position thermal plate system consisting of 5 by 6.5 cm of independent, electronically controlled, ceramic thermal modules with aluminum caps capable of producing constant temperatures (±1.0°C) from 5 to 40°C (device design modified from Burke and Mahan, 1993). Water vapor condensation on the fluorometer probe at temperatures <15°C was prevented by flushing the probe detector surface and the sample area with desiccated air that was prepared by forcing air through a column filled with silica gel stones (Griffith et al., 2000).

Approximately 10-mm-long leaf sections of the grasses were placed on moistened 3MM chromatography paper (Whatman, Maidstone, England); transferred to the temperature-controlled blocks; covered with CO2-permeable, transparent Glad Cling Wrap plastic film (First Brands Corp., Danbury, CT); and illuminated (high-pressure sodium lamp; 650 µmol m−2 s−1) for 10 min at 25°C. When all blocks reached the designated temperature, the lamp was turned off, the chlorophyll fluorescence measurements immediately begun (Time 0), and the response recorded at 3-min intervals for 33 min using a 10-s excitation period with 5 W m−2 light (procedure modified from

\[
k_i = \frac{x_i}{y_i} / \frac{x_j}{y_j} \tag{1}\]

where \(k_i\) is the RCC, \(\bar{x}\) is the mean yield per plant of species \(i\) in the mixture, \(\bar{y}\) is the mean yield per plant of species \(j\) in the mixture, \(\bar{x}_i\) is the mean yield per plant of species \(i\) in the pure stand, and \(\bar{y}_j\) is the mean yield per plant of species \(j\) in the pure stand (Harper, 1977). The RCC was calculated to determine the effect of both the dominant and lesser dominant grass on one another in a mixture. The grand RCC average was calculated over the four growing temperatures for each species. The seedling phytomass for each species in a mixture was plotted as a percentage of the average aboveground seedling phytomass grown in monoculture.

**Fluorescence Reappearance**

Plants of the three grasses and ‘Chuan Mai’ wheat (no. 18// JUP/DJP S) were grown from seed in 1-L pots in the greenhouse under 25 to 20°C (day and night, respectively) conditions with 12 h of supplemental lighting and were fertilized weekly as described above. The fluorescence reappearance experiments were conducted using fully expanded green-healthy leaves for ease of analysis. Wheat was included as a standard for comparison with results from previously published work (Burke, 1990).

Photosystem II variable fluorescence reappearance following illumination was measured using a Brancker SF 30 fluorometer (Richard Brancker Research, LTD, Ottawa, ON, Canada). Temperature was controlled using an eight-position commercial seed company.

1. The use of trade names in this publication does not imply endorsement of the products named nor criticism of similar ones not mentioned.
Burke, 1990). The plants were stored between experiments in a growth chamber at 25°C with supplemental lighting (260 μmol m⁻² s⁻¹).

The fluorescence reappearance ratios (FRRs) were calculated as:

\[ \text{FRR} = \frac{F_v}{F_0} \]  

where \( F_0 \) = initial fluorescence value at Time 0 and \( F_v \) = maximum fluorescence value. Measurements were taken over the range of temperatures from 5 to 40°C in 5°C increments and plotted as functions of time in darkness after illumination. The approximate temperature that achieved the highest FRRs in the shortest period of time was re-evaluated using ±2.5°C increments rather than 5°C increments to more accurately determine the optimal temperature. As a way to specifically quantify the optimal temperature that achieved the highest FRR, two quantification approaches were used instead of visual estimations. For the first, the temperature of peak FRR \((F_v/F_0/F_{peak})\) was estimated using the single greatest average FRR value for the twelve 3-min measurement intervals among the 10 temperature conditions.

The second estimated the average FRR of the twelve 3-min measurement intervals for all 10 temperature conditions.

**Glutathione Reductase Thermal Stability**

Glutathione reductase (EC 1.6.4.2) was extracted from the three grasses and the wheat standard, and the apparent Michaelis–Menten constant \( (k_m) \) of glutathione for GR was determined from assays using a fixed concentration of dihydronicotinamide adenine dinucleotide phosphate (NADPH; 100 μM) made in 5°C increments for 5 to 40°C (Griffith et al., 2000). The thermal stability of GR was determined as (i) the \( \Delta GR \), which is the sum of the changes in apparent \( k_m \) for each of the 5°C increments over the range of 5 to 40°C and is represented by:

\[ \Delta GR = \sum_{i=1}^{n} T_{i+1} - T_i \]  

where \( i \) represents each of the sequential temperatures \( (T) \) at which the \( k_m \) is determined and \( n \) is the number of temperatures examined; and (ii) the \( \Delta GR_{avg} \), which is the average change in apparent \( k_m \) for the \( \Delta GR \) over the range of temperatures tested and is represented by:

\[ \Delta GR_{avg} = \frac{\Delta GR}{n} \]  

**Statistical Methods**

Analysis of variance was determined for the grass monoculture treatments based on the dry mass per seedling. Species were used as treatments, and the five pots per species in each of the four temperatures were used as replications. Mean differences were determined using Fisher’s protected LSD test (Snedecor and Cochran, 1980). Based on the total mass of each species within a pot, an analysis of variance was used to test the effects of the two-species mixtures from the substitute competition assay for the three grass species grown at four temperatures and in five densities (Table 1). A modification of the nested hierarchical design was used because occurrence of competitors within species was restricted in a nested fashion (Anderson and McLean, 1974). The conservative competitors-within-species mean square was used to test the species source of variation for significance. The four-way interaction of species × temperatures × densities × replications was used as the error term to test the temperature and density main effects and their interactions with species. Based on the significant difference for the three-way interaction of species × temperature × density, the standard error of the mean was used to show differences among means in the substitutive competition assay.

Table 1. Nested factorial analysis of variance for the seedling dry mass of three grasses grown in growth chambers, with each of the other two as competitors in two-species combinations, at four temperatures and in four densities.

<table>
<thead>
<tr>
<th>Source</th>
<th>df</th>
<th>Sum of squares</th>
<th>Mean squares</th>
<th>F†</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Species (S)&lt;sup&gt;i&lt;/sup&gt;</td>
<td>2</td>
<td>2214.5</td>
<td>1107.3</td>
<td>25.7</td>
<td>*</td>
</tr>
<tr>
<td>Competitor (C)&lt;sup&gt;i&lt;/sup&gt;</td>
<td>3</td>
<td>129.3</td>
<td>43.1</td>
<td>None</td>
<td></td>
</tr>
<tr>
<td>Restriction error (δ&lt;sub&gt;ij&lt;/sub&gt;)&lt;sup&gt;†&lt;/sup&gt;</td>
<td>0</td>
<td>None</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Temperature (T&lt;sub&gt;k&lt;/sub&gt;)&lt;sup&gt;†&lt;/sup&gt;</td>
<td>3</td>
<td>2250.5</td>
<td>750.2</td>
<td>781.4</td>
<td>***</td>
</tr>
<tr>
<td>Density (D)&lt;sup&gt;i&lt;/sup&gt;</td>
<td>3</td>
<td>999.7</td>
<td>333.2</td>
<td>347.1</td>
<td>***</td>
</tr>
<tr>
<td>S × T (ST&lt;sub&gt;kl&lt;/sub&gt;)&lt;sup&gt;†&lt;/sup&gt;</td>
<td>6</td>
<td>1233.7</td>
<td>205.6</td>
<td>214.2</td>
<td>***</td>
</tr>
<tr>
<td>S × D (SD&lt;sub&gt;ij&lt;/sub&gt;)&lt;sup&gt;†&lt;/sup&gt;</td>
<td>6</td>
<td>57.8</td>
<td>9.6</td>
<td>10.0</td>
<td>**</td>
</tr>
<tr>
<td>S × T × D (STD&lt;sub&gt;ijn&lt;/sub&gt;)</td>
<td>18</td>
<td>26</td>
<td>4.6</td>
<td>4.8</td>
<td>**</td>
</tr>
<tr>
<td>S × T × D × Replicates (STD&lt;sub&gt;irn&lt;/sub&gt;)</td>
<td>72</td>
<td>69.1</td>
<td>1.0</td>
<td>None</td>
<td></td>
</tr>
<tr>
<td>Inconsequential§</td>
<td>366</td>
<td>1571.3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>479</td>
<td>6808.5</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Significant at the 0.05 level.
** Significant at the 0.01 level.
*** Significant at the 0.001 level.
† The \( F \) for the species source of variation is based on the mean square error from competitors nested in species. All other \( F \) statistics are based on the mean square error from the four-way interaction \((S \times T \times D \times R)\). The experimental design and analysis is adapted from Anderson and McLean (1974) and uses their convention for describing sources of variation. Subscripts indicate the number of variable levels in each source. ‡ The restriction error indicates the competitors were nested within the species, which did not allow random assignment. § All remaining sources of variation, including those with replications and competitors, are summarized here.

To determine the relationships among interspecific competition measures from the replacement series experiments and species physiologic responses, the MXCOMP command of NTSSYS program version 2.2 (Rohlf, 1997) was used to calculate product moment correlations \((r)\) from the normalized Mantel Z (Mantel, 1967). This approach allowed determination of relationships among variables with single- and multi-state characteristics. Because of the limited sample size (three grass species), the probability \((P)\) for \( r \) was based on critical values from the one-tailed test using the equation:

\[ t = [(n - 2)/(1 - r^2)]^{1/2} \]  

for one degree of freedom (Snedecor and Cochran, 1980). Because the number of degrees of freedom was small, relationships with \( P \leq 0.10 \) were accepted as significant.

To prepare the response data for analysis using the Mantel Z statistic, the symmetric Euclidean distance matrices were calculated from:

\[ A = \begin{bmatrix} a_{11} & a_{12} & \ldots & a_{1j} \\ a_{21} & a_{22} & \ldots & a_{2j} \\ \vdots & \vdots & \ddots & \vdots \\ a_{n1} & a_{n2} & \ldots & a_{nj} \end{bmatrix} \]  

where \( A \) is any \( i \times j \) matrix for \( a_{ij}; i = 1, 2, \) and 3, representing each of the three grass species; and \( j \) equaling the number of measurements observed for a response variable. All data for a measured response were entered into a row for each of the grasses and transposed; using the STATS/CORR/EUC-CLIDIAN function (SYSTAT 5.2.1 for the Macintosh; SPSS, Chicago, IL), the data produced the distance \((D)\) matrix:

\[ D = \begin{bmatrix} e_{11} & e_{12} & e_{13} \\ e_{21} & e_{22} & e_{23} \\ \vdots & \vdots & \vdots \\ e_{n1} & e_{n2} & e_{n3} \end{bmatrix} \]
The effect of higher temperature was not as pronounced as the average RCC increased (Fig. 1). At 10°C, tall fescue and sloughgrass produced more dry mass than did hairgrass. When grown at 20°C, all three grasses reached maximal dry mass production, but tall fescue produced more than sloughgrass, and sloughgrass more than hairgrass. Tall fescue grown in monoculture at 20 and 30°C produced more dry mass than at 5 and 10°C and more dry mass than the two native species. At 30°C, tall fescue produced less dry mass than at 20°C but still more than both native grasses. Dry mass production for sloughgrass was greater than that for hairgrass at 30°C.

Tall fescue was able to produce more dry mass when grown at 20 and 30°C than the two native grasses, and thus appeared to have an adaptive advantage by its capacity to fix C into dry mass at elevated temperatures. Both sloughgrass and hairgrass growth were not as robust at 30°C as at 20°C. Even though all three grasses did not perform as well at 30°C as at 20°C, the adverse effect of the higher temperature was not as pronounced for tall fescue (27% reduction) as it was for sloughgrass and hairgrass (62 and 81% reductions, respectively). Also, tall fescue produced more dry matter at 30°C than at 10°C, but both sloughgrass and hairgrass produced less at 30°C than at 10°C, further indicating the greater thermal tolerance of tall fescue compared with the two native grasses.

**RESULTS AND DISCUSSION**

**Monoculture Growth**

All three grasses grown in monoculture produced the same amount of seedling dry mass at 5°C, but differentiation among the species occurred as the growing temperature increased (Fig. 1). At 10°C, tall fescue and sloughgrass produced more dry mass than did hairgrass. When grown at 20°C, all three grasses reached maximal dry mass production, but tall fescue produced more than sloughgrass, and sloughgrass more than hairgrass. Tall fescue grown in monoculture at 20 and 30°C produced more dry mass than at 5 and 10°C and more dry mass than the two native species. At 30°C, tall fescue produced less dry mass than at 20°C but still more than both native grasses. Dry mass production for sloughgrass was greater than that for hairgrass at 30°C.

**Replacement Series Experiments**

The results from the replacement series experiments generally followed the Model IIa form (Harper, 1977), with the more dominant of two species having a greater interference effect on the less aggressive species than the lesser species on itself in monoculture (Fig. 2). Also, the influence of the less aggressive species on the more aggressive species was greater than the intraspecific effect of the lesser species on itself in monoculture. We did not conduct multiple density experiments to determine the influence of total density on replacement series experiment results (Jolliffe et al., 1984). However, plant density (3390 plants m⁻²) and seedling growth were sufficient for interference to occur among the three two-species combinations.

The general pattern of interspecific aggressiveness among the grasses at 10, 20, and 30°C was tall fescue > sloughgrass > hairgrass, as measured by the RCC (Fig. 2). The general pattern of species aggressiveness differed at 5°C from the other three temperatures in that tall fescue was less aggressive than sloughgrass and hairgrass, and sloughgrass was less aggressive than hairgrass. Generally, at no other temperature were the two native species more aggressive than tall fescue.

The RCC for tall fescue grown with sloughgrass increased with increasing temperature while the sloughgrass RCC declined. Hairgrass RCC also declined with increasing temperature when grown with tall fescue, but the tall fescue RCC reached a maximum at 20°C and then declined at 30°C. Sloughgrass was more aggressive than hairgrass as indicated by the lower average RCC for hairgrass than sloughgrass when grown with tall fescue (average RCC = 0.5 and 0.9, respectively), the rela-

---

**Table 2. The single-state physiologic responses of sloughgrass, hairgrass, and tall fescue grown at eight temperatures.**

<table>
<thead>
<tr>
<th>Species</th>
<th>Avg. FRR</th>
<th>Temperature of peak FRR</th>
<th>ΔGRavg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tall fescue</td>
<td>28.9</td>
<td>22.5</td>
<td>0.5</td>
</tr>
<tr>
<td>Sloughgrass</td>
<td>23.3</td>
<td>15.0</td>
<td>4.8</td>
</tr>
<tr>
<td>Hairgrass</td>
<td>15.9</td>
<td>20.0</td>
<td>6.3</td>
</tr>
<tr>
<td>LSD_{0.05}</td>
<td>3.6</td>
<td>2.3</td>
<td>0.5</td>
</tr>
</tbody>
</table>

† The single-state physiologic measurement are average fluorescence reappearance ratio (FRR), temperature of peak FRR, and average change in the apparent Michaelis–Menten constant (kₘ) of glutathione for glutathione reductase (ΔGRavg).

where cᵢ off of the diagonal is the Euclidean distance for each of the three grasses with the other two grasses for the variable measured. The distance matrices were used to compare variables using the MXCOMP command in NTSYSpc.

The multistate measurements for interspecific interference from the replacement series experiments were (i) dry mass of the seedlings grown in monoculture at the four temperatures (four measurements) and (ii) interference dry mass as a percentage of the monoculture control for each grass grown with its two competitors at three replacement series densities and four temperatures (24 measurements). The single- and multistate measures of species physiologic responses were (i) FRR using 12 measurements through time at eight temperatures (96 measurements); (ii) average FRR of the 12 time periods for each of the eight temperatures (one measurement; from Table 2); (iii) temperature of peak FRR (one measurement; from Table 2); (iv) GR thermal response, measured as the kₘ at eight temperatures (eight measurements); and (v) GR thermal stability, measured as ΔGR obtained by subtracting the kₘ for each temperature from the kₘ of the next higher temperature (seven measurements) and as ΔGR obtained by calculating the average of the seven ΔGR observations (one measurement; from Table 2).
Fig. 2. The effect of four temperatures on the seedling dry mass per pot of tall fescue, sloughgrass, and tufted hairgrass grown in monoculture and in two-species mixtures at three mixture ratios. The relative crowding coefficient (RCC) for each pair of grasses ($k_{fb}$, tall fescue with sloughgrass; $k_{bf}$, sloughgrass with tall fescue; $k_{fd}$, tall fescue with tufted hairgrass; $k_{df}$, tufted hairgrass with tall fescue; $k_{bd}$, sloughgrass with tufted hairgrass; and $k_{db}$, tufted hairgrass with sloughgrass) grown in mixtures is shown. Lines with $X$ indicate the combined species yields. All standard-error bars are smaller than the size of the symbols.

in a warmer temperature regime (28–22°C day and night, respectively) than at a lower regime (22–16°C day and night, respectively), for which barley and wild oat were more aggressive than foxtail (Wall, 1993). These findings further support the concept of temperature-compensating effects on the relative competitiveness of different grass species.

Evidence that maximal density was achieved for constant yield of individual tall fescue seedlings was shown by the similar dry mass amounts that resulted when tall fescue was grown in monoculture or with sloughgrass in the 75:25 mixture and by the modest seedling dry mass increase when grown with hairgrass. The advantage for tall fescue when grown with the less aggressive hairgrass at elevated temperatures was especially apparent in the 50:50 mixed population. Tall fescue seedlings grown in mixtures with the less competitive species experienced less intraspecific interference because fewer number of tall fescue seedlings took advantage of relatively more space afforded by the less aggressive species. Thus, tall fescue seedlings produced more dry mass when grown in mixtures with a less competitive species than when grown in monoculture.

At 5°C, tall fescue generally was less aggressive than sloughgrass and hairgrass in that the same amount of
Fig. 3. The effects of four temperatures on interspecific interference among tall fescue, sloughgrass, and tufted hairgrass grown in two-species combinations at three mixture ratios (25:75, 50:50, and 75:25). Data are presented as dry mass per seedling for each species as a percentage of the monoculture yield (equality with the monoculture control is represented by 0%) and each temperature-density condition. Symbol keys indicate the order of the species in the two-species mixture. The TF, SG, and HG indicate tall fescue, sloughgrass, and tufted hairgrass, respectively. Bars indicate the standard error of the mean, and ns indicates the yield is not different from the monoculture seedling mass at $P \leq 0.01$ according to Fisher's protected LSD test.

Fig. 4. The effect of temperature on the dark recovery of photosystem II (PS II) chlorophyll variable fluorescence from leaves of sloughgrass, hairgrass, and tall fescue following illumination at 25°C. Data are presented as fluorescence reappearance ratio (FRR). Each point is the mean of five or more replications. Bars indicate the standard error of the mean. Standard errors of the mean not shown are smaller than the size of the symbol.

Native Grass Aggressiveness

Sloughgrass, like tall fescue, was generally more aggressive than hairgrass at 10, 20, and 30°C when it comprised 25, 50, or 75% of the mixed population with hairgrass. The only exception was the 75:25 ratio at 10°C, in which seedling dry mass was the same as in its monoculture control even though at 5°C, tall fescue was less aggressive toward sloughgrass and hairgrass than the two natives toward tall fescue (Fig. 2).
the monoculture control. At 5°C, sloughgrass seedling growth was similar to tall fescue in all population ratios and produced larger seedlings than in monoculture when grown in the 25:75 and 75:25 population ratios. Particularly in the 75:25 population ratio, sloughgrass produced more seedling dry mass when mixed with tall fescue at 5 and 10°C than its monoculture control. This demonstrated that sloughgrass was at least as well adapted as tall fescue when grown under cool temperature conditions and could tolerate a greater seedling density than tall fescue. The greater sloughgrass seedling dry mass (concurrent with tall fescue seedling mass being equal to its monoculture control) may have been due to sloughgrass seedlings growing without intraspecific interference while tall fescue seedling growth may have been more physiologically limited at cooler temperatures. Sloughgrass seedling dry mass in the 25:75 mixture with tall fescue was 0.032 mg seedling⁻¹ ± 0.012 mg and in monoculture was 0.018 mg seedling⁻¹ ± 0.001 mg. The only conditions in which hairgrass had a greater seedling dry mass than its monoculture control were at 5°C in the 25:75 and 75:25 population ratios when grown with either tall fescue or sloughgrass and at 10°C in the 75:25 population ratio when grown with sloughgrass. There were no conditions in which hairgrass interference reduced seedling dry mass of tall fescue or sloughgrass compared with their monoculture controls even though at 5°C, the hairgrass RCC was greater than that for tall fescue and sloughgrass (Fig. 2).

These findings from the replacement series interference studies generally demonstrated the compensating effects of these grasses on their relative aggressiveness due to thermal adaptive differences, particularly at low temperatures.

**Fluorescence Reappearance and Glutathione Reductase Thermal Stability**

As with dry mass production, both the variable fluorescence reappearance response (Fig. 4) and apparent $k_m$ for glutathione (Fig. 5) varied for the three grasses over the range of temperatures used in the replacement series experiments. The two native grasses were less thermally stable than tall fescue as measured by the average change in apparent $k_m$ for glutathione over the range of four growing temperatures ($\Delta GR_{avg}$) (Table 2). The variable fluorescence reappearance peaked at 15°C for sloughgrass, 20°C for tufted hairgrass, and 22.5°C for tall fescue (Table 2). The average FRR was correlated with $\Delta GR$ and $\Delta GR_{avg}$ ($P < 0.10$) (Table 3), which generally confirms the utility of variable fluorescence for determining plant responses to thermal stresses (Mahanta et al., 1990; Burke, 1990; Burke, 1995; Burke and Oliver, 1993; Ferguson and Burke, 1991). The high association of temperature of peak FRR with the FRR provides an easy-to-determine estimator of overall variable fluorescence response over a range of temperatures (Table 3). However, unlike average FRR, temperature of peak FRR was not associated with any other response or predictor variables, which rules out further utility as an indicator of plant aggressiveness.

Both the average FRR and $\Delta GR$ of the three grasses were correlated with their seedling dry mass production.

Table 3. Product moment correlations ($r$) from the normalized Mantel Z statistics for comparisons of chlorophyll variable fluorescence and glutathione reductase (GR) activity measurements used to estimate the relative aggressiveness of three grasses based on their ability to produce seedling dry mass.

<table>
<thead>
<tr>
<th>Measurement†</th>
<th>FRR</th>
<th>Avg. FRR</th>
<th>Temp. of peak FRR</th>
<th>$k_m$</th>
<th>$\Delta GR$</th>
<th>$\Delta GR_{avg}$</th>
<th>Seedling dry mass</th>
<th>Interference dry mass</th>
<th>$r$</th>
</tr>
</thead>
<tbody>
<tr>
<td>FRR</td>
<td>1.00</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Avg. FRR</td>
<td>0.42</td>
<td>1.00</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Temp. of peak FRR</td>
<td>$-1.00$</td>
<td>0.33</td>
<td>1.00</td>
<td>$-0.51$</td>
<td>0.56</td>
<td>0.59</td>
<td>1.00</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$k_m$</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\Delta GR$</td>
<td>$-0.67$</td>
<td>0.96$^{**}$</td>
<td>$-0.60$</td>
<td>$-0.29$</td>
<td>1.00</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\Delta GR_{avg}$</td>
<td>0.21</td>
<td>0.97$^{**}$</td>
<td>$-0.11$</td>
<td>0.74</td>
<td>$-0.86$</td>
<td>1.00</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Seedling dry mass</td>
<td>0.67</td>
<td>0.96$^{**}$</td>
<td>$-0.59$</td>
<td>0.30</td>
<td>$-1.00$</td>
<td>0.86</td>
<td>1.00</td>
<td>0.76</td>
<td>0.98$^{**}$</td>
</tr>
<tr>
<td>Interference dry mass</td>
<td>0.79</td>
<td>0.89</td>
<td>$-0.73$</td>
<td>0.12</td>
<td>$-0.98$</td>
<td>0.76</td>
<td>0.98$^{**}$</td>
<td>1.00</td>
<td></td>
</tr>
</tbody>
</table>

† FRR, fluorescence reappearance ratios for eight temperatures at 12 times after illumination; $k_m$, apparent Michaelis–Menten constant of glutathione for glutathione reductase (GR) measured at eight temperatures; $\Delta GR$, total change in apparent $k_m$ over the range of measured temperatures; and $\Delta GR_{avg}$, average change in apparent $k_m$; Seedling dry mass is the weight of individual seedlings grown in monoculture at 5, 10, 20, and 30°C; and interference dry mass is the average seedling dry mass due to interference measured at four temperatures in three mixture ratios.

‡ Superscripts indicate the number of degrees of freedom, relationships with $P < 0.10$ were accepted as significant.
(r = 0.96 and −1.00, respectively). The ΔGR was also negatively correlated with interference dry mass production, which indicated that the more thermally stable GR is, the greater the production of seedling dry matter by the grasses in competition with one another over the range of temperatures tested (Table 3). The availability of objective quantitative measures of the variable fluorescence response curves (Fig. 4) average FRR and temperature of peak FRR may augment the visual FRR interpretations used in previous reports (Burke, 1990; Ferguson and Burke, 1991; Burke and Oliver, 1993). The repeatability of our FRR methods with those reported in a previous study (Burke, 1990) was demonstrated by our wheat standard results (data not shown).

These findings suggest differential responses to temperature by PS II, as indicated by the chlorophyll fluorescence response, and the stability of GR are possible predictors of the relative aggressiveness among grass species over a range of growing temperatures. The use of physiological responses for inferring interspecific interference is novel, and further research is needed to test their utility with other species.

CONCLUSIONS

This study provides insights into the relative aggressiveness of sloughgrass, hairgrass, and tall fescue seedlings during seedling development. Based on replacement series experiments, seedlings of sloughgrass and tufted hairgrass were less aggressive than tall fescue seedlings, particularly as temperature increased. The native species were less susceptible than tall fescue to intraspecific interference, so it may be possible to enhance native species establishment if seeds are planted at high densities during cool temperature periods. Our findings also suggest that the stability of GR and the efficiency of the PS II apparatus under elevated temperatures may be indicators of relative species aggressiveness. Tall fescue has possibly been able to dominate some temperate western USA landscapes because of greater PS II efficiency and GR stability under elevated temperatures than sloughgrass and hairgrass. The findings of this experiment are limited to early plant development of three grasses, so further research is needed to determine how different growing temperature affects plant interference in established stands or on seed production potential of these and other species.

ACKNOWLEDGMENTS

The authors thank Chris Poklemba, Don Streeter, and Brad Thompson for technical assistance with this research. Thank you also to Dr. John Burke for his assistance with methodology development and Dr. Mark Wilson for the first review of the manuscript and his helpful suggestions.

REFERENCES