AN ABSTRACT OF THE THESIS OF

Tim G. Brewer for the degree of Masters of Science in Crop Science presented on December 19, 1996. Title: Plant Growth, Thermal Stress Response, and Enzyme Kinetic Relationships in Native Wetland and Introduced Grasses.

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Jeffrey J. Steiner

Previous studies suggest that the thermal dependence of enzyme kinetic parameters and chlorophyll a variable fluorescence can be used as indicators of species thermal optima and tolerance limits. The effect of temperature on relative interspecific competition and its relationship to thermal dependence of the apparent Michaelis-Menten constant ($K_m$) for glutathione reductase (EC 1.6.4.2, GR) (oxidized glutathione as substrate) and post-illumination reappearance of chlorophyll a variable fluorescence ($F_v$) was investigated for two Pacific Northwest native wetland and one introduced grass. The apparent $K_m$ for GR extracted from leaves of slough grass (*Beckmannia syzigachne* Steud.), tufted hairgrass (*Deschampsia caespitosa* L.), tall fescue (*Festuca arundinaceae* Schreb. cv. Titan), and a corn (*Zea mays* L.) reference species was determined over the range of 1 to 40°C. For all species, minimum apparent $K_m$ for GR was observed at 1°C, and $K_m$ values increased as temperature increased. The enzyme from tufted hairgrass had the lowest apparent $K_m$ at < 15°C, followed in increasing order by slough grass, tall fescue, and corn. Enzyme kinetic data indicated that tufted hairgrass was the most cool-

temperature tolerant species, followed by slough grass and tall fescue, respectively. Reappearance of $F_v$ peaked at 15°C for slough grass, 17.5°C for tufted hairgrass and, 22.5°C for tall fescue. Seedlings of these grasses grown across a gradient of temperatures ranging from 5 to 32.5°C showed peaks of biomass production that were similar to Fv recovery peaks. Multiple replacement series experiments with species mixtures planted in the proportions: 0:1, 25:75, 50:50, 75:25, and 1:0 were conducted at 5, 10, 20, and 30°C. These results allowed ranking of slough grass, tufted hairgrass, and tall fescue according to relative competitiveness for those temperatures. Tall fescue was the most competitive of the three grasses and its aggressiveness, relative to slough grass and tufted hairgrass, increased with increasing temperature. Slough grass and tufted hairgrass ranked second and third, respectively, and were more aggressive at lower than higher temperatures. The response of $F_v$ reappearance to temperature and indications from enzyme kinetics were effective predictors of plant relative aggressiveness among these grasses when grown in mixtures at different temperatures.

# Plant Growth, Thermal Stress Response, and Enzyme Kinetic Relationships in Native Wetland and Introduced Grasses.

By

Tim G. Brewer

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Dean of Graduate School

I understand that my thesis will become part of the permanent collection of Oregon State University libraries. My signature below authorizes release of my thesis to any reader upon request.

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Tim G. Brewer, Author

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## CHAPTER 1

### General Introduction and Literature Review

The goals of this research project were to evaluate and employ two techniques that have been presented recently concerning the characterization of temperature dependencies of several plant physiological processes. The temperature dependent recovery of chlorophyll a variable fluorescence as an indicator of photosynthetic efficiency and temperature dependent variation in the Michaelis-Menten constant ($K_m$) of enzyme catalyzed reactions have been suggested as effective means for determining plant temperature-dependent growth characteristics. These characterizations were subsequently evaluated as to their usefulness to predict grass seedling growth and relative competitive ability when exposed to a wide range of ambient temperatures.

The subject of molecular level adaption of organisms to temperature has been vigorously studied in animal systems, especially ectothermic vertebrates. A recent focus has been on adaptive variations in protein structure and kinetic properties among homologous proteins from differently adapted species or differently acclimated populations of a species. Such studies conducted on plant systems are few in recent literature. Much of this research with plants was motivated by the desire to improve productivity of crop plant species adapted to specific thermal environments but grown in different climates. Identification of plant characteristics that limit productivity under thermal stress conditions may point the way to improving management practices that alter

the growing environment (i.e. irrigation schemes) or identify germplasm that is suited to specific environments. Understanding the adaptive characteristics that influence individual plant performance and determining the extent of their influence in moderating interspecific interactions (i.e. competition) can also help to determine the best management practices needed to maintain natural native plant ecosystems and rehabilitate native species in disturbed sites (Steiner and Greene, 1996).

Thermal dependence of enzyme-substrate affinity, the Michaelis-Menten Constant.

The rate of an enzymatic reaction is affected by the affinity of the enzyme to its substrate. The influence of temperature on this relationship is related to conformational changes of enzyme structure that alter the specific shape of the active site. The Michaelis-Menten constant ($K_m$) is numerically equal to the concentration of a substrate at which the rate of the reaction is half that of the maximal rate attained at saturation of the enzyme with the substrate. The $K_m$ value is inversely proportional to the strength of the enzymes' affinity to the substrate because the greater the affinity the lower the substrate concentration needed to achieve the maximum reaction rate. When a substrate is available in excess, changes in $K_m$ values will only slightly affect the rate of the reaction. However, when substrate concentrations are low, as is usually the case for live cells, a decrease or increase in the $K_m$ will enhance or suppress the rate of the reaction, respectively. Consequently, as reaction rates increase with temperature, reduced enzyme-substrate affinity, as shown by increases in apparent $K_m$, serves as a regulatory mechanism. Conversely, as reaction rates decreased with temperature, increased

enzyme-substrate affinity, as shown by decreases in $K_m$, serves as a positive modulator of the retarding affect of cooling. This rate-compensating mechanism is defined as positive thermal modulation (Somero 1975, 1978).

The influence of temperature on enzyme system adaption has been actively studied, especially in comparative and environmental ectothermic animal physiology (Somero, 1995). Because these kinds of animals must maintain metabolic control at different temperatures, the influence of temperature on enzyme-ligand affinities is of great importance. It is believed that these systems have evolved under specific temperature-constrained environments. The value of $K_m$ as an important rate moderating mechanism has been shown by Hochachka and Somero (1984) and Somero (1995). The influence of temperature on homologous lactic dehydrogenases from animals from contrasting thermal environments was determined from 0 to 45°C (Hochachka and Somero, 1984). Values of the apparent $K_m$ increase as a positive function of temperature with their curves displaced along the temperature axis. These responses are reflective of the temperatures an organism normally experiences within its physiologically adaptive range. The value of apparent $K_m$ at each species' physiological temperature is conserved within a narrow range. Apparent $K_m$ values for enzymes from warm-adapted species are lower at common assay temperatures than for cold-adapted species. Enzymes from eurythermal species (organisms that can live in a wide range of temperatures) retain stable apparent $K_m$ over the wide temperature ranges characteristic of their habitats. Rapid increases in $K_m$ with increasing temperature are characteristic of enzymes from

stenotherms (organisms that can live only in a narrow range of temperatures) (Somero, 1995).

Recent investigations with plant enzymes generally show similar results. Positive thermal modulation by change in apparent $K_m$ is described for $NADP^+$-malate dehydrogenases (EC 1.1.1.82) from: *Lathyrus japonicus* Willd. (Simon, 1979), two species of *Aster* and their hybrid (Brouillet and Simon, 1980), *Arabidopsis thaliana* L. (Simon et al., 1983), and two ecotypes of *Echinochloa crus-galli* L. (Lapointe et al., 1989; Simon and Vairinhos, 1991). It is also evident for glutathione reductases (EC 1.6.4.2, GR) from: *Pinus strobus* L. (Anderson et al., 1992), *Picea rubens* Sarg. (Hausladen and Alscher, 1994) and *Zea mays* L. cultivars with contrasting thermotolerances (Turner et al., 1994).

Although the enzymes from most plants analyzed produce apparent $K_m$ to temperature plots that are consistent with the positive thermal modulation model, others such as some transaminases show flat responses that indicate stability of enzyme function over a wide range of temperatures (Simon et al., 1983). Results with $NADP^+$-malic enzyme (EC 1.1.1.40) and phosphoenolpyruvate carboxylase ($PEP_C$) (EC 4.1.1.31) from two populations of *Echinochloa crus-galli* L. collected in contrasting climates (Dubuc et al., 1988; Simon et al., 1984) show negative thermal modulation (Hochachka and Somero, 1984). Such a response indicates improved enzyme-substrate affinities at warm temperatures that are optimal for *Echinochloa crus-galli* L. and other warm-temperature-adapted species (Simon et al., 1984).

Another kind of $K_m$-temperature response is where the apparent $K_m$ is at a minimum around the organisms physiological temperature and include: $PEP_C$ from *Bryophyllum fedttschenkoi* at 25°C (Jones et al., 1978), and phemylalanine ammonia lyase from sweet potato at 20°C (Tanaka and Uritani, 1977).

Glutathione reductase is a commonly investigated enzyme and has been utilized to quantify the temperature range of optimum enzyme function. Mahan et al. (1990), modifying results of Terri et al. (1978), developed the concept of a thermal kinetic window (TKW) as an indicator of thermal optima and tolerance limits. They defined TKW as the temperature range where the apparent $K_m$ of NADPH ($K_{m(NADPH)}$) was within 200% of the minimum apparent $K_m$ observed for a species. By these criteria, an enzyme would have its maximal insensitivity to temperature within this range (Kidambe et al., 1990; Mahan et al., 1990; Anderson et al., 1992; Burke and Oliver, 1993; Burke, 1995; Burke and Upchurch, 1995).

Haulsladen and Alsher (1994) theorize that enzymes from plants adapted to climates of moderate temperatures are more likely to produce apparent $K_m$ to temperature profiles with minimums around physiological temperatures and unstable values as temperature extremes are approached. Conversely, plants adapted to harsh climates are more likely to retain stable apparent $K_m$ values throughout a wide range of temperatures. Furthermore, although their results were inconclusive, they suggest modulating mechanisms such as increasing isozyme concentration serves to stabilize the systems.

A recent investigation (Turner et al., 1994), demonstrates that reduced enzyme-substrate affinity at high temperatures, as shown by increases in apparent $K_m$, may be

offset by increased $V_{max}$ values. Therefore, *in vivo* enzyme function may not always be impaired by temperature-dependent changes in apparent Km. Glutathione reductase was shown to function efficiently over a wide temperature range with no significant differences in $K_m$-temperature responses identified among maize cultivars adapted to contrasting thermal environments. These authors conclude that deriving physiologically important information from apparent $K_m$-temperature profiles is difficult to interpret from *in vitro* experiments.

Thermal dependence of recovery of chlorophyll variable fluorescence

The recovery of chlorophyll photosystem II (PSII) variable fluorescence ($F_v$) following illumination has been shown to be an effective *in vivo* indicator of photosynthetic efficiency (Papageorgiou, 1975; Krause and Weis, 1984). Recent studies and reviews have described a method to identify optimal temperature characteristics of species through the measurement of the thermal response of induced variable fluorescence reappearance following illumination across a range of temperatures (Burke, 1990; Ferguson and Burke, 1991; Burke and Oliver, 1993; Burke, 1995). These reports propose that different thermally adapted species show unique variable fluorescence recovery characteristics that are reflective of physiologically optimal temperatures. High temperature acclimation of plants reduces $F_v$ absolute values but does not alter the temperatures of optimum recovery (Ferguson and Burke, 1991).

Variable fluorescence recovery is presented as the ratio $F_v/F_o$, where $F_o$ = initial fluorescence and $F_v$ = (F maximum - $F_o$). Since $F_o$ changes little throughout experiments,

increases in the ratio demonstrates increases of the $F_v$ component. When experiments are conducted across a range of temperatures and the $F_v/F_o$ ratios are plotted against recovery time in darkness, the temperature at which the ratio reaches the highest peak in the least amount of time is indicative of the optimal temperature for that species (Burke, 1990).

Relative interspecific competition

The relative competitiveness of different species can be evaluated using a substitutive, or replacement series experimental design (deWit, 1960). This design is considered to be effective for assessing the relative aggressiveness of different species because it separates the effects of density and proportion by holding total mixture density constant while species proportions are varied. Inter- and intra-specific competition effects can be separated because each species is also grown alone. The concept of density-independent yield (law of constant final yield) is the basic premise of this design and an important assumption is that yields in mixtures can be established from monoculture yields.

The level of interference between species in a replacement series is established by comparing deviations from expected yields. A competitive interaction model occurs when one species contributes more than expected to the total yield while the second species contributes less than expected. Such an interaction suggests that the two species are utilizing a common limited resource and that the species have different competitive abilities (Harper, 1977). Since emergence timing is an important factor in determining

dominance and greatly influences competitiveness (Ross et al., 1972), it is critical to establish stands of same-age and same-size seedlings.

Jolliffe et al. (1984) have addressed the criticisms and limitations of replacement series experiments and demonstrated that the influence of total mixture density is an important limitation of replacement series experiments. It was recommended that multiple experiments be conducted over a range of densities to separate the effects of total density on the interactions in mixtures. However, when the research goal is to assess relative competitive ability, Taylor and Aarssen (1989) argued that it is sufficient to conduct experiments at a single density providing that the planting density is high enough to achieve monoculture yields that are independent of density (constant final yield). Their results suggest that density dependence in mixture experiments is restricted to experimental designs where the density of the mixtures is below the density required to provide a constant final yield.

Although we have not found literature on the subject, it is plausible that by conducting multiple replacement series experiments over a range of temperatures, the influence of temperature on relative competitive ability of different species with different optimal temperature adaptions can be evaluated.

# CHAPTER 2

## Thermal Dependence of the Apparent $K_m$ of Glutathione Reductase from Three Wetland Grasses

Abstract

The thermal dependence of enzyme kinetic parameters has been presented as an indicator of species thermal optima and tolerance limits. Previous studies suggest the relationship between temperature and the apparent Michaelis-Menten constant ($K_m$) of an enzyme system can be used to predict whole-plant success at specific temperatures. The apparent $K_m$ for glutathione reductase (EC 1.6.4.2, GR) (oxidized glutathione as substrate) extracted from leaves of slough grass (*Beckmannia syzigachne* Steud.), tufted hairgrass (*Deschampsia caespitosa* L.), tall fescue (*Festuca arundinaceae* Schreb. cv. Titan), and the reference species corn (*Zea mays* L.) was determined over the range of 1 to 40°C. For all species, minimum apparent $K_m$ for GR was observed at 1°C, and $K_m$ values increased as temperature increased. The apparent $K_m$ values differed in stability among all species from 1 to 15°C, but became more similar at higher temperatures. The enzyme from tufted hairgrass had the lowest apparent $K_m$ at < 15°C, followed in increasing order by slough grass, tall fescue, and corn. Our experimental system failed to reproduce 'thermal kinetic window' profiles similar to those reported by others when cuvette condensation was eliminated from the spectrophotometer reaction chamber by the introduction of desiccated air. Cuvette condensation increased experimental error and skewed $K_m$ values at low temperatures. Enzyme kinetic data indicated that tufted hairgrass was the most cool-temperature tolerant species, followed by slough grass and tall fescue, respectively.

Introduction

The rate of an enzymatic reaction is affected by the affinity of the enzyme to its substrate. The influence of temperature on this relationship is related to conformational changes of enzyme structure that alter the specific shape of the active site. The Michaelis-Menten constant ($K_m$) is numerically equal to the concentration of a substrate at which the rate of the reaction is half that of the maximal rate attained at saturation of an enzyme with the substrate. The $K_m$ value is inversely proportional to the strength of an enzymes' affinity to the substrate because the greater the affinity the lower the substrate concentration needed to achieve the maximum reaction rate. When a substrate is available in excess, changes in $K_m$ values will only slightly affect the reaction rate. However, when substrate concentrations are low, as is usually the case in plant systems, a decrease or increase in the $K_m$ will enhance or suppress the rate of the reaction, respectively. Consequently, as reaction rates increase with temperature, reduced enzyme-substrate affinity, as shown by increases in $K_m$, serves as a regulatory mechanism. Conversely, as reaction rates decreased with temperature, increased enzyme-substrate affinity, as shown by decreases in $K_m$, serves as a positive modulator of the retarding affect of cooling. This rate-compensating mechanism is defined as positive thermal modulation (Somero 1975, 1978).

The influence of temperature on enzyme system adaption has been actively studied in comparative and environmental ectothermic animal physiology (Somero, 1995). Since some animals must maintain metabolic control at different temperatures, the influence of temperature on enzyme-ligand affinities is of great importance. It is

believed that enzyme systems have evolved under the specific constraints of the temperature environment experienced by these species. The importance of $K_m$ as a rate moderating mechanism has been shown by Hochachka and Somero (1984) and Somero (1995). The influence of temperature on homologous lactic dehydrogenases from animals of contrasting thermal environments was determined from 0 to 45°C. It was shown that the apparent $K_m$ increased as a positive function of temperature and, the value of apparent $K_m$ at each species' physiological temperature was conserved within a narrow range (Hochachka and Somero, 1984). Apparent $K_m$ values for enzymes from warm-adapted species were lower at common assay temperatures than for cold-adapted species. Enzymes from eurythermal species (organisms that can live in a wide range of temperatures) retain stable apparent $K_m$ over wide temperature ranges characteristic of their natural habitats. Rapid increases in $K_m$ with increasing temperature are characteristic of enzymes from stenotherms (organisms that can live only in a narrow range of temperatures) (Somero, 1995).

Recent investigations with most plant enzymes generally show results similar to those discussed for animal systems. Positive thermal modulation by change in apparent $K_m$ is described for $NADP^+$-malate dehydrogenases (EC 1.1.1.82) from *Lathyrus japonicus* Willd. (Simon, 1979), two species of *Aster* and their hybrid (Brouillet and Simon, 1980), *Arabidopsis thaliana* L. (Simon et al., 1983), and two ecotypes of *Echinochloa crus-galli* L. (Lapointe et al., 1989; Simon and Vairinhos, 1991). It was also evident for glutathione reductases (EC 1.6.4.2, GR) from *Pinus strobus* L. (Anderson et

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In another kind of $K_m$-temperature response, $PEP_C$ from *Bryophyllum fedttschenkoi* was shown to have a minimum apparent $K_{m(PEP)}$ around 25 °C (Jones et al., 1978), and the $K_{m(phenylalanine)}$ of phenylalanine ammonia lyase from sweet potato at a minimum at approximately 20 °C (Tanaka and Uritani, 1977).

Glutathione reductase is a commonly investigated enzyme and has been utilized to quantify the temperature range of optimum enzyme function. Mahan et al. (1990), modifying results of Terri et al. (1978), developed the concept of a 'thermal kinetic window' (TKW) as an indicator of thermal optima and tolerance limits. They defined TKW as the temperature range where the apparent $K_m$ of NADPH ($K_{m(NADPH)}$) is within 200% of the minimum apparent $K_m$ observed for a species. By these criteria, an enzyme

has its maximal insensitivity to temperature within this range (Kidambe et al., 1990; Mahan et al., 1990; Anderson et al., 1992; Burke and Oliver, 1993; Burke, 1995; Burke and Upchurch, 1995).

Glutathione is an essential metabolite in plants and has several important roles including prevention of enzyme and membrane system oxidation and the regulation of gene expression associated with environmental stress responses (Creissen et al., 1991). Glutathione reductase (NAD(P)H:oxidized-glutathione oxidoreductase) catalyzes the NAD(P)H-dependent reduction of oxidized glutathione (GSSG) to reduced glutathione (GSH). In higher plants, GR is present in high concentrations in the chloroplasts and maintains high levels of GSH, an important detoxificant during conditions of photoxidative stress (Halliwell and Foyer, 1978).

Haulsladen and Alsher (1994) theorized that enzymes from plants adapted to moderate temperature climates were more likely to produce apparent $K_m$ to temperature profiles with minimums around physiological temperatures and unstable values as temperature extremes were approached. Conversely, plants adapted to harsh climates were more likely to retain stable apparent $K_m$ values over a wide range of temperatures. Although their results were inconclusive, they suggested that modulating mechanisms such as increasing isozyme concentration serves to stabilize the systems.

Slough grass (*Beckmannia syzigachne* Steud.), a summer annual, and tufted hairgrass (*Deschampsia caespitosa* L.), a perennial, are two native wetland grasses adapted to the moderate climate of the U.S. Pacific Northwest (PNW). Interest in their

conservation has raised the question of their ability to re-seed and persist in the presence of non-native competitors. Tall fescue (*Festuca arundinaceae* Schreb.), a widely adapted perennial species, is among several introduced grasses that are common in PNW wetlands. Decisions concerning best management practices to use in natural stands and

rehabilitated sites may be influenced by better understanding the adaptive mechanisms that influence plant performance, including identification of thermal optima.

The objective of this study was to determine the thermal optima for GR from slough grass, tufted hairgrass, and tall fescue. Apparent $K_m$s for the substrate GSSG were determined. In addition, the apparent $K_m$s for the substrate NADPH was also measured for corn to compare our results with those of Mahan et al. (1990).

## Materials and methods

### *Plant Material*

Seeds of slough grass (*Beckmannia syzigachne* Steud.) were collected from wild populations near Corvallis, OR (44.33N,123.15W). Tufted hairgrass (*Deschampsia caespitosa* L.) seeds were obtained from the USDA-NRCS, Plant Materials Center, Corvallis, Oregon. Tall fescue (*Festuca arundinaceae* Schreb. cv. Titan) and corn (*Zea mays* L. cv. Early Sunglow) seeds were obtained from a commercial source. Plants were grown in the greenhouse under 25/20° C day/night conditions with 12 hr supplemental lighting and fertilized weekly with a complete nutrient solution containing

473 mg $L^{-1}$ N. Fresh leaf material was sampled from unstressed juvenile plants and frozen at -70°C prior to GR extraction.

*Enzyme Extraction*

Glutathione reductase (EC 1.6.4.2, GR) was extracted from frozen leaves that were ground to a powder in liquid $N_2$ and suspended in 0.1M Hepes (pH 8.0) that contained 1% polyvinyl-polypyrrolidone (PVPP) (1.33 mg fresh weight · $mL^{-1}$ buffer). The slurry was centrifuged at 10,000*g* for 60 min. Dry ammonium sulfate was added to the supernatant to 50% of saturation with constant stirring. The mixture was centrifuged at 10,000*g* for 60 min and ammonium sulfate added to the supernatant to 70% saturation. After a third and final centrifugation, the supernatant was discarded and the pellet resuspended in 0.1M Hepes buffer (pH 8.0). Further purification was deemed unnecessary (Turner and Pollock, 1993).

*Enzyme Assay*

Enzyme assays and kinetic analyses were conducted on a Shimadzu UV-2101 spectrophotometer (Shimadzu Scientific Instruments, Columbia, MD) equipped with a water flow-through cell holder to maintain constant reaction temperatures. The 1.0 mL assay cuvette mixture contained: 125 mM Hepes buffer (pH 8.0), 100 µM NADPH, 1 of 10 concentrations of GSSG ranging from 30 to 165 µM and GR extract (approximately 0.02 units). A unit of activity was defined as the quantity of enzyme that catalyzed the reduction of 1.0 µmol GSSG $min^{-1}$ at each assay temperature. Enzyme activity was

determined from a decrease in absorbance at 340 nm. Each reaction temperature was achieved by preincubating the reaction mixture in a waterbath. The range of reaction temperatures evaluated were from 1 to 40° C (±0.5 °C) at 5° C increments. The use of Hepes buffer, which has a $\Delta$pKa of -0.014 units $°C^{-1}$ (Gueffroy, 1975), allowed pH to vary with temperature and approximated intra-cellular pH response to changing temperature (Reeves, 1977; Patterson and Graham, 1987). A linear least squares function was used to calculate initial reaction velocity and the slope of the line was converted to $\Delta A_{340}$ $min^{-1}$. A function derived from the regression fit of absorbance values to NADPH gradient standards was used to convert $\Delta A_{340}$ $min^{-1}$ to µmol GSSG reduced $min^{-1}$.

*Apparent Michaelis-Menten Constants*

The apparent Michaelis-Menten constant ($K_m$) for GSSG was determined at a fixed concentration of NADPH (100 µM). At this concentration NADPH was saturating and non-inhibiting to enzyme function. Initial velocity was recorded over a range of 10 concentrations of GSSG (30 to 165 µM), which was approximately 0.5 to 5.0 times $K_m$. After addition of GR extract, the cuvette was sealed with parafilm, inverted several times to initiate the reaction, wiped with tissue to remove moisture and immediately monitored for enzyme activity in the spectrophotometer. The progress of the reaction was monitored for 15 s at a rate of 602 observations $min^{-1}$. Assays were replicated a minimum of three times each for each substrate (GSSG) concentration combination at each temperature. The apparent $K_m$ was calculated using the Eadie-

Hofstee linear transformation plot and the method of least squares.

Water vapor condensation on the reaction cells at temperatures lower than 15° C was prevented by introducing desiccated air into the reaction chamber. The desiccated air was prepared by forcing air through a column of silica gel stones. To test the effect of water condensation on $K_m$ calculations, enzyme activity was measured with and without desiccated air introduced into the reaction chamber.

Results

The apparent $K_{m(GSSG)}$ of GR from slough grass, tufted hairgrass, and tall fescue increased as temperature increased, with a minimum $K_m$ recorded at 1 °C for each of the three species (Fig. 2.1). The absolute apparent $K_m$s for the three species were different at lower temperatures (1-15 °C), but became more similar as temperature increased. Glutathione reductase from tufted hairgrass had the lowest apparent $K_m$ values at lower temperatures, followed by slough grass and tall fescue, respectively. Apparent $K_mS_{(GSSG)}$ of GR obtained at low temperatures (less than 15 °C) were exaggerated and erratic when $K_m$ calculations were based upon measurements taken when desiccated air was not introduced into the reaction chamber (Fig. 2.2).

The apparent $K_{m(GSSG)}$ of GR from corn increased as temperature increased, with a minimum recorded at 10 °C (Fig. 2.3A). The apparent $K_mS_{(GSSG)}$ for corn across a range of temperatures were most similar to those of tall fescue. When apparent $K_m$s of corn GR were also measured using NADPH, a similar relationship to temperature was observed as for GSSG, with a minimum at 5 °C (Fig. 2.3B).

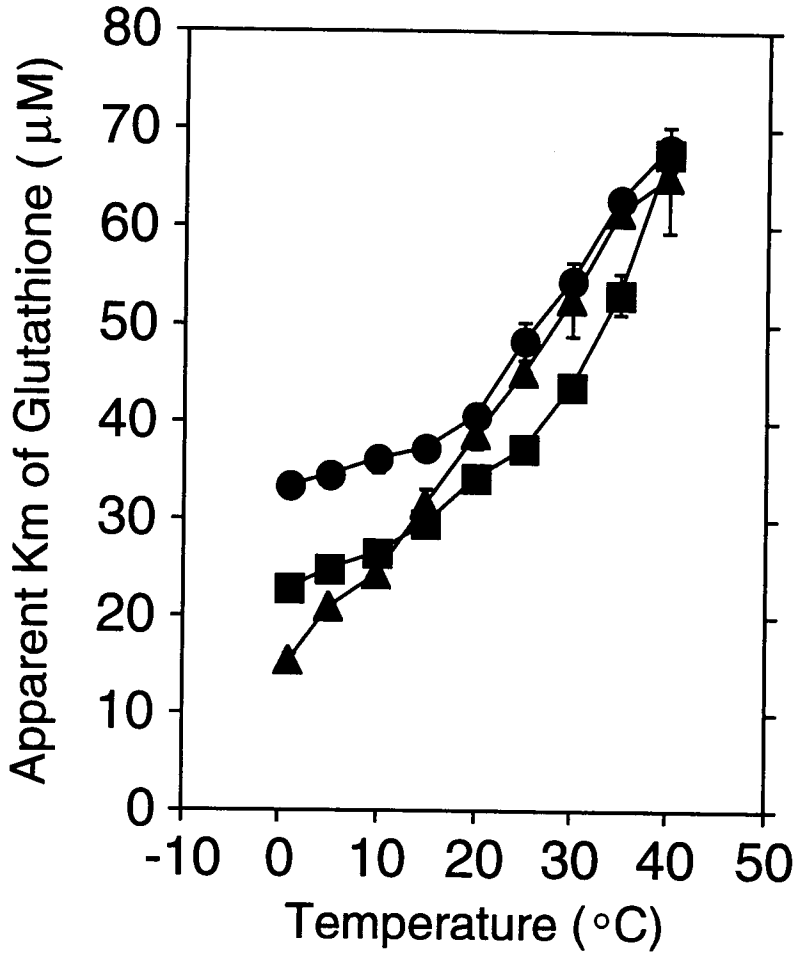


Figure 2.1 Effects of temperature on the Michaelis-Menten constant ($K_m$) of glutathione for glutathione reductase of ● tall fescue (*Festuca arundinacea* Schreb. cv. Titan), ■ slough grass (*Beckmannia syzigachne* Steud.), and ▲ tufted hairgrass (*Deschampsia caespitosa* L.). Cold temperature experiments (≤ 15°C) were conducted with the inclusion of desiccated air into the spectrophotometer reaction chamber to prevent condensation buildup on cuvette surfaces. Each point is the mean of five or more replications ± one standard error of the mean.

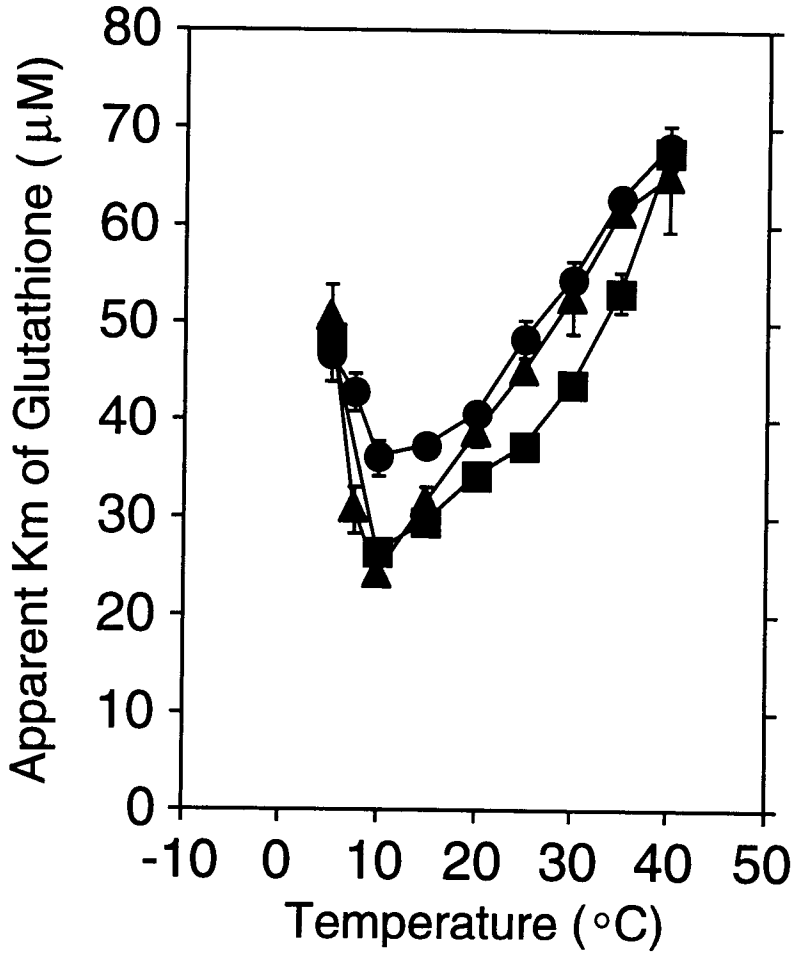


Figure 2.2 Effects of temperature on the Michaelis-Menten constant ($K_m$) of glutathione for glutathione reductase of ● tall fescue (*Festuca arundinacea* Schreb. cv. Titan), ■ slough grass (*Beckmannia syzigachne* Steud.), and ▲ tufted hairgrass (*Deschampsia caespitosa* L), measured without the introduction of desiccated air into the spectrophotometer reaction chamber. Values below 10°C are artifactually inflated due to water vapor condensate effects on measurements of initial velocity. Each point is the mean of five or more replications ± one standard error of the mean.

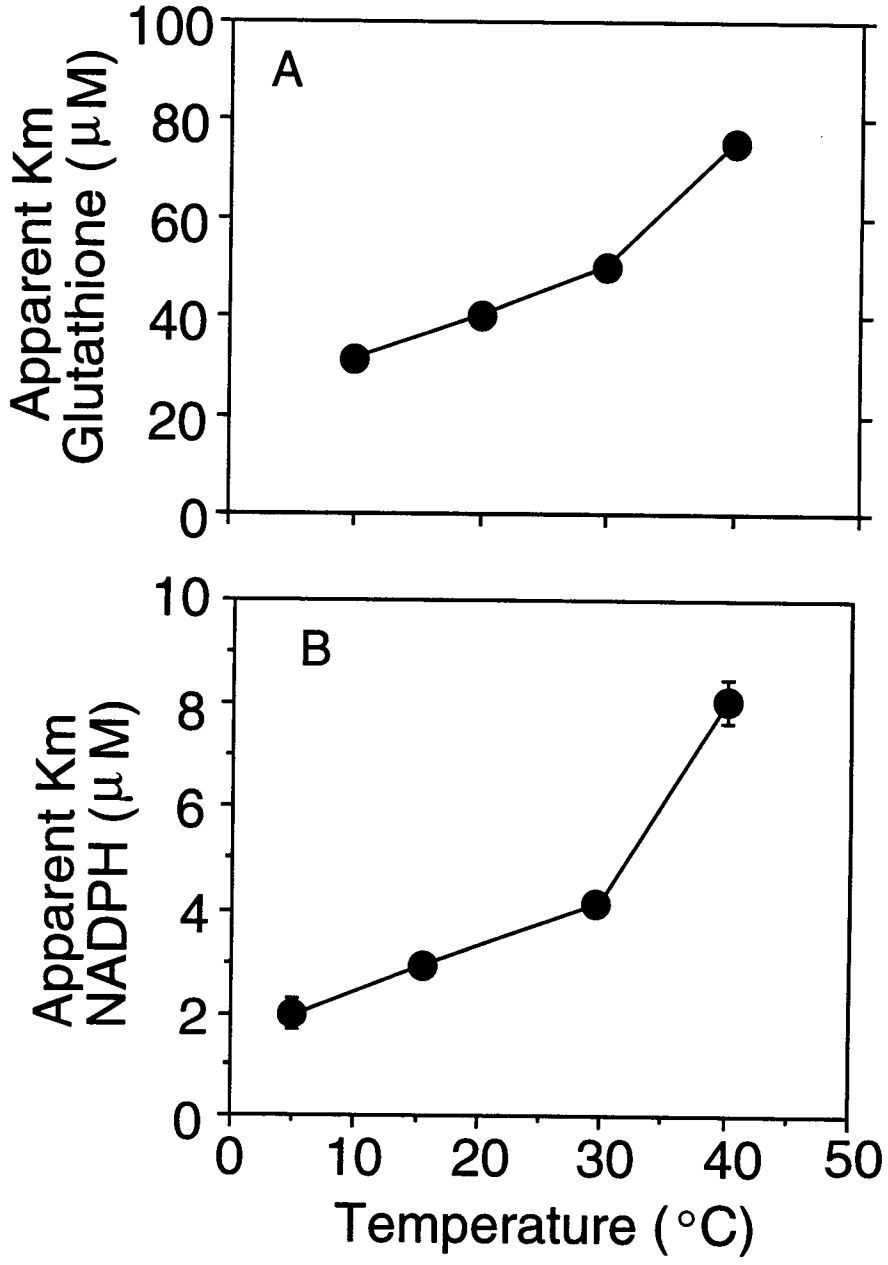


Figure 2.3 Effects of temperature on the Michaelis-Menten constant ($K_m$) of glutathione (A) and NADPH (B), for glutathione reductase of corn. Each point is the mean of five or more replications ± one standard error of the mean.

Discussion

Apparent $K_mS_{(GSSG)}$ of GR from slough grass, tufted hairgrass, and tall fescue increased as positive functions of temperature and are conserved within a narrow range at each species physiological temperature (Brewer et al., 1996). This response is similar to that reported for several enzyme systems in ectothermic animals (Hochachka and Somero, 1984; Somero, 1978; Coppes and Somero, 1990; Dahlhoff and Somero, 1993; Lin and Somero, 1995) and plants (Simon, 1979; Brouillet and Simon, 1980; Simon et al., 1983; Lapointe et al., 1989; Simon and Vairinhos, 1991; Anderson et al., 1992; Hausladen and Alscher, 1994; Turner et al., 1994). At common assay temperatures, the apparent $K_mS_{(GSSG)}$ of GR determined for these species are consistent with those reported for GRs from non-hardened red spruce (*Picea rubens* Sarg.) (Hausladen and Alscher, 1994), eastern white pine (*Pinus strobus* L.) (Anderson et al., 1992) and corn cultivars with contrasting adapted thermotolerances (Turner et al., 1994).

Since GSH has been implicated as an important antioxidant under conditions of low temperature induced photoxidative stress, it is expected that the affinity of GR for GSSG should increase with decreasing temperatures. Our results show that the affinity of GR for GSSG increases as assay temperatures decrease as shown by decreasing apparent $K_m$s (Fig. 2.1). Rapid increases of apparent $K_{m(GSSG)}$ of GR for tufted hairgrass suggests it is stenothermic. Conversely, GR from tall fescue, a widely adapted species, and slough grass, a summer annual, maintain more stable apparent $K_mS_{(GSSG)}$ over a wide temperature range that is consistent with eurythermic adaption (Somero, 1995).

Under the conditions of these experiments, few differences in temperature dependence of apparent $K_{m(GSSG)}$ of GR were found between slough grass, tufted hairgrass, and tall fescue at temperatures above 20°C. However, considering the assumption that low $K_m$ values are an advantage for enzyme function (which is true only at low substrate concentrations), the differences at lower temperatures (1-15°C) may allow ranking by correlation with grass growth success at cool temperatures. By this criterion, with its low apparent $K_{m(GSSG)}$ values, tufted hairgrass was the most cool-temperature tolerant species, followed by slough grass and tall fescue, respectively. Ranking according to thermal dependence of GR was supported by whole plant biomass production (Brewer et al., 1996). Compared to slough grass and tall fescue, tufted hairgrass produced more biomass at cold temperatures (< 20°C) relative to optimal production at 20°C. Slough grass and tall fescue ranked second and third, respectively.

The thermal dependence of the apparent $K_{m(GSSG)}$ of GR from corn was most similar to tall fescue, with apparent $K_m$s increasing as a positive function of temperature. Using GR from corn, Mahan et al. (1990) reported a minimum apparent $K_{m(NADPH)}$ at 30°C with values increasing as temperatures decrease or increase. In contrast, our results for GR extracted from corn using $K_{m(NADPH)}$ and $K_{m(GSSG)}$ produced curvilinear profiles related to increasing temperature which is consistent with the positive thermal modulation model (Hochachka and Somero, 1973). At common assay temperatures, our apparent $K_m$s using $K_{m(NADPH)}$ and $K_{m(GSSG)}$ for corn GR are consistent with those reported for a GR from eastern white pine (Anderson et al., 1992) and for GRs ($K_{m(GSSG)}$)

from corn cultivars with contrasting adapted thermotolerances (Turner et al., 1994). Because we observed the minimum apparent $K_m$ for each species at the lowest assay temperature used (1 °C), a TKW could not be defined.

Many factors can affect *in vitro* $K_m$ determinations (Burke, 1995). Determination of thermal dependent apparent $K_m$ profiles using the small $K_m$s typical for NADPH is difficult since small errors greatly confound results (Burke and Oliver, 1993). Isozymes with different affinities for substrates can accumulate in response to the environment (Guy and Carter; 1984) and affect enzyme kinetics characterizations (Smith et al., 1989). Inconsistencies between our results for corn and those previously reported (Mahan et al., 1990) may be due to the use of different species, varieties, or differences in acclimation temperatures affecting isozyme expression. Acclimation experiments can identify whether more complex interactions associated with corn GR temperature dependent kinetics are influenced by isozyme expression (Simon, 1979).

We found the use of desiccated air to eliminate water condensation on the spectrophotometer reaction cells was important for two reasons. First, when initial velocity determinations were delayed to allow condensate clearing, measured values were depressed and subsequent calculated apparent $K_m$s were markedly higher than those measured immediately after reaction initiation. Second, condensate on reaction cell walls interfered with true absorption measurements, so as clearing occurred, the initial velocity measurements were exaggerated.

The technique described to eliminate condensation has been routinely applied but not reported in previous studies of thermal dependence of enzyme kinetic parameters (Somero, personal communication, 1995). The introduction of dried air into the reaction chamber allows accurate spectrophotometer measurements across a broad range of cold assay temperatures. We strongly recommend reevaluation of experiments conducted at low temperatures to ensure that water vapor condensation does not occur on cuvette surfaces and thus bias absorbance measurements.

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# CHAPTER 3

## Thermal dependence of Chlorophyll a Variable Fluorescence and Interspecific Competition Among Native Wetland and Introduced Grasses

Abstract

Previous studies suggest that the thermal dependence of chlorophyll a variable fluorescence can be used to characterize optimal plant temperatures for growth. The effect of temperature on relative interspecific competition and its relationship to post-illumination reappearance of chlorophyll a variable fluorescence was investigated for two Pacific Northwest native wetland and one introduced grass. Dark recovery of the variable component ($F_v$) of PS II fluorescence at temperatures ranging from 5 to 40° C was measured in native slough grass (*Beckmannia syzigachne* Steud.), and tufted hairgrass (*Deschampsia caespitosa* L.), and for tall fescue (*Festuca arundinaceae* Schreb. cv. Titan) a non-native grass species. Reappearance of $F_v$ peaked at 15°C for slough grass, 17.5°C for tufted hairgrass, and 22.5°C for tall fescue. Seedlings of these grasses grown across a gradient of temperatures ranging from 5 to 32.5 °C showed optimal peaks of biomass production similar to $F_v$ recovery peaks. Multiple replacement series experiments with species mixtures planted in the proportions: 0:1, 25:75, 50:50, 75:25, and 1:0 were conducted at 5, 10, 20, and 30°C. These results allowed ranking of slough grass, tufted hairgrass, and tall fescue according to relative competitiveness for the four temperatures. Peak temperatures for PS II $F_v$ recovery and seedling growth were predictive of shifts in levels of relative aggressiveness in response to temperature. Tall fescue was the most competitive of the three grasses and its aggressiveness, relative to slough grass and tufted hairgrass, increased with increasing temperature. Slough grass and tufted hairgrass ranked second and third, respectively,

and were more aggressive at lower than higher temperatures. The response of $F_v$ reappearance to temperature was an effective predictor of plant relative aggressiveness among these grasses when grown in mixtures at different temperatures.

## Introduction

Slough grass (*Beckmannia syzigachne* Steud.), an annual, and tufted hairgrass (*Deschampsia caespitosa* L.), a perennial, are two native wetland species of the Pacific Northwest (PNW). Interest in native habitat conservation has raised the question of whether these grasses have the capacity to persist and reseed in the presence of introduced competitors. Tall fescue (*Festuca arundinaceae* Schreb.) is a widely adapted introduced perennial grass that is common in PNW wetlands. Understanding the adaptive characteristics that influence individual plant performance and relative interspecific competition will help determine the best management practices needed to maintain natural stands and to rehabilitate disturbed sites.

A substitutive, or replacement series experimental design is effective for assessing the relative aggressiveness of different species because 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 intra-specific plant competition effects can be separated because each species is also grown alone. The concept of density-independent yield (law of constant final yield) is the basic premise for this design, and an important assumption is that yields in population mixtures can be estimated from monoculture yields.

The level of interference between species in a replacement series is determined by comparing deviations from expected yields. A competitive interaction occurs when one species contributes more than expected to the total yield while the other species contributes less. Interactions suggest the two species are utilizing a common limited resource and have different competitive abilities (Harper, 1977). Since emergence time can be an important factor that initially determines dominance and greatly influences competitiveness (Ross et al., 1972), it is critical to establish stands of same-age and same-size seedlings. To our knowledge there is no literature reporting the influence of temperature on relative competitive ability of different species.

The thermal response of photosystem II (PS II) variable fluorescence ($F_v$) reappearance following illumination has been described as a method to identify optimal temperature characteristics of species (Burke, 1990; Ferguson and Burke, 1991; Burke and Oliver, 1993; Burke, 1995). These reports propose that different thermally adapted species show unique variable fluorescence recovery characteristics that are reflective of physiologically optimal temperatures. The objectives of this study were to define the optimal growth temperatures for two native and one introduced grass species grown over a range of temperatures and to compare these results with optimums measured by the thermal dependence of PS II $F_v$ recovery. These results will be used to predict the effect of temperature on relative interspecific competitive ability.

Materials and methods

*Substitutive Competition Assay.*

Seeds of slough grass (*Beckmannia syzigachne* Steud.) were collected from wild populations near Corvallis, OR (44.33N,123.15W). Tufted hairgrass (*Deschampsia caespitosa* L.) seeds were obtained from the USDA-NRCS, Plant Materials Center, Corvallis, Oregon. Tall fescue (*Festuca arundinaceae* Schreb. cv. Titan) seeds were obtained from a commercial source.

All seeds were germinated on blotter paper (7 days in 0.2% $KNO_3$ at 5 °C) and systematic mixtures of 60 same age/size seedlings transplanted into 15 cm top-diameter standard plastic pots (3,390 plants $m^{-2}$) in the proportions: 0:1, 25:75, 50:50, 75:25, and 1:0. Planting density was derived from preliminary experiments to determine the density at which a constant final yield was obtained within seven weeks at 5 °C. Plants were provided non-limited amounts of soil (standard potting mixture), water (saturated every two days), and fertilizer (applied weekly with a complete nutrient solution containing 473 mg $L^{-1}N$). Injured and dead plants were replaced soon after initial planting with surplus same age/size seedlings to maintain the initial population density. There were five replicates of each monoculture and mixture at each temperature. Pots were placed in growth chambers under fluorescent and incandescent light and their positions randomized weekly. Light intensity was monitored weekly and averaged 450 $\mu mol\ m^{-2}\ s^{-1}$. To provide vertical support and improve illumination to lower plant parts, common aluminum foil was attached around each pot to three inches above the rim.

Constant thermal treatments of 5, 10, 20 and 30° C were maintained in the growth chambers. Forty-nine days after transplanting, the plants were harvested by species, dried at 70°C, and weighed to determine total above-ground dry mass. Expected yield contribution from each species in a mixture was derived from monoculture data (Harper, 1977). The total dry weight contributed by each species in a mixture was plotted as relative yield (percent of maximum). Replacement series results were analyzed using the method of Harper (1977).

We did not conduct multiple density experiments as discussed by Jolliffe et al. (1984) who suggest that the influence of total density is an important limitation of replacement series experiments. There was sufficient plant density (3390 plants $m^{-2}$) and growth for interference to occur.

*Fluorescence Reappearance.*

Plants of the three wetland species were grown in the glasshouse under 25/20° C day/night conditions with 12 hr. supplemental lighting and fertilized weekly with a complete nutrient solution containing 473 mg $L^{-1}N$. Fluorescence reappearance experiments were conducted using fully matured plant material. Wheat (*Triticum aestivum* L. *cv.* Chuan Mai #18//JUP/DJP "S") was also included as a standard for comparison with previously published results.

Variable fluorescence was measured using a Brancker SF 30 fluorometer (Richard Brancker Research, LTD, 27 Monk St., Ottawa, Canada, K1S3Y7. Temperature control was achieved using an eight-position thermal plate system

consisting of 5 X 6.5 cm independently 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). Leaf sections of the grasses were placed on moistened 3MM chromatography paper, transferred to the temperature controlled blocks, covered with $CO_2$ permeable transparent plastic film (Glad® Cling Wrap, First Brands Corporation, Danbury, CT), and illuminated (high pressure sodium lamp; 650 μmol $m^{-2} s^{-1}$) for 10 min. at 25° C. When each block reached a prescribed temperature, the lamp was turned off and chlorophyll fluorescence measurements immediately initiated and recorded thereafter at 3 min. intervals for 33 min. using a 10s excitation period with 5 W $m^{-2}$ light (procedure modified from Burke 1990). Between experiments, plants were stored in a growth chamber at 25°C with supplemental lighting (260 μmol $m^{-2}$ $s^{-1}$).

The fluorescence transients ratios $F_v/F_o$ ($F_o$ = initial fluorescence; $F_v$ = F maximum - $F_o$) measured from 5 to 40° C (5° C increments) were plotted as functions of time. The range of temperatures that achieved the highest $F_v/F_o$ ratios in the shortest period of time were reevaluated using 2.5 C° increments.

Water vapor condensation on the fluorometer probe at temperatures lower than 15°C was prevented by flushing the probe and sample area with desiccated air that was prepared by forcing air through a column of silica gel stones. To test the effect of condensation on measurements of fluorescence recovery, experiments at temperatures

lower than 15°C were conducted without the desiccated air treatment and compared with those using desiccated air.

*Thermal Gradient Table.*

Pregerminated seeds (7 days in 0.2% $KNO_3$ at 5°C) of slough grass, tufted hairgrass, and tall fescue were sown in rows at a noncompetitive density on two layers of wetted blotter paper placed on the surface of a thermal gradient table (Peters, 1990). The blotter paper was wetted daily to maintain uniform moisture and the table was provided supplemental lighting (300 μmol $m^{-2}$ $s^{-1}$). The seedlings were allowed to establish root contact with the blotter paper at room temperature (22.0°C) for two days prior to imposition of the thermal treatments. The thermal gradient ranged from 5 to 32.5° C. Seedlings were harvested after 14 days of growth with the imposed temperatures, dried, weighed, and the weights plotted against temperature.

## Results

*Substitutive Competition Assay.*

In all but one case, a single species was identified as more competitive because it contributed a disproportionate share to the total yield while the yield of the other species was suppressed. These results are consistent with the negative interference model proposed by deWit (1960). At temperatures greater than 5°C, tall fescue was more competitive than slough grass (Fig. 3.1). At 5°C, responses of both species did not deviate from the null line. As temperatures increased from 10 to 30°C, tall fescue

growth was increasingly enhanced and slough grass growth suppressed. Tall fescue was more competitive than tufted hairgrass at all temperatures (Fig. 3.2). Tall fescue yields did not deviate from the null line at 5°C, but a competitive effect was evident because tufted hairgrass was suppressed. At 10°C, tall fescue growth was enhanced and tufted hairgrass suppressed. The magnitude of the competitive effect of tall fescue with tufted hairgrass increased with increasing temperature. At temperatures greater than 5°C, tall fescue achieved a maximal yield in the 75:25 proportion when mixed with tufted hairgrass.

Slough grass was more competitive than tufted hairgrass at all temperatures (Fig. 3.3). Slough grass growth was enhanced and tufted hairgrass was suppressed beginning at 5°C, and the effect increased with temperature to 20°C. Slough grass competition with tufted hairgrass was less at 30°C than at the other temperatures.

*Fluorescence Reappearance Experiment.*

Variable fluorescence reappearance peaked at 15.0°C for slough grass, 17.5°C for tufted hairgrass and, 22.5° C for tall fescue (Figures 3.4-3.6). Peak $F_v/F_o$ ratios were highest in tall fescue followed by slough grass and tufted hairgrass, respectively. Our results of $F_v$ recovery for wheat are in agreement with those reported in a previous study (Burke, 1990), both in peak recovery temperature and absolute values of $F_v/F_o$ (data not shown).

At temperatures lower than 15°C, water vapor condensation on the fluorometer probe created artifactually high $F_v/F_o$ ratios with large standard errors when desiccated

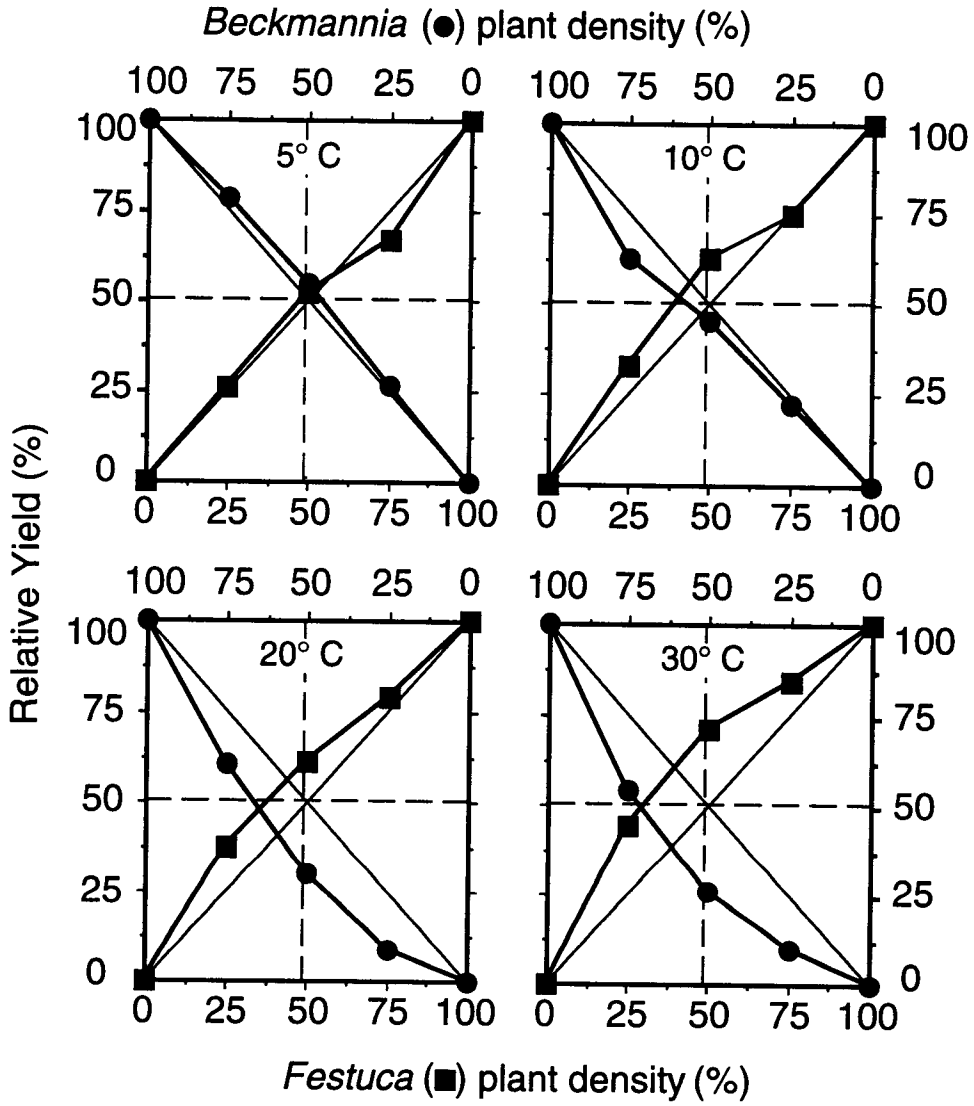


Figure 3.1. The effect of temperature (°C) on the intensity of relative interspecific competitive ability between tall fescue (*Festuca arundinacea* Schreb. cv. Titan) and slough grass (*Beckmannia syzigachne* Steud.). Data presented as dry weight yeild of each species relative to monoculture yield (100%). Expected yields (null line) are representd by the dotted line.

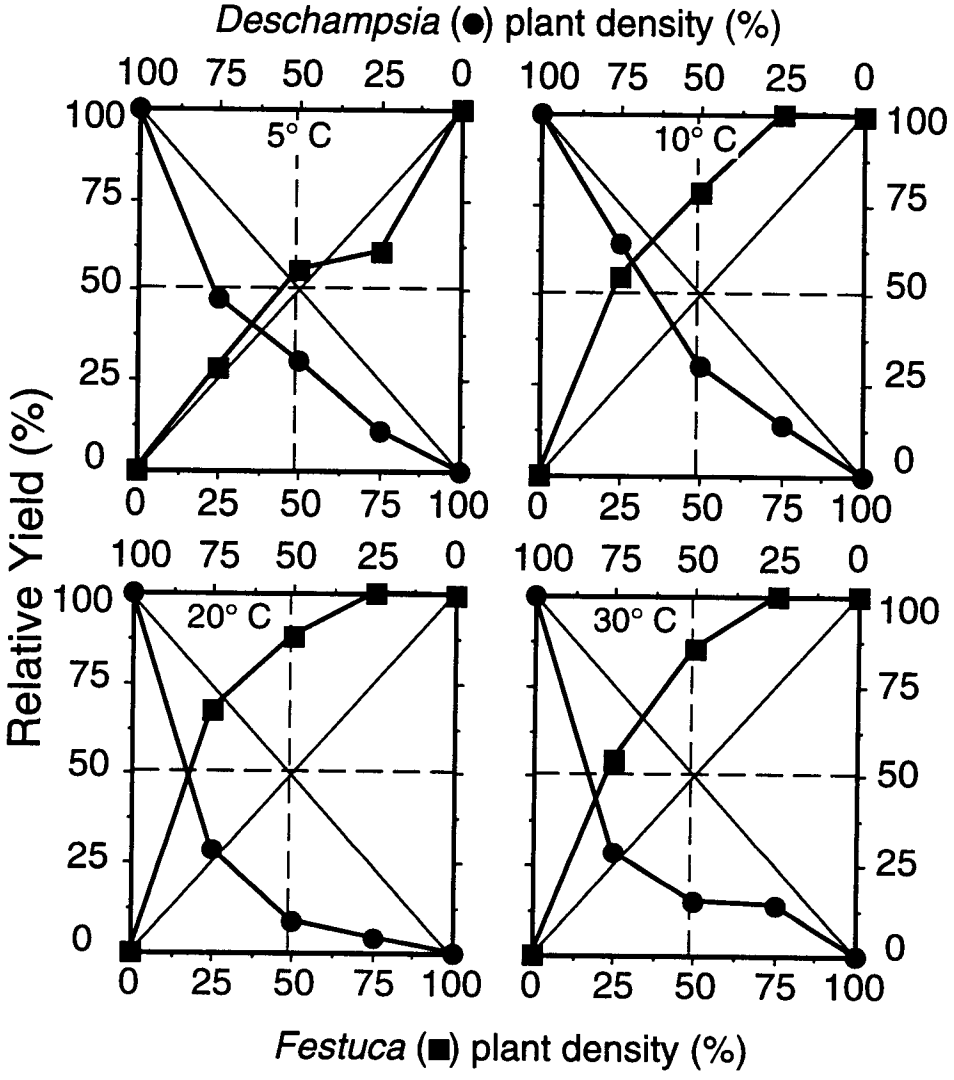


Figure 3.2 The effect of temperature (°C) on the intensity of relative interspecific competitive ability between tall fescue (*Festuca arundinacea* Schreb. cv. Titan) and tufted hairgrass (*Deschampsia caespitosa L.*). Data presented as dry weight yeild of each species relative to monoculture yield (100%). Expected yields (null line) are representd by the dotted line.

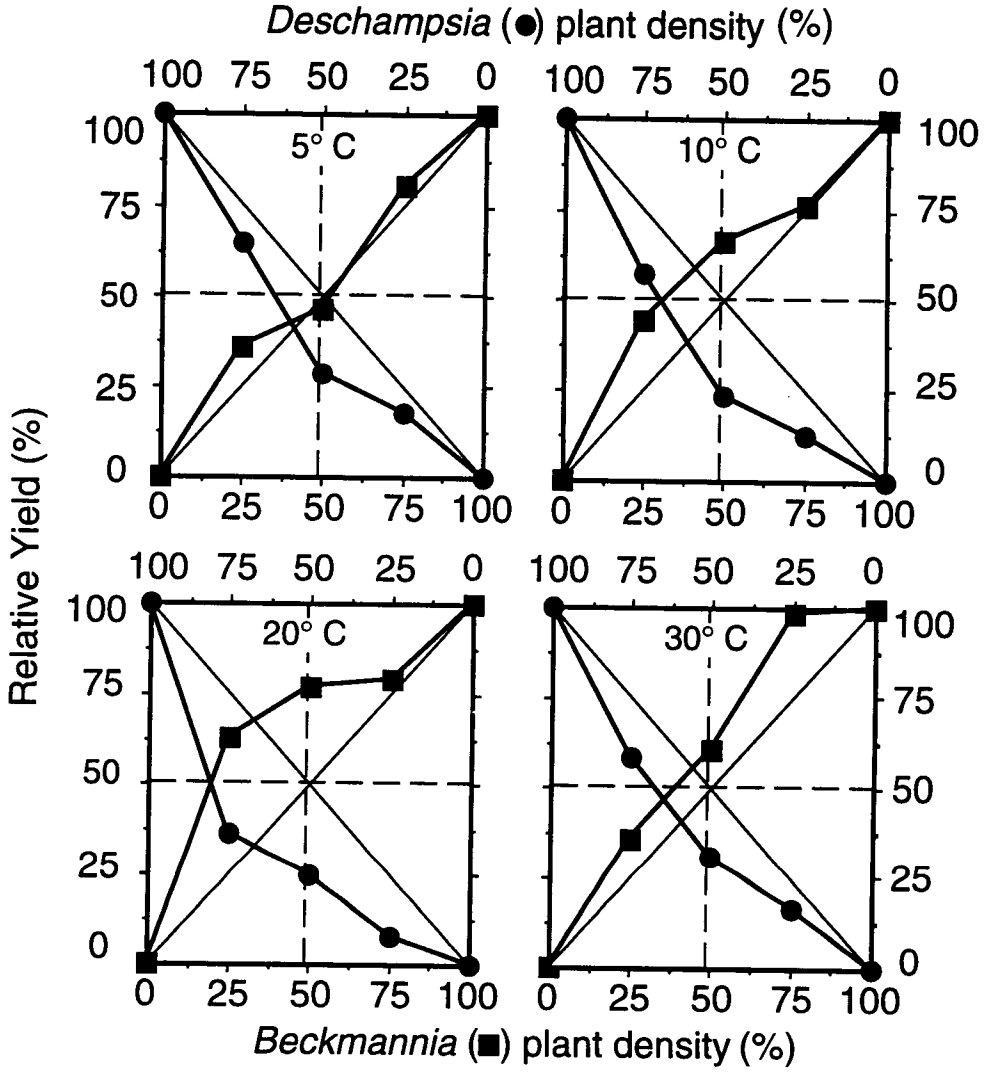


Figure 3.3 The effect of temperature (°C) on the intensity of relative interspecific competitive ability between slough grass (*Beckmannia syzigachne* Steud.) and tufted hairgrass (*Deschampsia caespitosa L.*). Data presented as dry weight yeild of each species relative to monoculture yield (100%). Expected yields (null line) are representd by the dotted line.

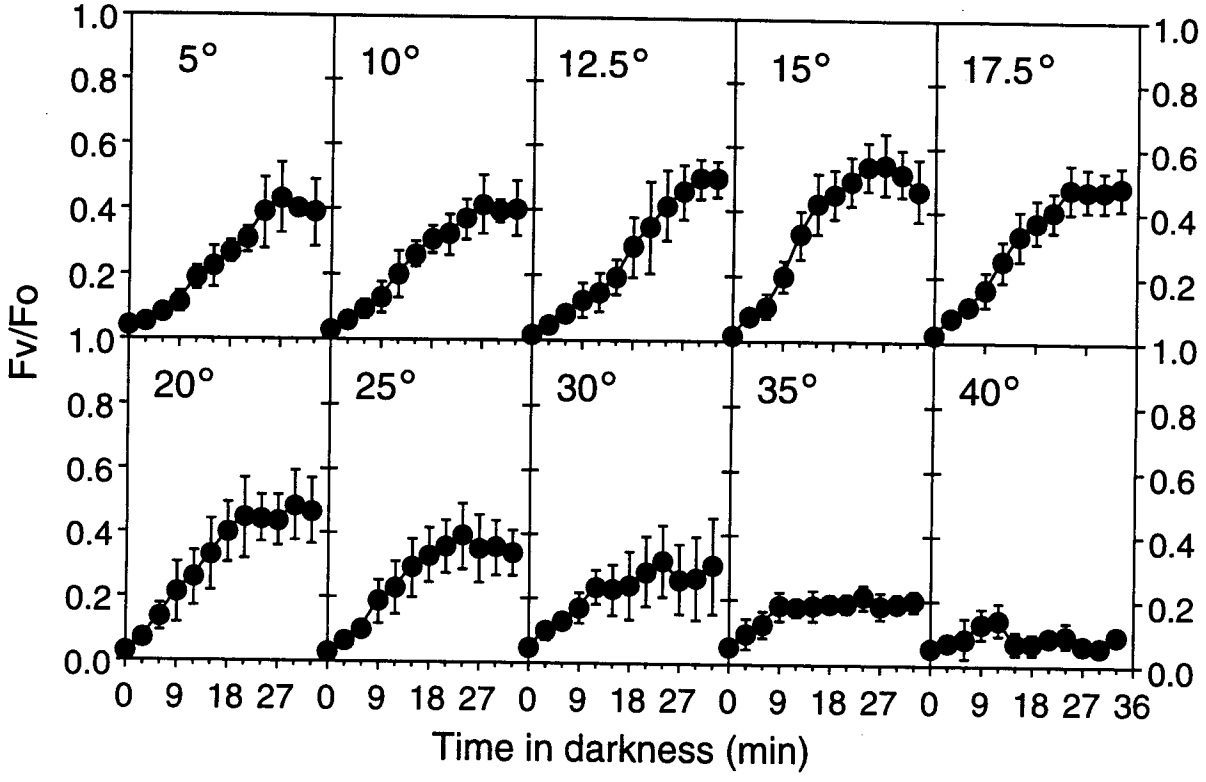


Figure 3.4 The effect of temperature on the dark recovery of photosystem II chlorophyll variable fluorescence from leaves of slough grass (*Beckmannia syzigachne* Steud.) following illumination at 25°C. Data presented as the ratio $F_v/F_o$ ($F_o$ = initial fluorescence; $F_v$ = F maximum - $F_o$). Each point is the mean of five or more replications ± one standard error of the mean.

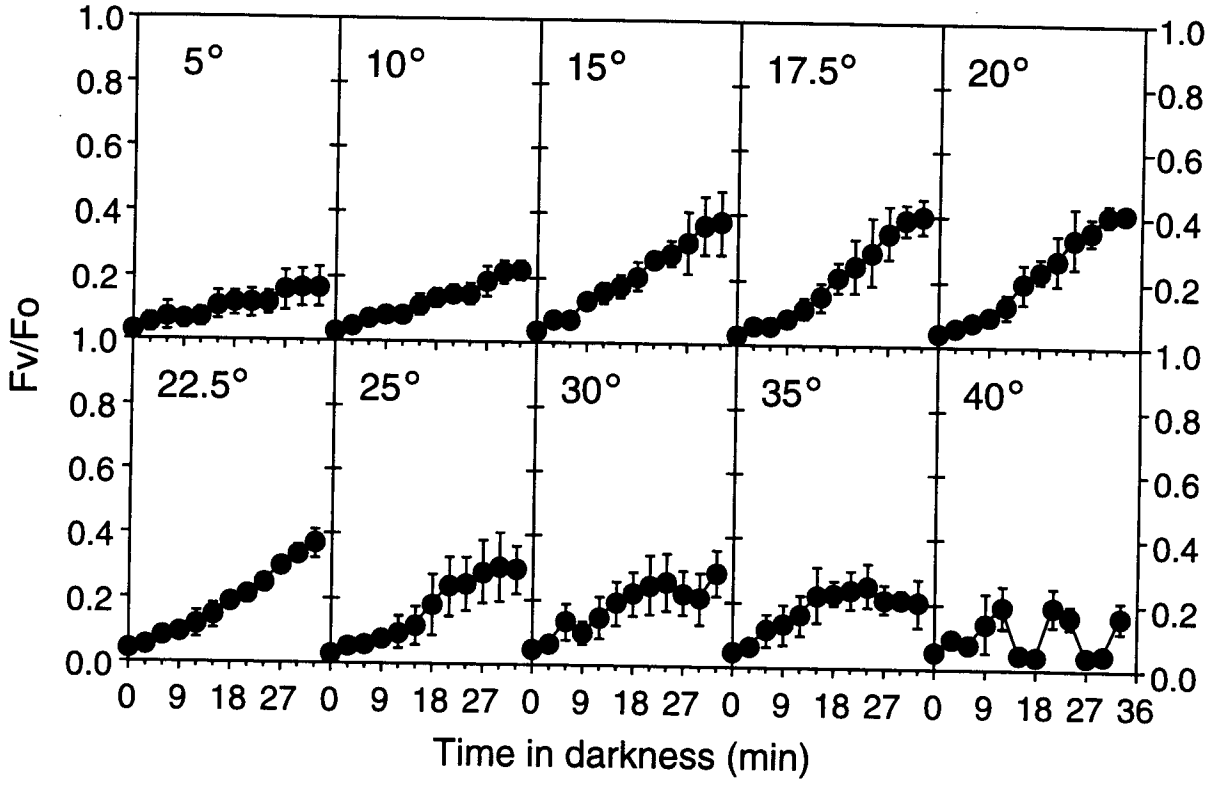


Figure 3.5 The effect of temperature on the dark recovery of photosystem II chlorophyll variable fluorescence from leaves of tufted hairgrass (*Deschampsia caespitosa* L.) following illumination at 25°C. Data presented as the ratio $F_v/F_o$ ($F_o$ = initial fluorescence; $F_v$ = F maximum - $F_o$). Each point is the mean of five or more replications ± one standard error of the mean.

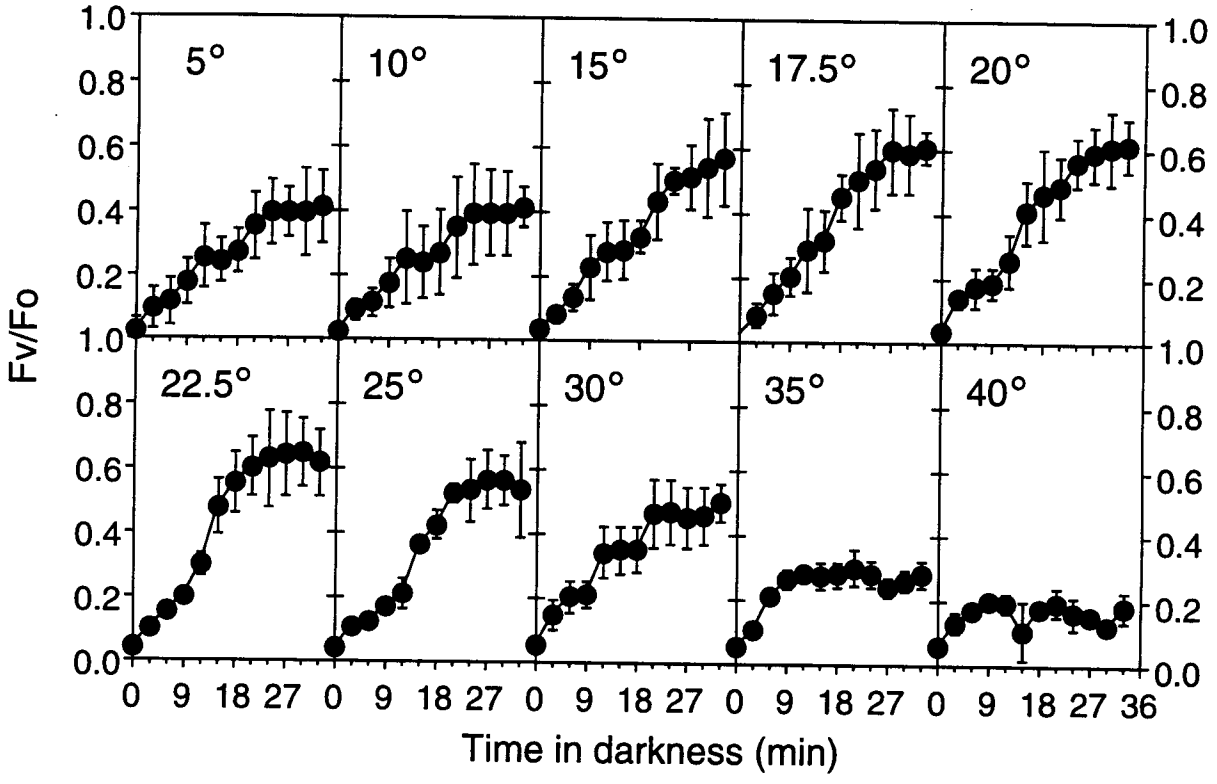


Figure 3.6 The effect of temperature on the dark recovery of photosystem II chlorophyll variable fluorescence from leaves of tall fescue (*Festuca arundinacea* Schreb. cv. Titan) following illumination at 25°C. Data presented as the ratio $F_v/F_o$ ($F_o$ = initial fluorescence; $F_v$ = F maximum - $F_o$). Each point is the mean of five or more replications ± one standard error of the mean.

air was not used to dry the probe and sample area (data not shown). Huner et al. (1992) report a similar problem and recommended application of a thin film of glycerol on fluorometer probes as a solution.

*Thermal Gradient Table.*

Tall fescue seedlings produced more biomass compared to tufted hairgrass and slough grass at all temperatures (Fig.3.7). Peak production for tall fescue was at 25.4°C. The peaks for seedling biomass production for slough grass and tufted hairgrass were 19.9 and 19.8°C, respectively, and were less defined with lower relative productivity reductions at the temperature extremes. The prediction equations for temperature of peak biomass production for each species were as follows:

1. Tall Fescue: $y = 0.4925 - 0.0155x + 0.0065x^2 - 0.0002x^3$; $r^2 = 0.969$ ($P \leq .0001$)
2. Slough grass: $y = 0.1566 + 0.0207x - 0.0005x^2$; $r^2 = 0.948$ ($P \leq .0001$)
3. Tufted hairgrass: $y = 0.117 + 0.023x - 0.0006x^2$; $r^2 = 0.952$ ($P \leq .0001$)

Discussion

Among slough grass, tufted hairgrass, and tall fescue, there was a similarity between the temperature dependent recovery of PS II $F_v$ following illumination and physiological temperature optima determined by seedling biomass production across a thermal gradient. Tall fescue peak of $F_v/F_o$ and maximum seedling biomass production occurred at 22.5 and 25.4°C, respectively. Peak $F_v/F_o$ for slough grass and tufted hairgrass were 15 and 17.5°C, respectively. Maximal seedling biomass production for

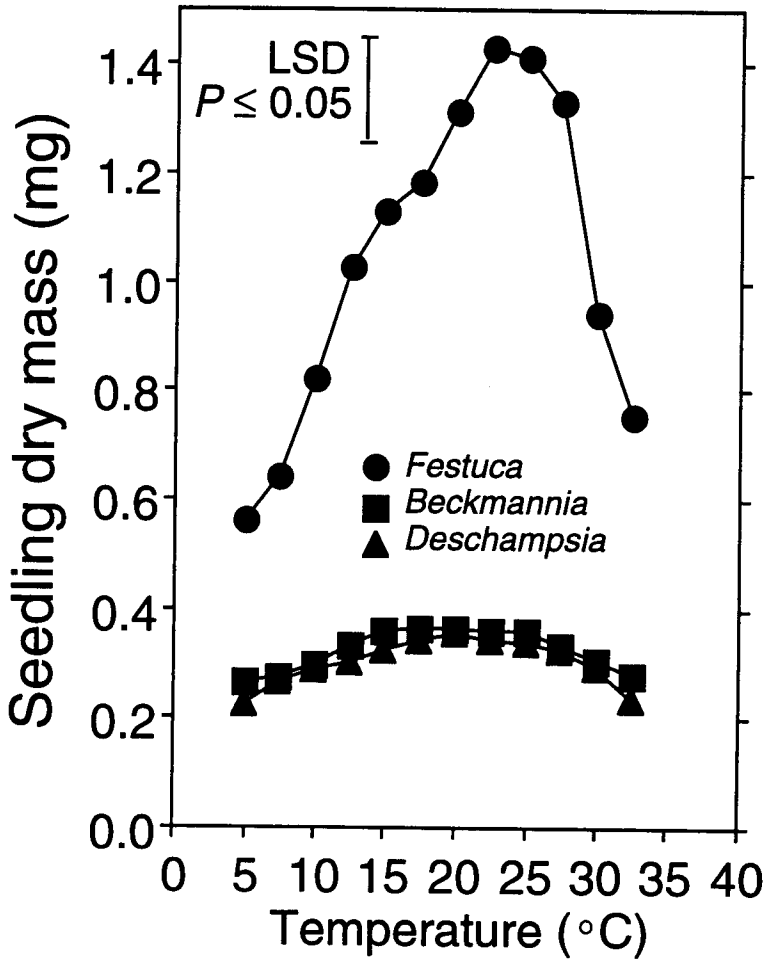


Figure 3.7 Biomass production of tall fescue (*Festuca arundinacea* Schreb. cv. Titan), slough grass (*Beckmannia syzigachne* Steud.), and tufted hairgrass (*Deschampsia caespitosa L.*) grown across a range of temperatures provide by a thermal gradient table.

slough grass and tufted hairgrass was between 17.5 and 20°C and with less of a well-defined optimal peak than for tall fescue. These results suggest that tall fescue is adapted to warm temperatures while slough grass and tufted hairgrass are adapted to cool temperatures.

The scope of this study was to determine the temperature optimums for the three species and evaluate the predictiveness of those results concerning the influence of temperature on relative interspecific competitive rankings. Tall fescue was more competitive than both slough grass and tufted hairgrass, and its aggressiveness increased with increasing temperature (Figs. 3.1 and 3.2). However, tall fescue and slough grass yields did not deviate from the expected total yield contributions at 5°C (Fig 3.1). The reasons for this may be either growth for these species at 5°C may have been insufficient to cause interference or the two species have an equal ability to interfere with the other at 5°C. Although growth rates for tall fescue and slough grass were low at 5°C, the planting density was sufficiently high for interference to occur. We suggest that slough grass and tall fescue have near equivalent competitive ability when grown in a mixture at temperatures approaching 5°C with cooler temperatures generally favoring slough grass growth.

Slough grass had a competitive advantage over tufted hairgrass when grown in a mixture, but the effect was diminished at 30°C (Fig 3.3). This suggests that both slough grass and tufted hairgrass growth are inhibited at temperatures approaching 30°C, and the reduction in growth at this high temperature resulted in a reduction in interference.

Tufted hairgrass was not competitive with either tall fescue or slough grass in any mixture proportion or at any temperature (Figs 3.2 and 3.3). Tufted hairgrass growth was suppressed more in a mixture with tall fescue than with slough grass. Tall fescue achieved constant final yield in the 75:25 proportion when mixed with tufted hairgrass at temperatures greater than 5°C. When mixed with slough grass, tall fescue did not reach a constant final yield at any proportion or temperature. This suggests that intraspecific competition was important for limiting tall fescue growth when in mixtures. Interspecific competition was more important in limiting tall fescue growth when mixed with slough grass than tufted hairgrass.

These results from the replacement series experiments at these temperatures allowed ranking of these species according to their relative competitive ability. Tall fescue became more competitive as temperature increased. Slough grass competitiveness was similar to tall fescue at 5°C, but its success decreased as temperatures increased. Tufted hairgrass was the least competitive of the three species, and its success decreased with increasing temperatures.

The relative competitiveness of these grasses over these temperatures was consistent with their rankings based on chlorophyll $F_v$ reappearance. We suggest that thermal dependent responses of chlorophyll $F_v$ reappearance can be predictors of relative competitive ability over a range of temperatures.

Seedlings of slough grass and tufted hairgrass will be most successful in competing with tall fescue if established during cool months. Early establishment, high

planting density, and moist/cool conditions should favor these native species. In managing existing stands, our results highlight the importance of maximizing soil moisture content. Diverting or draining water from wetland areas may reduce the cooling effect of soil moisture and would favor growth of more thermophilic species such as tall fescue in preference to the native slough grass and tufted hairgrass.

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# CHAPTER 4

## Common conclusion

Recovery of $F_v$, as a measure of photosynthetic efficiency, was an effective tool for characterization of species-specific thermal optima and, was an effective descriptor of temperature specific genetic diversity. Thermal dependent $F_v$ recovery results were indicative of whole plant responses to temperature treatments. The method appears to be sensitive enough to detect minor differences among species that are adapted to, or are adapting to similar environments.

Although Burke (1995) suggests that the best evidence for the validity of TKW is the supporting evidence provided by chlorophyll $F_v$ recovery, our results did not support this conclusion. Our results of thermal dependence of GR apparent $K_m$ did not fit the basic premises of the TKW concept and were consistent with positive thermal modulation model. In accord with previous authors we found this technique to be of limited value for determining the temperature characteristics of these grass species and that GR may be functioning efficiently across a wide range of temperatures. However, under the assumption that low $K_m$ values are advantageous to enzyme function, the enzymes of the three grasses can be ranked according to cool-temperatures tolerance. Tufted hairgrass was the most cool-temperature tolerant species followed by slough grass and tall fescue, respectively. This conclusion was consistent with results of $F_v$ recovery, seedling growth, and replacement series experiments across a gradient of temperatures.

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